VRIJE UNIVERSITEIT AMSTERDAM

VU Research Portal

Biological aging in major depressive disorder

Han, Laura Kim Mae

2021

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Han, L. K. M. (2021). *Biological aging in major depressive disorder*. s.n.

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 13. Dec. 2021

Biological Aging
in
Major Depressive Disorder

Laura Kim Mae Han

Colofon

The infrastructure of the Netherlands Study of Depression and Anxiety (NESDA) study (https://www.nesda.nl) is funded through the Geestkracht program of the Netherlands Organization for Health Research and Development (Zon-Mw, grant number 10-000-1002) and is further supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, Leiden University, GGZ Rivierduinen, University Medical Center Groningen, University of Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Rob Giel Onderzoekscentrum). DNA methylation research described in this thesis was supported by NIMH grant R01MH099110, and the EMGO+, Amsterdam Public Health, and Center for Biomarker Research & Precision Medicine travel grants. ENIGMA work was in part supported by the Endeavour Research Leadership award by the Department of Education of the Government of Australia. Telomere length assaying was supported through a NWO-VICI grant (number 91811602). Metabolomics data were generated within the framework of the BBMRI Metabolomics Consortium funded by BBMRI-NL, a research infrastructure financed by the Dutch government (NWO, grant nr 184.021.007 and 184033111). Gene expression data were funded by the US National Institute of Mental Health (RC2MH089951).This thesis was prepared at the Department of Psychiatry, Amsterdam University Medical Centers and GGZ inGeest, Amsterdam, within the Amsterdam Neuroscience Research Institute.

Financial support for publication and distribution of this thesis was kindly provided by: Department of Psychiatry, Amsterdam University Medical Centers & VU University Amsterdam.

ISBN: 978-94-93184-91-6

Cover design: Lucas Gijsberts
Illustrations: Luna Deckers & Joachim Rubinstein
Layout & Print: proefschrift-aio

Copyright © 2020, L.K.M. Han

All rights reserved. No part of this thesis may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval without prior permission from the author or publishers of the included papers.

VRIJE UNIVERSITEIT

BIOLOGICAL AGING IN MAJOR DEPRESSIVE DISORDER

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op vrijdag 4 juni 2021 om 13.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Laura Kim Mae Han

geboren te Amstelveen

promotor: prof.dr. B.W.J.H. Penninx

copromotor: dr. M. Aghajani

Beoordelingscommissie

Prof. Dr. Meike Bartels (VU)
Prof. Dr. Neeltje van Haren (Erasmus MC)
Prof. Dr. Odile van den Heuvel (Amsterdam UMC, locatie VUmc)
Prof. Dr. Eline Slagboom (Leiden UMC)
Prof. Dr. Guido van Wingen (Amsterdam UMC, locatie AMC)

TABLE OF CONTENTS

CHAPTER 1	General introduction	**9**
CHAPTER 2	Accelerating Research on Biological Aging and Mental Health: Current Challenges and Future Directions Published in *Psychoneuroendocrinology* 2019	**31**
CHAPTER 3	Epigenetic Aging in Major Depressive Disorder Published in *The American Journal of Psychiatry* 2018	**81**
CHAPTER 4	Do Psychiatric Problems, Lifestyle Variables and Adversities Accelerate Epigenetic Aging? Submitted for publication	**113**
CHAPTER 5	Brain Aging in Major Depressive Disorder: Results from the ENIGMA Major Depressive Disorder Working Group Published in *Molecular Psychiatry* 2020	**147**
CHAPTER 6	Contributing Factors to Advanced Brain Aging in Depression and Anxiety Disorders Submitted for publication	**203**
CHAPTER 7	An Integrative Study of Five Biological Clocks in Somatic and Mental Health Published in *eLife* 2021	**237**
CHAPTER 8	Summary and general discussion	**271**
	Dutch Summary | Nederlandse Samenvatting	**304**
	Acknowledgements | Dankwoord	**316**
	Curriculum Vitae	**320**
	Publication list	**321**
	Dissertation series	**325**

CHAPTER 1

General introduction

MAJOR DEPRESSIVE DISORDER

The Size And Burden Of Depression

Major depressive disorder (MDD) is a common and debilitating mental disorder that affects more than 264 million people of all ages worldwide [1]. This psychiatric disorder occurs about twice as often in females than males and is a leading cause of global disability, with persistent pessimistic projections into 2030 [2]. At present, it is considered the second largest contributor to the overall global burden of disease in terms of "years lived disability" [3]. Importantly, next to the total direct and indirect costs of depression, this mental disorder poses a serious personal burden and negatively impacts an individual's quality of life. At worst, depression may lead to suicide, and, sadly, this is currently one of the most common causes for death in young individuals 15-29 years old.

Clinical And Biological Characteristics of Depression

MDD is diagnosed when a person has a depressive episode that persists at least two weeks, involving obvious signs of disturbances in mood, and interests and pleasure, present nearly every day. The Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) [4] states that an individual must show five (or more) of the nine symptoms, at least including one of the two core symptoms of depressed mood and/or diminished interest, to fulfill the diagnostic criteria for clinical depression. Important to mention is that 227 possible combinations of symptoms can thus lead to the same diagnosis. In other words, it is possible for two persons with the same diagnosis of MDD to show only one overlapping symptom. Moreover, divergent symptoms, for instance, increased but also decreased appetite, can both contribute to the diagnosis. By design, depression is thus a clinically diverse and heterogeneous disorder, including many different symptom profiles.

To date, the etiology and pathophysiology of depression still remains complex and relatively unknown, perhaps in part due to its clinical heterogeneity. However, twin-based studies typically find a genetic contribution of approximately 35% [5]. The latest genome-wide association study (GWAS) involving >246,000 MDD patients shows evidence for 87 independently associated variants mapping onto 269 genes and complex biological pathways related to neurotransmission, response to stimuli, and the prefrontal brain regions [6]. Epigenome-wide association studies have identified depression

associated methylation sites in overlapping blood and brain tissue, mapping onto biological pathways important for brain development and function [7]. Beyond biological risk factors, psychosocial factors matter as well. For instance, personality traits have shown to influence the likelihood of depression. Particularly neuroticism has repeatedly and robustly been linked to current [8] or future depressive episodes [9]. Neuroticism is a personality trait that renders one susceptible to distress, negative affect, and heightened responses to threat, loss, and frustration [10]. While it is evident that there are substantial trait-like contributions to depression, there also is abundant room for social and environmental factors to play a role.

A wide range of environmental risk factors have shown to influence the risk and outcome of depression. Broadly speaking, they can be categorized into sociodemographic, environmental, and lifestyle factors [11, 12]. Strongest effects are commonly reported for childhood trauma exposure including physical and sexual abuse, psychological neglect, but also victimization, exposure to violence, and other exposures of threat and deprivation, as they predispose, increase the risk of, and predict poorer course of depression later in life [13–16]. Similarly, negative or personal life events contribute to the disease and even suicidal risk [17]. In terms of lifestyle, convergent evidence indicates that physical inactivity, smoking, poor diet, and poor sleep are major disease contributors [18]. Together, these factors represent commonly observed determinants of depression, with some of these links being bidirectional and mutually reinforcing.

From a biological perspective, several systems appear to be dysregulated in depression [19, 20], specifically those implicated in or sensitive to stress. A cluster of metabolic risk factors often occurs, including abdominal obesity, increased blood glucose (i.e. hyperglycemia), elevated blood pressure, increased triglycerides and decreased HDL cholesterol. From these components, abdominal obesity and lipid disturbances are most consistently associated with depression [21]. Another line of research indicates that depression is associated with dysregulated (innate) inflammation [22], with elevated levels of proinflammatory cytokines such as interleukin (IL)-6, and C-Reactive Protein (CRP), but no significant longitudinal associations between tumor necrosis factor-alpha and depressive symptoms [23]. Yet another line of research relates depression to autonomic dysregulations, presumably due to disturbed activations of the sympathetic nerves and reduction of parasympathetic nerve activity essential to prepare the body for a fight or flight response. However, this evidence is relatively inconsistent, might be dependent on the type of stressor [24], and seems to be confounded by

antidepressant use [25]. Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis has been reliably implicated in depression, with prolonged stress resulting in cortisol releases into the blood. Finally, structural neuroimaging studies have consistently found the prefrontal cortex and hippocampus to be reduced in volume in depression compared controls [26, 27], as well as thinner cortical gray matter in the orbitofrontal cortex, anterior and posterior cingulate, insula, and temporal lobes [28, 29].

Furthermore, the clinical heterogeneity of MDD can also be observed on an environmental and biological level. The existence of different subtypes of depression is supported by studies reporting differential roles of HPA-axis function, inflammation, and metabolic syndrome in depressed persons with melancholic/typical features, compared to those with atypical depression [30]. Melancholic depression is more characterized by decreased appetite and weight loss, whereas the atypical subtype of depression is characterized by overeating and weight gain. For example, childhood adversities, smoking and HPA-axis hyperactivity seem to be more related to typical depression, while metabolic syndrome components and higher inflammation are more specifically linked to atypical depression [31]. MDD subtypes also seem to be characterized by partially distinct polygenic liabilities [32] and differential structural brain correlates in both youth [33] and adults [34, 35], further highlighting that differential clinical representation may map onto a distinct biological profile. Importantly, this is suggested to, at least partly, explain blurred effect sizes in pathophysiological research, as well as explain why some treatment strategies that work well for some patients, might not work for others [36, 37]. More research is needed to further characterize subtypes of depression and to get the right treatment, to the right patient group, at the right time.

Comorbidity With Anxiety Disorders & Life Course Perspective

To date, it is well established from a variety of studies that MDD is commonly accompanied by anxiety symptoms. Since this group of patients has increased likelihood of presenting suicidal ideation and antidepressant treatment resistance (i.e. different clinical representation), the DSM also introduced an “anxious distress” specifier for MDD. However, there is not only an overlap in symptoms, there are also other significant similarities between MDD and anxiety disorders (social phobia, panic disorder, agoraphobia, and generalized anxiety disorder) in terms of genetics [38], neurobiological correlates [39], shared risk factors [14], and physiological dysregulations [19]. It is therefore

not surprising that comorbidity levels of depression and anxiety disorders can be as high as 75% [40, 41], showing that both mental disorders appear to be closely linked [42]. Collectively, the current literature outlines why depression and anxiety disorders are or should typically be studied together, as they are difficult to fully disentangle, and should perhaps be grouped under the broader family of internalizing disorders.

When one considers MDD and frequently co-occurring disorders such as dysthymia, (hypo)mania, and anxiety over a long-term course, patients tend to move in and out of diagnoses and recovery rates become more pessimistic and illness trajectories more chronic [43]. From a life-course perspective on mental disorders, diagnoses may even frequently shift among different disorders outside of depression and anxiety. Caspi et al. (2020) refer to this as “the ebb and flow” of mental disorders over decades [44], demonstrating that internalizing (e.g. depression and anxiety), externalizing (e.g. substance use disorders), and thought disorder (e.g. obsessive compulsive disorder) “families” also frequently co-occur, and presence of one disorder may often increase the risk of developing another disorder. To illustrate, at ages 11-15 years, approximately 32% of the participants with one disorder had a comorbid disorder, but at age 45, this percentage was more than 2.5 times as high increasing to as much as 85%, suggesting that with longer follow ups, participants often seem to have accumulated comorbid mental disorders.

HUMAN AGING

The Importance of Studying Our Aging Society

Aging has long been a question of scientific interest in a wide range of fields, possibly because it is almost a universal trait affecting most species on earth with the exception of some bacteria, plants or simple animals [45]. In humans, medical and public health improvements have contributed to the global trend of extended lifespan. More specifically, the human life expectancy has increased more than 35 years over the last 100 years [46]. Our current society thus experiences a steep increase in people living into old age. However, while some age-related changes such as graying of hair can be considered relatively benign, others are more objectively disadvantageous. For example, aging is also considered a critical risk factor for chronic diseases such as cardiometabolic problems, type 2 diabetes, cancer [47], and chances of death [48]. Thus, although human life expectancy has increased, parallel increases

have been observed in terms of chronic disease and years lived with disability [1]. This is partly explained by the fact that diseases can now be treated and become non-fatal, even though they still negatively impact a person's quality of life. Still much is unknown about the nature of why we age and the biological mechanisms that underpin aging processes. If we can better understand the biology of aging and its impact on disease, we may potentially find a way to more successful aging, adding more healthy and happy years to our lifespan. Within the field of psychiatry, studying aging has been instrumental in understanding the somatic consequences and medical comorbidities of depression.

AGING PHENOTYPES IN DEPRESSION

Somatic Symptoms Of Depression

Interestingly, the chronic diseases commonly observed at older ages, also frequently present themselves in depression [49]. Previous research has established that the impact of depression extends much further than the mere presence of psychological symptoms. The Inventory of Depression Symptoms (IDS) is a reliable instrument to measure signs of depression and provides an indication of illness severity. The instrument comprises 50% of items related to mood/cognition, but the other 50% of items tap into somatic symptoms such as sleeping problems, weight and/or appetite changes, physical energy, psychomotor agitation/retardation, and aches, pains or other bodily problems [50]. This already suggests that depression is by definition not only considered a "mind problem" but also a "body problem".

Age-related Comorbidities

A large and growing body of literature has shown that depression increases the risk of cardiovascular diseases, including, but not limited to, coronary heart disease, peripheral heart disease, and cerebrovascular disease [51]. In turn, cardiovascular problems also seem to increase the risk of depression, suggestive of a bidirectional relationship. But depression is even further associated with poorer somatic health outcomes beyond cardiovascular disease. Convergent evidence has been found for chronic diseases in depression, illustrated by increased risks of type 2 diabetes, obesity, stroke [52, 53], hypertension [54], and metabolic disorders. Moreover, longitudinal

evidence indicates that depression also increases the risk of age-related comorbidities like dementia [55], Alzheimer's disease [56], and, in some cases even cancer [57]. Overall, these findings provide convincing evidence that depression has a major adverse impact on somatic health.

Alternatively, studies have suggested that unhealthy lifestyles and poorer (self) care may offer an explanation as to why poorer somatic health can be observed in depression. Indeed, research linking depression and poorer health is limited by fact that behavioral, psychiatric, and somatic conditions frequently co-occur, and thus may have confounding effects. For instance, longer duration and higher frequencies of smoking, alcohol, and substance use have been observed in psychiatric patients compared to controls [58]. In addition, obesity, poor diet, and physical inactivity are common depression characteristics [51]. However, some studies show that a poorer lifestyle cannot fully explain the link between depression and adverse health consequences, as statistical models that correct for lifestyle differences show only slightly lower effect sizes between depression and e.g. cardiovascular disease [59].

Nevertheless, the most important consequence to consider from the established literature is that depression is associated with excess mortality [60–63] and life-years lost [64], regardless of the direct or indirect effects of the disease itself, including shared and unique associations with poorer lifestyle and poorer somatic health. The increased risk of developing aging-related conditions [65] is a fundamental association of depression that further increases the burden of the disease through decreased quality of life [66] and increased health care utilization [67]. There are thus two global challenges we are currently facing, as psychiatric illness and aging populations are both increasing worldwide, highlighting the urgent need to address both simultaneously in the same studies.

Cellular Aging In Depression

To examine the age-related somatic conditions in depression, previous studies have mainly focused on measures of cellular aging. At the conception of this thesis (April 2016), most of the literature on biological aging in depression was focused on telomere length, a marker that becomes progressively shorter with increasing age [68]. Robust associations have been found between shorter telomere length and depression [69], but also anxiety disorders [70], in at-risk [71], adolescent [72], and adult populations [73]. Since then, several technological advances have led to more "modern" measures of biological aging that will be discussed in the following paragraphs. The most popular

algorithms of biological aging rely on epigenetics, and are called "epigenetic clocks". Several epigenetic clocks that accurately track age exist, of which the Horvath and Hannum epigenetic clocks are most extensively used and validated [74, 75].

COMING OF AGE: TECHNOLOGICAL ADVANCES

Big Data, Machine Learning & High-performance Cluster Computing

The past decades have seen increasingly rapid advances in the field of neuroscience. This section provides some background to the molecular and brain imaging developments that were essential for the type of data and methods used in the current thesis. In 1975, scientists first found that DNA methylation could alter gene expression [76, 77]. In the following three decades, the importance of DNA methylation in the context of health and disease could be studied with steadily increasing coverage of the epigenome. Dramatic technological improvements have made it possible to perform high-throughput screening of the whole epigenome with declines in costs and expansion of computational power for processing. At present, we can measure the methylation statuses of almost all 28 million so-called CpG sites in human blood (see [78] for a detailed timeline of methods and applications) using cost-effective approaches [79]. This has promoted a shift of focus from hypothesis- and theory-driven discovery to data-driven and hypothesis-free approaches [80]. Thus, whereas the previous decades were dominated by candidate studies with promising leads derived from animal work, today we can basically associate all CpG sites that can be methylated in the blood epigenome with certain traits to examine the underlying biological patterns.

In the 1990s, the "Decade of the Brain" was designated in the U.S. to promote public awareness of beneficial discoveries from brain research [81]. Since then, data collection of (structural) magnetic resonance imaging (MRI) scans accelerated its pace due to the wide adoption of this method across the globe. Statistical software (e.g. FreeSurfer)[82], brain atlas templates, and various other toolboxes were developed to accommodate and sometimes automate the analyses of complex neuroimaging data. However, the field has also been greatly challenged by low statistical power, software errors, and notorious flexibility and researcher degrees of freedom in data analysis [83]. Scientists therefore also began to acknowledge the need to harmonize data collection, standardize analysis pipelines, and pool multiple datasets

to obtain better-powered studies in pursuance of robust findings that would replicate. Data sharing thus became critically important for creating research opportunities, with added benefits of crowdsourcing costly research data. To that aim, increasing numbers of international consortia emerged to promote team science and scientific collaborative efforts.

Finally, to model the complexity of vast and heterogeneous amounts of biological data, intelligent data analysis was needed. Machine learning algorithms use statistics to learn correlated patterns in large sample sizes with high-dimensional data (e.g. structural brain scans commonly include >35,000 voxels or 3D pixels). Cutting-edge scientific infrastructures were built for data storage and to allow sufficient power to compute complex calculations. Today, we are thus amidst exciting times, specifically in the field of neuroscience. We have access to unprecedented sample sizes, advanced machine learning methods, and high-performance cluster computing. Together, these technological advances have increased both the possibility to apply, as well as matured state-of-the-art statistical methods to examine high-dimensional data and the problem of age prediction.

QUANTIFICATIONS OF AGING

Concepts Of Chronological And Biological Aging

As mentioned before, aging is one of the strongest "risk factors" of chronic disease, loss of functional capacity, and, perhaps obviously, likelihood of mortality [84]. Across the lifespan we can distinguish two different concepts of aging, namely, chronological aging on one hand and biological aging on the other, although there is no agreed definition on what constitutes biological aging [85]. While our chronological age is basically an answer to the question of how many candles we can put on our birthday cake, our biological age can be quantified in many different ways and is more elusive. The chronological age is invariable and is merely based on the passage of time, whereas the biological age reflects the functional and biological state of our body, and may fall behind or outpace chronological age. The concept of the biological age thus aims to explain the inter-individual aging rates between two people of the same chronological age, or, to put it bluntly, why two people born on the same date may die (from "natural" causes) on diverging moments in time.

Given that it is not possible to obtain a complete picture of the biological state of an individual, biological age indicators may provide an approximation.

Biological age indicators can be derived from functional, neuroanatomical, cellular or molecular measures that are correlated with age, or, “hallmarks of aging” [86]. Definitions of biological age indicators often reflect loss of function, increased risk to medical conditions and diseases, and closer proximity to death. Importantly, a biological age indicator should be a better predictor of these reflections than chronological age. The current thesis is mainly focused on epigenetic and brain-based proxies of biological aging.

Modern Tools To Quantify Biological Aging

Normal aging is accompanied by brain atrophy, cortical thickness reductions, and ventricle enlargements [87]. Similarly, stochastic, environmental, and individual-specific methylation changes occur during the course of healthy aging [88]. Leveraging the natural aging-related biological changes that are reasonably consistent across individuals, we can use statistical tools to capture these specific patterns and develop predictive algorithms that are able to accurately predict one’s chronological age from these patterns. For a general overview of the biological age estimation procedure see **Figure 1**.

Central to this procedure is to use a sufficiently large sample of participants of which the chronological age is known such that a “supervised” machine learning method can be used. Generally, supervised machine learning refers to the approach that a machine learns correlated patterns in the data from which the individual’s chronological age is known (i.e. training data), before predicting the correct chronological age for newly presented data (i.e. test data). The current thesis mostly focused on epigenetic (i.e. CpG sites) and brain structure data (i.e. gray matter cortical thickness, surface area, and subcortical volumes) to obtain predicted biological age estimates. Importantly, by contrasting an individual’s predicted biological age to their chronological age, it can be studied whether individuals are biologically younger or older than expected on the basis of chronological age. To illustrate, if a person of 40 years old (i.e. chronological age) is predicted to be 42 years old (i.e. predicted biological age), that individual’s biological age based on their biological state outpaces their chronological age by +2 years (i.e. predicted age difference or biological aging effect).

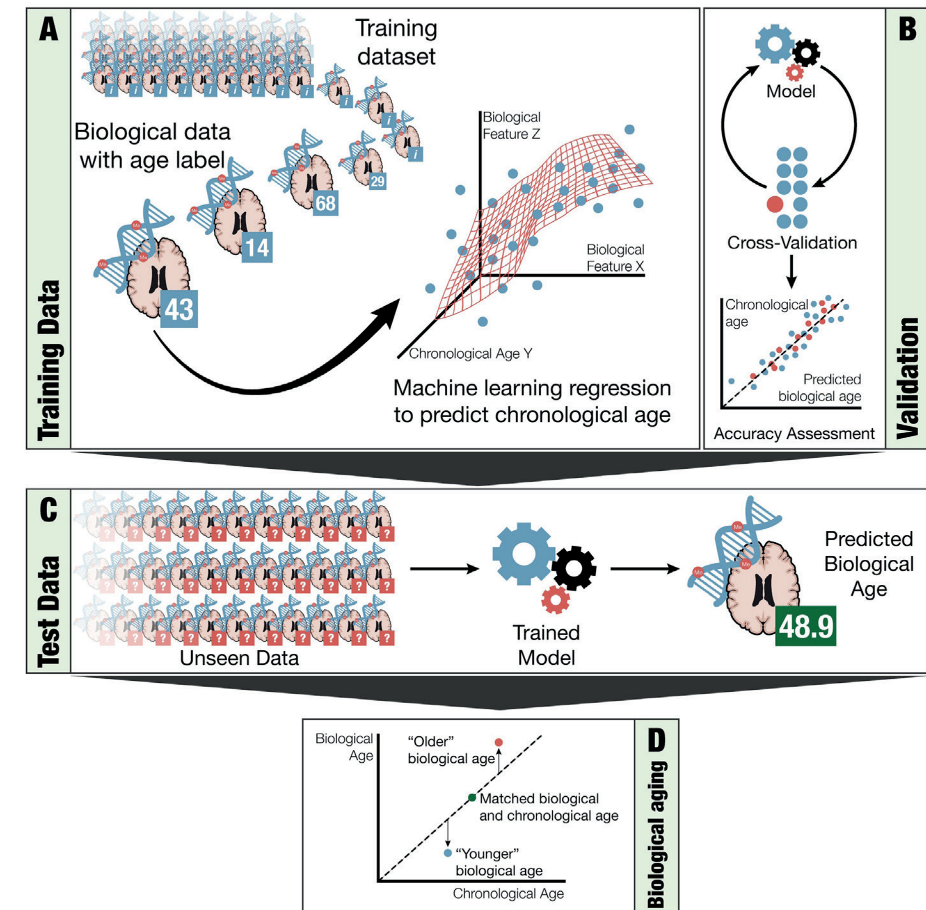


Figure 1. Biological age estimation procedure. Several general methodological steps are needed to predict an individual’s age from biological data (e.g. DNA methylation patterns or brain scans) to result in a predicted biological age. Biological data usually undergoes quality checking and some sort of dimension reduction in order to obtain a selection of “features” that relate to aging. (**A**) The obtained features are then used as predictors in a regression model with chronological age as “target” or outcome in the training data. (**B**) The validity of the model must then be established by its ability to generalize to test data that was not used to fit the model. The weights learned from the training data will be applied to the test data to obtain biological age predictions for each individual. (**C**) Once an optimal prediction accuracy is obtained through cross-validation, the model may also further be used on completely new and independent individuals to test true generalization power. However, this step is dependent on the available data and aim of the study. (**D**) Predicted biological age is compared to chronological age to obtain the predicted age difference or the “biological aging effect”.

Application of Biological Age Prediction Models to Depression

The purpose of building a biological age prediction model is to subsequently apply it to patient populations with depression and/or anxiety disorders to test the hypothesis whether their biological age outpaces chronological aging. The difference between one's chronological and biological age can then also be related to other disease characteristics. Understanding the link between biological aging and depression will help identify and predict who carry measurable risk for increased vulnerability to age-related health problems. It is important to mention that age prediction models are not sensitive to depression. In other words, biological age prediction models can be applied to the general population or other disease populations to examine individual deviations from "normal" aging. However, this dissertation primarily seeks to specifically explain the biological aging patterns observed in persons with depression.

DATASETS STUDIED IN THIS THESIS

The current thesis primarily used empirical data drawn from three main sources: 1) the Netherlands Study of Depression and Anxiety (NESDA)[89], 2) the Great Smoky Mountains Study (GSMS)[90], and 3) the MDD working group of the Enhancing Neuroimaging Genetics through Meta-analysis (ENIGMA) consortium [91]. First, the NESDA cohort is an ongoing longitudinal study that investigates the course and consequences of depression and anxiety disorders, therefore including in-depth assessments of clinical and biological characteristics. Between 2004 and 2007, 2,981 participants (78% met criteria for depression and/or anxiety disorders) were included that were recruited from the general population, primary care, and specialized mental health care. A subset of 1,130 participants (18-64 years old) underwent whole methylome profiling. A (partly overlapping) subsample of 301 participants (18-55 years old) took part in the neuroimaging study. Second, the GSMS is a unique longitudinal population-based cohort study of 1,420 children recruited from 11 predominantly rural counties of North Carolina, the United States of America. In 1993, participants were aged 9 to 13 years old at intake, and provided detailed assessments of health factors, and blood samples during annual assessments until 2015. Participants are now in their early 30s, and the current thesis used data from 539 participants (9-35 years old) including a total of 1,029 measurements. Third, the ENIGMA consortium is a worldwide neuroscience alliance of >1,400 scientists across 43 countries that aims to examine fundamental questions in neuroscience and genetics [92]. The structure of the consortium comprises different working groups, with the ENIGMA MDD working group being the main data source used for this thesis. Data from more than 19 cohorts including over 6,900 participants (18-75 years old) with 38.3% of patients with MDD were included in a pooled mega-analysis.

AIMS AND OUTLINE OF THESIS

This thesis will examine multisystem quantifications of the biological age in MDD in pursuance of a better understanding of the complex interplay between mental health and biological aging. The overall structure of the thesis takes the form of eight chapters, ending with a summary and discussion of the main findings. The first aim was to highlight important guideposts for researchers interested in making advances in the field of stress, psychopathology, and biological aging. **Chapter 2** therefore provides a literature overview of the link between biological aging and mental health, as well as a description of this rapidly expanding field of research, including limitations, current challenges, and future recommendations.

The second aim of the current thesis was to conduct experimental work to examine biological aging in peripheral tissues. The following two chapters operationalize biological aging as measured by DNA methylation, or, epigenetic patterns. More specifically, **Chapter 3** describes whether major depression is cross-sectionally associated with older appearing epigenetic patterns in blood, whether depression characteristics have a further impact on these patterns, and whether findings replicate in brain tissue. The work in **Chapter 4** examined longitudinal epigenetic aging patterns from childhood and adolescence into young adulthood, and describes whether changes in various health risks result in changes in epigenetic aging, a relationship that should be observed if the health risk has a causal effect on the epigenetic biological age indicator.

The third aim of this thesis was to examine whether premature or advanced biological aging observed in peripheral tissues could also be observed in the brain as measured from MRI scans. The next two chapters were therefore concerned with brain-based biological age indicators. **Chapter 5** describes the development of a multi-site brain age prediction model and examines brain aging in over 6,900 individuals from the ENIGMA consortium, including

exploratory associations with several basic harmonized clinical characteristics. To further build on those findings, we then applied the developed brain age prediction to controls and both depression and anxiety disorder patients from the NESDA cohort in **Chapter 6**, and associated the brain age indicator with more detailed clinical, psychological, and biological factors.

The fourth and final aim of the current thesis was to examine multiple biological age indicators and to combine and integrate them in one study. **Chapter 7** therefore investigates intercorrelations between five biological clocks based on telomeres and four omics levels, and examines their unique and shared associations with a wide range of somatic and mental health risks. Finally, a summary of the main findings, together with its discussion and (clinical) implications, is provided in **Chapter 8**.

REFERENCES

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1789–1858.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:e442.
3. Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386:743–800.
4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub; 2013.
5. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry. 2000;157:1552–1562.
6. Howard DM, Adams MJ, Clarke T-K, Hafferty JD, Gibson J, Shirali M, et al. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nat Neurosci. 2019;22:343–352.
7. Aberg KA, Dean B, Shabalin AA, Chan RF, Han LKM, Zhao M, et al. Methylome-wide association findings for major depressive disorder overlap in blood and brain and replicate in independent brain samples. Mol Psychiatry. 2018. 21 September 2018. https://doi.org/10.1038/s41380-018-0247-6.
8. Kotov R, Gamez W, Schmidt F, Watson D. Linking ‘big’ personality traits to anxiety, depressive, and substance use disorders: a meta-analysis. Psychol Bull. 2010;136:768–821.
9. Goldstein BL, Perlman G, Eaton NR, Kotov R, Klein DN. Testing explanatory models of the interplay between depression, neuroticism, and stressful life events: a dynamic trait-stress generation approach. Psychological Medicine. 2019:1–10.
10. Lahey BB. Public health significance of neuroticism. Am Psychol. 2009;64:241–256.
11. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. Major depressive disorder. Nature Reviews Disease Primers. 2016;2:16065.
12. Patel V, Chisholm D, Parikh R, Charlson FJ, Degenhardt L, Dua T, et al. Addressing the burden of mental, neurological, and substance use disorders: key messages from Disease Control Priorities, 3rd edition. Lancet. 2016;387:1672–1685.
13. Teicher MH, Samson JA. Childhood maltreatment and psychopathology: A case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. Am J Psychiatry. 2013;170:1114–1133.
14. Hovens JGFM, Giltay EJ, Spinhoven P, van Hemert AM, Penninx BWJH. Impact of childhood life events and childhood trauma on the onset and recurrence of depressive and anxiety disorders. J Clin Psychiatry. 2015;76:931–938.
15. Hovens JGFM, Giltay EJ, Wiersma JE, Spinhoven P, Penninx BWJH, Zitman FG. Impact of childhood life events and trauma on the course of depressive and anxiety disorders. Acta Psychiatr Scand. 2012;126:198–207.

16. Humphreys KL, LeMoult J, Wear JG, Piersiak HA, Lee A, Gotlib IH. Child maltreatment and depression: A meta-analysis of studies using the Childhood Trauma Questionnaire. Child Abuse Negl. 2020;102:104361.
17. Oquendo MA, Perez-Rodriguez MM, Poh E, Sullivan G, Burke AK, Sublette ME, et al. Life events: a complex role in the timing of suicidal behavior among depressed patients. Mol Psychiatry. 2014;19:902–909.
18. Firth J, Solmi M, Wootton RE, Vancampfort D, Schuch FB, Hoare E, et al. A meta-review of 'lifestyle psychiatry': the role of exercise, smoking, diet and sleep in the prevention and treatment of mental disorders. World Psychiatry. 2020;19:360–380.
19. Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 2013;11:129.
20. Kiecolt-Glaser JK, Derry HM, Fagundes CP. Inflammation: depression fans the flames and feasts on the heat. Am J Psychiatry. 2015;172:1075–1091.
21. Xu Q, Anderson D, Lurie-Beck J. The relationship between abdominal obesity and depression in the general population: A systematic review and meta-analysis. Obes Res Clin Pract. 2011;5:e267–e360.
22. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16:22–34.
23. Mac Giollabhui N, Ng TH, Ellman LM, Alloy LB. The longitudinal associations of inflammatory biomarkers and depression revisited: systematic review, meta-analysis, and meta-regression. Mol Psychiatry. 2020. 17 August 2020. https://doi.org/10.1038/s41380-020-00867-4.
24. Hu MX, Lamers F, de Geus EJC, Penninx BWJH. Differential Autonomic Nervous System Reactivity in Depression and Anxiety During Stress Depending on Type of Stressor. Psychosom Med. 2016;78:562–572.
25. Hu MX, Milaneschi Y, Lamers F, Nolte IM, Snieder H, Dolan CV, et al. The association of depression and anxiety with cardiac autonomic activity: The role of confounding effects of antidepressants. Depress Anxiety. 2019;36:1163–1172.
26. Price JL, Drevets WC. Neural circuits underlying the pathophysiology of mood disorders. Trends Cogn Sci. 2012;16:61–71.
27. Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat Neurosci. 2007;10:1116–1124.
28. Schmaal L, Hibar DP, Sämann PG, Hall GB, Baune BT, Jahanshad N, et al. Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group. Mol Psychiatry. 2016;22:900.
29. Schmaal L, Veltman DJ, van Erp TGM, Sämann PG, Frodl T, Jahanshad N, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry. 2015:1–7.
30. Lamers F, Vogelzangs N, Merikangas KR, de Jonge P, Beekman ATF, Penninx BWJH. Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. Mol Psychiatry. 2013;18:692–699.
31. Lasserre AM, Glaus J, Vandeleur CL, Marques-Vidal P, Vaucher J, Bastardot F, et al. Depression with atypical features and increase in obesity, body mass index, waist circumference, and fat mass: a prospective, population-based study. JAMA Psychiatry. 2014;71:880–888.
32. Milaneschi Y, Lamers F, Peyrot WJ, Abdellaoui A, Willemsen G, Hottenga J-J, et al. Polygenic dissection of major depression clinical heterogeneity. Mol Psychiatry. 2015;21:516–522.
33. Toenders YJ, Schmaal L, Harrison BJ, Dinga R, Berk M, Davey CG. Neurovegetative symptom subtypes in young people with major depressive disorder and their structural brain correlates. Transl Psychiatry. 2020;10:108.
34. Simmons WK, Burrows K, Avery JA, Kerr KL, Taylor A, Bodurka J, et al. Appetite changes reveal depression subgroups with distinct endocrine, metabolic, and immune states. Mol Psychiatry. 2020;25:1457–1468.
35. Simmons WK, Burrows K, Avery JA, Kerr KL, Bodurka J, Savage CR, et al. Depression-Related Increases and Decreases in Appetite: Dissociable Patterns of Aberrant Activity in Reward and Interoceptive Neurocircuitry. Am J Psychiatry. 2016;173:418–428.
36. Köhler O, Benros ME, Nordentoft M, Farkouh ME, Iyengar RL, Mors O, et al. Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. JAMA Psychiatry. 2014;71:1381–1391.
37. Raison CL, Rutherford RE, Woolwine BJ, Shuo C, Schettler P, Drake DF, et al. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. JAMA Psychiatry. 2013;70:31–41.
38. Consortium C-DG of TPG, Cross-Disorder Group of the Psychiatric Genomics Consortium. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nature Genetics. 2013;45:984–994.
39. Ionescu DF, Niciu MJ, Mathews DC, Richards EM, Zarate CA Jr. Neurobiology of anxious depression: a review. Depress Anxiety. 2013;30:374–385.
40. Lamers F, van Oppen P, Comijs HC, Smit JH, Spinhoven P, van Balkom AJLM, et al. Comorbidity patterns of anxiety and depressive disorders in a large cohort study: the Netherlands Study of Depression and Anxiety (NESDA). J Clin Psychiatry. 2011;72:341–348.
41. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch Gen Psychiatry. 1994;51:8–19.
42. Penninx BWJH. Depression and anxiety: their insidious dance. Lancet Psychiatry. 2015;2:479–480.
43. Verduijn J, Verhoeven JE, Milaneschi Y, Schoevers RA, van Hemert AM, Beekman ATF, et al. Reconsidering the prognosis of major depressive disorder across diagnostic boundaries: full recovery is the exception rather than the rule. BMC Med. 2017;15:215.
44. Caspi A, Houts RM, Ambler A, Danese A, Elliott ML, Hariri A, et al. Longitudinal Assessment of Mental Health Disorders and Comorbidities Across 4 Decades Among Participants in the Dunedin Birth Cohort Study. JAMA Netw Open. 2020;3:e203221.
45. Kirkwood TBL, Austad SN. Why do we age? Nature. 2000;408:233–238.
46. Roser M, Ortiz-Ospina E, Ritchie H. Life expectancy. Our World in Data. 2013. 2013.

47. Niccoli T, Partridge L. Ageing as a risk factor for disease. Curr Biol. 2012;22:R741–R752.
48. Harman D. The aging process: major risk factor for disease and death. Proc Natl Acad Sci U S A. 1991;88:5360–5363.
49. Moussavi S, Chattereji S, Verdes E. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet [Internet]. 2007; 370: 851-8.
50. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. Psychol Med. 1996;26:477–486.
51. Penninx BWJH. Depression and cardiovascular disease: Epidemiological evidence on their linking mechanisms. Neurosci Biobehav Rev. 2016. 2016. https://doi.org/10.1016/j.neubiorev.2016.07.003.
52. Whooley MA. Depression and Cardiovascular Disease. JAMA. 2006;295:2874.
53. Dong J-Y, Zhang Y-H, Tong J, Qin L-Q. Depression and risk of stroke: a meta-analysis of prospective studies. Stroke. 2012;43:32–37.
54. Meng L, Chen D, Yang Y, Zheng Y, Hui R. Depression increases the risk of hypertension incidence: a meta-analysis of prospective cohort studies. J Hypertens. 2012;30:842.
55. Byers AL, Yaffe K. Depression and risk of developing dementia. Nat Rev Neurol. 2011. 2011.
56. Green RC, Adrienne Cupples L, Kurz A, Auerbach S, Go R, Sadovnick D, et al. Depression as a Risk Factor for Alzheimer Disease: The MIRAGE Study. Arch Neurol. 2003;60:753–759.
57. Currier MB, Nemeroff CB. Depression as a risk factor for cancer: from pathophysiological advances to treatment implications. Annu Rev Med. 2014. 2014.
58. Viron MJ, Stern TA. The Impact of Serious Mental Illness on Health and Healthcare. Psychosomatics. 2010;51:458–465.
59. Nicholson A, Kuper H, Hemingway H. Depression as an aetiologic and prognostic factor in coronary heart disease: a meta-analysis of 6362 events among 146 538 participants in 54 observational studies. Eur Heart J. 2006;27:2763–2774.
60. Harris EC, Barraclough B. Excess mortality of mental disorder. Br J Psychiatry. 1998;173:11–53.
61. Laursen TM, Munk-Olsen T, Nordentoft M, Mortensen PB. Increased mortality among patients admitted with major psychiatric disorders: a register-based study comparing mortality in unipolar depressive disorder, bipolar affective disorder, schizoaffective disorder, and schizophrenia. J Clin Psychiatry. 2007;68:899–907.
62. Wahlbeck K, Westman J, Nordentoft M, Gissler M, Laursen TM. Outcomes of Nordic mental health systems: life expectancy of patients with mental disorders. Br J Psychiatry. 2011;199:453–458.
63. Lawrence D, Hancock KJ, Kisely S. The gap in life expectancy from preventable physical illness in psychiatric patients in Western Australia: retrospective analysis of population based registers. BMJ. 2013;346:f2539.
64. Erlangsen A, Andersen PK, Toender A, Laursen TM, Nordentoft M, Canudas-Romo V. Cause-specific life-years lost in people with mental disorders: a nationwide, register-based cohort study. Lancet Psychiatry. 2017;4:937–945.
65. Momen NC, Plana-Ripoll O, Agerbo E, Benros ME, Børglum AD, Christensen MK, et al. Association between Mental Disorders and Subsequent Medical Conditions. N Engl J Med. 2020;382:1721–1731.
66. Brettschneider C, Leicht H, Bickel H, Dahlhaus A, Fuchs A, Gensichen J, et al. Relative impact of multimorbid chronic conditions on health-related quality of life--results from the MultiCare Cohort Study. PLoS One. 2013;8:e66742.
67. Bock J-O, Luppa M, Brettschneider C, Riedel-Heller S, Bickel H, Fuchs A, et al. Impact of Depression on Health Care Utilization and Costs among Multimorbid Patients – Results from the MultiCare Cohort Study. PLoS ONE. 2014;9:e91973.
68. Blackburn EH. Structure and function of telomeres. Nature. 1991;350:569–573.
69. Schutte NS, Malouff JM. The association between depression and leukocyte telomere length: A meta-analysis. Depress Anxiety. 2015;32:229–238.
70. Darrow SM, Verhoeven JE, Révész D, Lindqvist D, Penninx BWJH, Delucchi KL, et al. The Association Between Psychiatric Disorders and Telomere Length: A Meta-Analysis Involving 14,827 Persons. Psychosom Med. 2016;78:776–787.
71. Gotlib IH, LeMoult J, Colich NL, Foland-Ross LC, Hallmayer J, Joormann J, et al. Telomere length and cortisol reactivity in children of depressed mothers. Mol Psychiatry. 2014:1–6.
72. Henje Blom E, Han LKM, Connolly CG, Ho TC, Lin J, LeWinn KZ, et al. Peripheral telomere length and hippocampal volume in adolescents with major depressive disorder. Transl Psychiatry. 2015;5:e676.
73. Verhoeven JE, Révész D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. Mol Psychiatry. 2013:1–7.
74. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
75. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol Cell. 2013;49:359–367.
76. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. Science. 1975;187:226–232.
77. Riggs AD. X inactivation, differentiation, and DNA methylation. Cytogenet Cell Genet. 1975;14:9–25.
78. Harrison A, Parle-McDermott A. DNA methylation: a timeline of methods and applications. Front Genet. 2011;2:74.
79. Aberg KA, Mcclay JL, Nerella S, Xie LY, Clark SL, Hudson AD, et al. MBD-seq as a cost-effective approach for methylome-wide assocciation studies: demonstration in 1500 case-control samples. Epigenomics. 2012;4:605–621.
80. Hägg S, Belsky DW, Cohen AA. Developments in molecular epidemiology of aging. Emerging Topics in Life Sciences. 2019;3:411–421.
81. Jones EG, Mendell LM. Assessing the decade of the brain. Science. 1999;284:739.
82. Fischl B. Automatically Parcellating the Human Cerebral Cortex. Cerebral Cortex. 2004;14:11–22.
83. Poldrack RA, Baker CI, Durnez J. Scanning the horizon: towards transparent and reproducible neuroimaging research. Nat Rev. 2017. 2017.
84. Fernandes M, Wan C, Tacutu R, Barardo D, Rajput A, Wang J, et al. Systematic analysis of the gerontome reveals links between aging and age-related diseases. Hum Mol Genet. 2016;25:4804–4818.
85. Xia X, Chen W, McDermott J, Han J-DJ. Molecular and phenotypic biomarkers of aging. F1000Res. 2017;6:860.
86. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–1217.
87. Apostolova LG, Green AE, Babakchanian S, Hwang KS, Chou Y-Y, Toga AW, et al. Hippocampal atrophy and ventricular enlargement in normal aging, mild cognitive impairment (MCI), and Alzheimer Disease. Alzheimer Dis Assoc Disord. 2012;26:17–27.

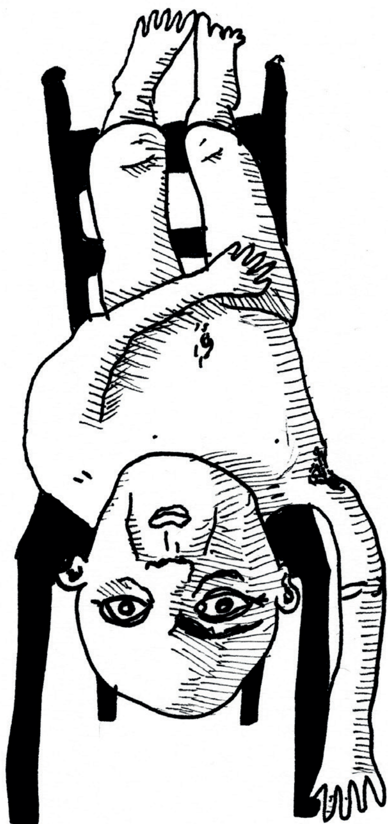
88. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. Aging Cell. 2015;14:924–932.
89. Penninx BWJH, Beekman ATF, Smit JH, Zitman FG, Nolen WA, Spinhoven P, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale , objectives and methods. 2008;17:121–140.
90. Costello EJ, Angold A, Burns BJ, Stangl DK, Tweed DL, Erkanli A, et al. The Great Smoky Mountains Study of Youth. Goals, design, methods, and the prevalence of DSM-III-R disorders. Arch Gen Psychiatry. 1996;53:1129–1136.
91. Schmaal L, Pozzi E, C Ho T, van Velzen LS, Veer IM, Opel N, et al. ENIGMA MDD: seven years of global neuroimaging studies of major depression through worldwide data sharing. Transl Psychiatry. 2020;10:172.
92. Thompson PM, Jahanshad N, Ching CRK, Salminen LE, Thomopoulos SI, Bright J, et al. ENIGMA and global neuroscience: A decade of large-scale studies of the brain in health and disease across more than 40 countries. Transl Psychiatry. 2020;10:100.

CHAPTER 2

Accelerating Research on Biological Aging and Mental Health: Current Challenges and Future Directions

Published in *Psychoneuroendocrinology* 2019, 106, 293-311

Laura K.M. Han
Josine E. Verhoeven
Audrey R. Tyrka
Brenda W.J.H. Penninx
Owen M. Wolkowitz
Kristoffer N.T. Månsson
Daniel Lindqvist
Marco P. Boks
Dóra Révész
Synthia H. Mellon
Martin Picard



ABSTRACT

Aging is associated with complex biological changes that can be accelerated, slowed, or even temporarily reversed by biological and non-biological factors. This article focuses on the link between biological aging, psychological stressors, and mental illness. Rather than comprehensively reviewing this rapidly expanding field, we highlight challenges in this area of research and propose potential strategies to accelerate progress in this field. This effort requires the interaction of scientists across disciplines - including biology, psychiatry, psychology, and epidemiology; and across levels of analysis that emphasize different outcome measures - functional capacity, physiological, cellular, and molecular. Dialogues across disciplines and levels of analysis naturally lead to new opportunities for discovery but also to stimulating challenges. Some important challenges consist of 1) establishing the best objective and predictive biological age indicators or combinations of indicators, 2) identifying the basis for inter-individual differences in the rate of biological aging, and 3) examining to what extent interventions can delay, halt or temporarily reverse aging trajectories. Discovering how psychological states influence biological aging, and vice versa, has the potential to create novel and exciting opportunities for healthcare and possibly yield insights into the fundamental mechanisms that drive human aging.

LIST OF ABBREVIATIONS

BD	Bipolar Disorder
BDNF	Brain-derived Neurotrophic Factor
Brain-PAD	Brain-predicted age difference
ccf-mtDNA	Circulating cell-free mitochondrial DNA
DNAm	DNA methylation
MDD	Major Depressive Disorder
MHI	Mitochondrial Health Index
mtDNA	Mitochondrial DNA
mtDNAcn	Mitochondrial DNA copy number
nDNA	Nuclear DNA
PTSD	Post-traumatic Stress Disorder
SCZ	Schizophrenia
SSRI	Selective Serotonin Reuptake Inhibitor
TL	Telomere length

INTRODUCTION

Aging is the strongest risk factor for many chronic illnesses, loss of functional capacity, and mortality [1]. It is associated with complex biological changes, but there is no consensus on the very definition of aging, nor on the best methods to quantify it biologically [2]. Chronological age is based on the passage of time and is invariable. But biological age may fall behind or else outpace chronological age – it is modifiable. Based on specific molecular and other measures discussed below, the rate of biological aging has been reported to vary substantially between individuals [2, 3], although the causes of such inter-individual differences are mostly unclear. In particular, a major gap in knowledge is reflected in our ignorance of the mechanisms for the transduction of psychological states, and of psychopathology, into changes in biological aging (**Figure 1**). How do "mind" states influence biological aging and vice versa?

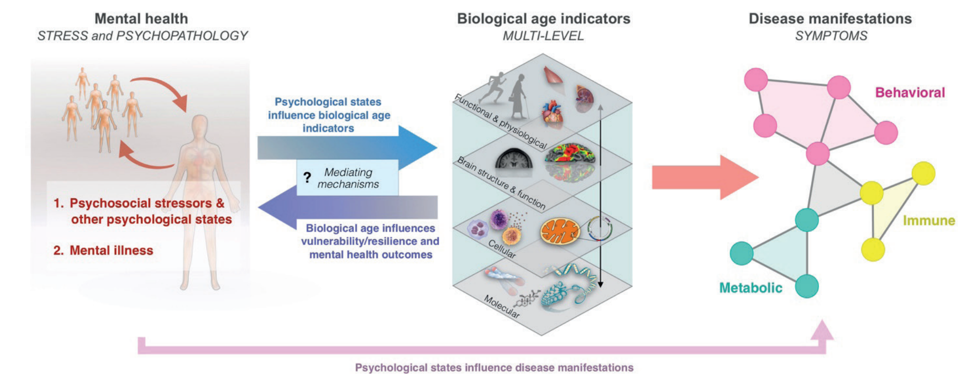


Figure 1. Integrative model for the transduction of mental health into biological aging and downstream disease manifestations. (**Left**) Two main domains of mental health are considered: 1. Acute and chronic psychosocial stressors, which include distress and other subjective experiences; 2. Mental illness and clinical psychopathology (e.g., depression, anxiety, schizophrenia, bipolar disorder, etc). (**Middle**) These factors are transduced into biological age indicators, which span functional and physiological, brain structure and function, cellular, and molecular levels of analysis. In turn, the reverse association may transduce increased biological age into increased vulnerability and resilience to life stressors. The mechanisms responsible for the bi-directional flow of information between psychological states, psychopathology, and biological age indicators largely remain to be defined. (**Right**) Increased biological aging reflected in individual or combinations of biological age indicators manifest in symptoms across multiple interconnected systems, represented here as a functional network. Mental health domains can also directly contribute to disease manifestations (bottom arrow). Molecular indicators refer to components that are inert when in isolation (e.g. DNA, proteins) whereas cellular indicators refer to animated "living" components (e.g. breathing mitochondria, dividing/secreting cells).

This psycho-biological problem is a unique opportunity to make scientific progress on two main fronts: First, it is an opportunity to develop new measurements and technical approaches to capture meaningful, valid, and reproducible measures of biological aging. Second, this interdisciplinary problem requires dialogue across research and clinical domains. We see the intersection of experiential, psychological, and biological aging processes as a platform for the development of new (and possibly radically different) concepts and measures that will most faithfully capture human health and the aging process. Currently, although we have some quantifiable measures of biological aging in humans - biological age indicators - we still know little about their causal role in the aging process, and about their modifiability by psychological states and psychopathology.

One important shared goal towards enhancing well-being across the lifespan is to understand aging as dynamic trajectories determined by a variety of factors. Some determinants of biological aging are pre-programmed ("intrinsic"; e.g., genetic), while others are affected by the environment ("extrinsic"; e.g., diet, adversity) [4]. Most definitions of biological aging include loss of function, increased propensity to certain diseases, and closer proximity to death [5]. Certain objective biological measures (or "clocks") may also track biological aging. Development and validation of biological age indicators and clarification of their mediators and moderators are high priorities, since they may lead to a better understanding of the underpinnings of healthy and unhealthy aging trajectories. These indicators may also present proximal outcomes, or "early warning signs" that portend disease development and may provide a more sensitive platform to detect – and intervene upon – meaningful interactions between psychological, social, and bio-behavioral factors that influence aging trajectories and health outcomes.

Biological age indicators currently being investigated include telomere length (TL), epigenetic changes, alterations of mitochondrial function and mitochondrial DNA (mtDNA), age-related brain structure and function, and transcriptomic, metabolomic, and proteomic changes, among others (see, [2, 3, 6] for recent reviews). Current topics of investigation include the nature of the inter-relationship of these biological age indicators, whether they measure the same or different aspects of biological aging, whether they are causally involved in the aging process, whether they have a causal role in disease and disorders, and the best ways to assess them. The possibility that the aging process is accelerated by chronic psychological stress and that it plays a role in the pathophysiology of some mental illnesses has been supported by observations that chronically stressed or psychiatrically ill individuals are at increased risk of acquiring specific age-related diseases and have a reduced

life expectancy [7–11]. But certain conceptual and methodological obstacles are impeding growth in this field and hinder replication of findings across laboratories.

Rather than comprehensively reviewing this rapidly expanding field, here we focus on highlighting various challenges and arising opportunities for this interdisciplinary endeavor. We conclude by proposing strategies to accelerate the progress of this field towards a predictive science that can enhance our understanding of the psychobiological factors that influence the aging process and lifespan.

The concept of biological aging: Definitions and obstacles

Chronological age is strictly quantitative and requires no more than a calendar to measure. Biological age is more elusive as it reflects the functional and biological condition of an individual. The difference between biological age and chronological age can indicate whether the individual's biological state is "older" or "younger" than would be expected for a given chronological age. This is often referred to as "accelerated" or "slowed" aging, respectively. However, cross-sectional assessments of biological age do not allow to determine aging rates, or to distinguish between "accelerated" and "premature" or "advanced" aging (**Figure 2**). The rate of increase in biological aging over time may also exhibit nonlinear behavior, particularly in early life where the measured rate of aging may be more rapid than across adult life [3, 12].

Since it is not possible to directly assess the total biological state of a person, biological age indicators serve as proxies. Biological age *indicators* are functional, anatomical, biochemical, cellular or molecular measures that are correlated with age and that may reflect the health status of specific cell types and/or organ systems. The term *biomarker*, in contrast to indicators as defined here, is best used in the context of specific disease or health outcomes. By definition, biomarkers must exhibit both sensitivity and specificity in relation to the outcome we design them to predict [13]. For aging, a still broadly defined process compared to a disease that can be ascertained with certainty, the term indicator is rendered more appropriate. Although some biological age indicators have undoubtedly established their sensitivity to chronological age, few have convincingly demonstrated their specificity - that changes in their value occurs specifically in response to the aging process and not in response to other pathophysiological process. Some biological age indicators are indeed modified by disease states independent of aging, and some as discussed below may notably be sensitive to psychological states, namely stress and psychopathology.

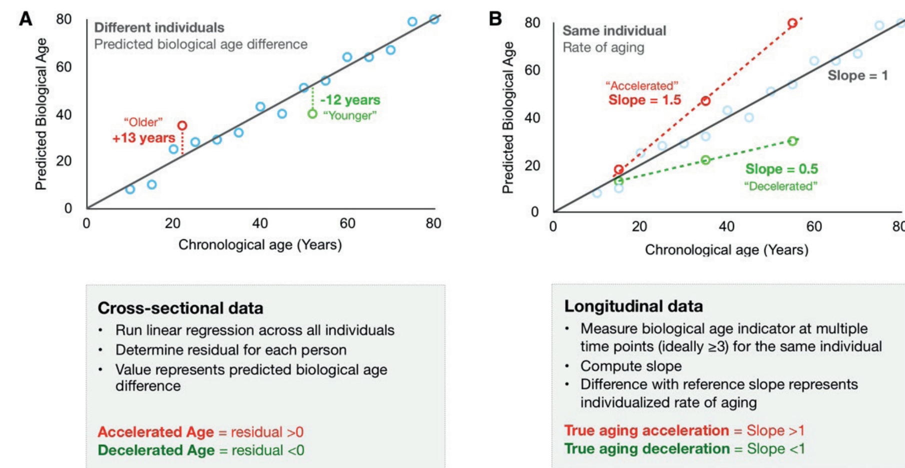


Figure 2. Computing age acceleration using cross-sectional and longitudinal data. (**A**) From cross-sectional data, accelerated aging is established when biological age is over-predicted relative to the chronological age reference (group regression line). (**B**) In longitudinal data, the rate of aging is directly determined from multiple measurements in the same person (also same tissue and cell type). The slope for each individual can be compared to the theoretical slope of 1 to ascertain true aging acceleration or deceleration.

Biological age indicators

Aging is a multifaceted and complex process that manifests across multiple levels. In recent decades, measurements spanning each of these levels have been developed, reflecting our prevailing reductionist scientific approach to biomedical sciences. Here, rather than providing an exhaustive overview that can be found in recent reviews [2, 6, 14–17], we provide a selective overview of aging indicators commonly studied in relation to mental health (**Table 1**). This table, which is illustrative rather than comprehensive, also includes some emerging biological age indicators that reflect the development of omics technologies and of computational approaches to integrate multiple metrics into composite indices, as also discussed in *Section 5*. Due to space limitation, we do not cover self-reported age [18], self-reported perception of aging [19], more specific brain measures [20], or inflammatory markers [21], which have also been associated with lifespan. We then discuss the practical limitations and conceptual challenges commonly associated with these measurements.

General limitations common to biological age indicators

There are limitations inherent to existing biological age indicators: they rely on specific organs or tissue types, can be confounded by cell type heterogeneity, and represent static measures of dynamic states (**Figure 3**). These limitations apply to most molecular biological age indicators and should represent the foundation from which we design research projects, and interpret findings. However, these limitations are often not well understood and, instead, only considered post-hoc once data is collected and is being analyzed. Here, to be consistent with the logic whereby limitations inform research design, method development, and data interpretation, we discuss these challenges prior to the literature review. Recommendations to overcome some of these limitations are also discussed in *Section 5*.

Organs and tissue types

A common limitation of biological age indicators is that they are generally measured in one particular tissue and then used as a general age estimator for the person from whom the sample was obtained. However, it is unlikely that every tissue - peripheral and central - are entirely synchronized and that one tissue accurately reflects the biological age of all other tissues. Tissues from multiple organs have been found to age at different rates in terms of epigenetic age [87]. With age, mtDNA mutations also accumulate differently between brain regions [88–90] and even between cells of a given organ [91]. Within the brain, TL also varies between different cortical areas [92]. This limitation may also apply to measures of functional capacity. For example, there is relatively poor agreement between muscle strength measured from handgrip or knee extension, suggesting that the most commonly used metric of muscle strength - handgrip strength - is not a proxy for overall muscle strength [93]. Developing approaches to measure and perhaps capitalize on the heterogeneous nature of aging dynamics across tissues requires further research.

Cellular composition and heterogeneity

Tissues such as the brain, heart, muscles, and the blood are composed of multiple different cell types. Although all cell types have the same genome, they show unique epigenetic, morphological, functional, and molecular differences relevant to biological age indicators. For example, different blood cell types have different epigenomes [94], telomerase activity and TL [42, 95, 96], and mitochondrial respiratory capacity [97]. These intrinsic differences are inevitable and may introduce bias and confound findings when assessed

Table 1. Selective list of biological age indicators.

Category	Biological age indicator	Method	Correlation with chronological age	Evidence for mental health association	Representative survival or method-related references
Functional & physiological measures	Walking speed/ gait	Timed distance walked Self-report	Inverse	Impaired in depression [22, 23]	[24–27]
	Hand-grip strength	Dynamometer	Inverse	Lower in depression [28, 29]	[24, 30, 31]
	Lung function	Spirometer	Inverse	Impaired in depression [29]	[32]
Brain measures	Brain age$^{\Delta}$	T1-MRI	Positive/high	Higher in SCZ [33], MDD, BPD, and psychosis (risk) [34, 35] Inconsistent in BD [36, 37] Higher with more negative fateful life events [38]	[3, 39]
Cellular measures	Respiratory capacity	Mitochondrial enzymatic activities Respiratory capacity in fresh cells	Inverse	Enzymatic activities: correlated with previous day positive mood [40] Cellular respiration: associations with early life adversity [41] and MDD [42, 43]	[44–46]
Molecular measures	Telomere length	qPCR Southern blot Q-FISH	Inverse	Shorter in MDD [47], anxiety disorders [48], SCZ [49], PTSD [50] N.S. in BD	[51–54]

Table 1. Continued

Category	Biological age indicator	Method	Correlation with chronological age	Evidence for mental health association	Representative survival or method-related references
Molecular measures	Epigenetic age$^{\Delta}$	Microarray and sequencing	Positive/high	Increased in response to traumatic stress [55], life stress [56], BD [57], MDD [58, 59] N.S. in SCZ [60]	[61–66]
	Transcriptomic age$^{\Delta}$	RNA-seq	Positive/moderate to high	NA	[67, 68]
	Proteomic age$^{\Delta}$	Mass spectrometry	Positive/high	NA	[69, 70]
	Metabolomic age$^{\Delta}$	Mass spectrometry	Positive/high	NA	[71, 72]
	Glycomics$^{\Delta}$	Microarray and mass spectrometry	Positive/moderate to high	Altered in MDD [73, 74] GlycoAge Test was higher in PTSD [75]	[76–79]
	ccf-mtDNA	qPCR	Positive	Higher in plasma of suicidal patients [80] and MDD [81] Acutely elevated with induced psychological stress in plasma [82] and serum (Trumpff et al., 2019)	[83]
	mtDNAcn	qPCR (whole blood)	Inverse	Higher in BD [57] No evidence for an association with depression Increased in mixed psychiatric disorders	[84–86]

Abbreviations: BD, bipolar disorder; BPD, borderline personality disorder; ccf-mtDNA, circulating cell-free mitochondrial DNA; MDD, major depressive disorder; MRI, magnetic resonance imaging; mtDNAcn, mitochondrial DNA copy number; NA, not applicable; N.S., not significant; PTSD, post-traumatic stress disorder; qPCR, quantitative polymerase chain reaction; Q-FISH, quantitative fluorescent in situ hybridization; SCZ, schizophrenia. Note that correlation coefficients (r) for biological age indicators and chronological age is dependent on the age range within each dataset and should not be use as sole metric of accuracy. Criteria used for size of r: High: 0.7-0.9 (-0.7 to -0.9); Moderate: 0.5-0.7 (-0.5 to -0.7); Low: 0.3-0.5 (-0.3 to -0.5); Negligible: 0 - 0.3 (-0.3 to 0). Effect size reflects correlation coefficient reported in the original publications of the multivariate composites, indicated by$^{\Delta}$. Systematic reviews and meta-analytic studies are indicated by†.

individually. Similar limitations of cellular heterogeneity also apply to saliva, skin, and any tissue such as placenta, brain, and others. However, in many cases, the relative cellular composition of these tissues is poorly characterized or methods may not be available to effectively disentangle cellular composition effects, relative to the true biological aging signal.

Static indicators of dynamic processes

Most biological age indicators reflect the current state of the organism, and the biological age of the sampled tissue and cells, at the moment of collection. In many cases, it is unknown how dynamic these markers are. In other words, how much they change from day-to-day, across the day (i.e., diurnal variation), or sometimes even within minutes, as is the case for neuroendocrine mediators and blood-based metabolites. It is generally assumed that most biological age indicators (and the specific measures that compose some of them) are largely stable, changing slowly over the course of years, but that assumption has gone untested or proven false for most indicators listed in **Table 1**. Unrecognized, unmeasured, or uncontrolled variability of biological age indicators due to regular or irregular changes over time has two undesirable effects: it introduces noise that cannot be accounted for, and possibly limits our interpretation of the downstream result. Biological age indicators may follow different trajectories over time and may theoretically be differentially sensitive to behaviors such as sleep, exercise, diet, meditation, and others. Studies with frequently – over hours, days, months, and years – repeated measures of biological age indicators will be necessary to establish the temporal kinetics for existing and new biological age indicators.

Specific limitations to measurements of biological aging

Limitations of functional capacity and physiological measures

A major advantage of functional capacity and physiological measures is the high efficiency in terms of costs and collection as well as their integrative informativeness, specifically compared to blood-based, molecular and DNA-based measurements. However, the predictive value of objective functional and physiological measures on mortality has mostly been reported in older populations (Cooper et al. 2010) and in middle-aged populations [98]. Except for some data reporting associations between handgrip strength and mortality in male adolescents [26], whether functional capacity measures such as walking speed are sensitive to aging and predictive of morbidity and mortality in younger populations largely remain to be established.

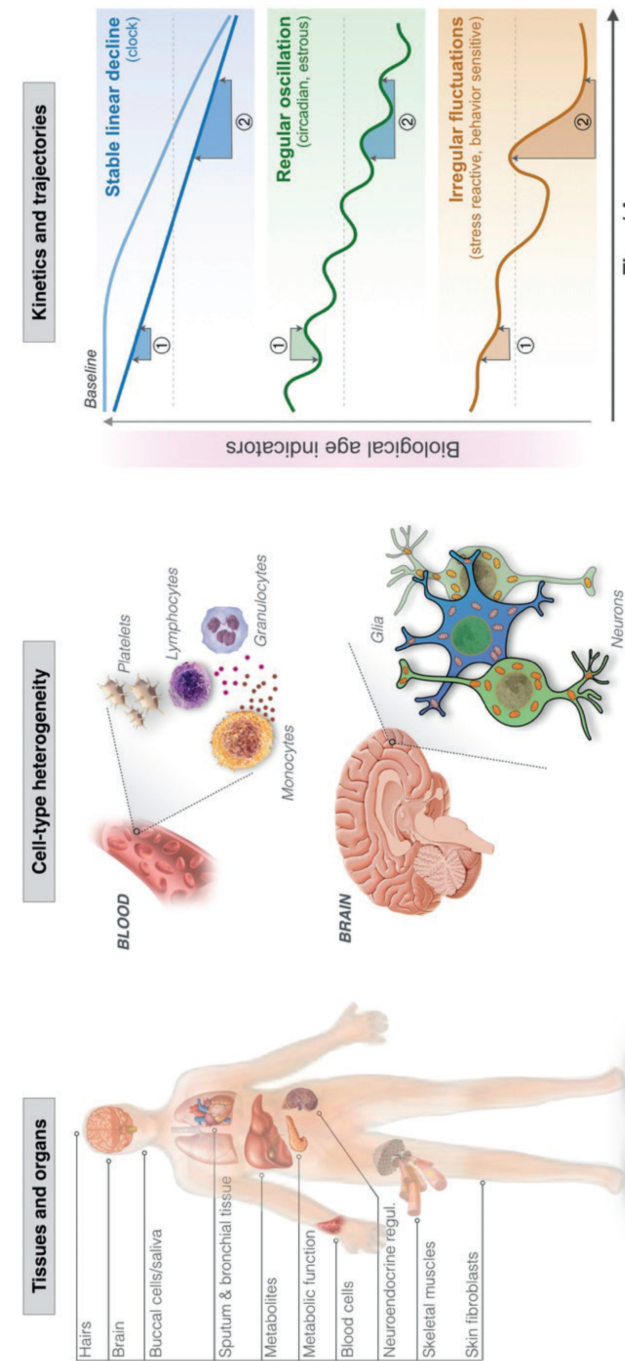


Figure 3. Major limitations of biological age indicators. (**Left**) Biological age indicators are measured in samples from different tissues and organs of the body. (Middle) Individual tissues such as blood and the cortical regions of the brain also exhibit substantial heterogeneity marked by relative abundance of different cell-types. (**Right**) Three hypothetical kinetics for biological age indicators are shown: The first shows a stable decline, which is typically assumed, but not necessarily accurate for most biological age indicators. The second illustrates an indicator subject to either circadian regulation or monthly estrous cycle, thus exhibiting regular oscillations. One such example is cortisol. Knowledge of this oscillatory pattern can be used to adequately schedule time of sampling (e.g., morning or evening) and derive useful parameters (e.g., cortisol awakening response). The third indicator shows irregular fluctuations, which could arise from sensitivity to acute stress mediators or to behavior (exercise, sleep, or other). Note the two hypothetical pairs of assessments on each trajectory. Mis-timing of measurement (1) for the regularly oscillating measure leads to pseudo-reversal of the biological aging indicator. Assessment (2) shows an exaggerated decline in the irregular fluctuation.

Limitations of brain function- and structure-based measures

Normal aging is accompanied by brain atrophy and loss of brain tissue volume, which can be quantified non-invasively with magnetic resonance imaging (MRI). Voxel-based morphometry and surface-based analysis are two commonly used image preprocessing techniques, which may yield divergent results [99, 100]. Moreover, the macroscopic volumetric changes observed in T1- and T2-weighted MR imaging reflect microscopic changes at the tissue and cellular levels, and in many circumstances possibly represent an aggregate of multiple cellular mechanisms related to synapses, neurons, and glial cells [101]. Thus, what changes in brain volume represent is not fully understood.

Age-related differences in brain function can also be detected with functional connectivity [102] and novel analytics on brain response are also available. For example, Garrett and colleagues [103] showed that the age-predictive power of the brain's signal variability was five times higher than that of the conventional method of assessing the average signal across time. But functional MRI data has low signal-to-noise ratio, and movement artifacts are one source of such noise. Because individuals of different ages might move differently in response to assessments, movement artifacts are a possible confounder in many study designs. Implementing methods to systematically review individual participants' images and manually separating noise from signal [104] could be a useful technique to minimize artifacts. There is evidence that manually cleaned BOLD-fMRI data, compared to data preprocessed with conventional automatic methods, better predicts chronological age [103], emphasizing the importance of data quality and pre-processing procedures in conclusions derived from brain-based age indicators.

A popular approach in neuroscience is to use statistical approaches to translate complex whole-brain multivariate patterns of aging into a single outcome [105], the so-called "brain age" (see [106] for details). Brain age algorithms [33, 107–111] generate accurate individual age predictions in healthy controls, but show greater prediction errors when applied to patient groups [3]. Within this framework, neuropathology may be reflected by the trajectory of aberrant normal aging, rather than a *different* deteriorating pattern of pathology. Gutierrez Becker and colleagues [112] show that Gaussian Process uncertainty in age estimation may yield a better separation between cases and healthy individuals than the prediction error. Nevertheless, brain age models have high reliability in terms of test-retest performance at both same and different scanners [110, 113], and have shown biologically meaningful associations with health, clinical, and neuropsychiatric phenotypes [3].

Limitations of cellular measures

Although cellular measures of aging have been used widely in the laboratory setting, they are seldom applied to human (clinical, epidemiological) research. For instance, replicative senescence, a cellular measure of aging, involves monitoring cells grown in culture, and counting the number of cells over time (e.g., [114]. This enables the investigator to count the number of times cells divide (i.e., total population doublings or "hayflick limit"), and determine the time required per cell division (i.e., population doubling time), which increases as cells age and divide more slowly. It should be noted that although senescence - defined as the loss of the ability of a cell to grow or divide - is associated with aging, it is not equivalent and can be dissociated from chronological age. Indeed, other factors such as irradiation can specifically induce senescence, even in chronologically young cells, thus reflecting biological aging.

The assessment of cellular bioenergetics, particularly mitochondrial content and functions, represents another domain of cellular aging measures. These indicators reflect the ability of cells to generate energy through oxygen-dependent mechanisms (for a review, see [115]. Respiration can be measured in whole cells [42] where it mostly reflects cellular energy demand, or in permeabilized cells [116] and isolated mitochondria where the intrinsic function of the organelle can be directly assessed independent of cellular contributions [44]. A major limitation of functional measures on intact cells and mitochondria is that measurements must be performed rapidly after blood sampling (within minutes to hours), which limits throughput and increases technical variability between samples, requiring exceptional standardization of procedures.

Other approaches relying on lysates (homogenized cells or mitochondria) from frozen samples allow measurement of enzymatic activity (e.g., telomerase, mitochondrial respiratory chain complexes) for multiple samples at once or in large batches [40]. A ubiquitous limitation to all measures of biological *activity* (as opposed to inert molecules) is the degradation of cellular and enzymatic activities over time when samples are stored under suboptimal conditions. An important unknown in this field is the degree to which storage conditions, and especially the length of time a sample resides in a freezer, contributes to changes in assay results. This issue is worthy of applied investigation, since there is commonly a trade-off between freezing samples for shorter periods of time vs. freezing samples for longer periods of time to minimize inter-assay variability in assaying sequential frozen batches. In contrast to molecular analytes that are mostly or fully preserved at -80ºC, samples destined for functional measurements should be stored in liquid nitrogen (< -150ºC).

Limitations of molecular measures

A large fraction of the most widely used biological age indicators are molecular in nature. They include DNA methylation (DNAm), metabolites, proteins, TL, mtDNAcn, circulating cell-free mtDNA (ccf-mtDNA), mtDNA damage, and others. One important consideration to all molecular measures is that inadequate handling of fresh samples can alter the concentration of various analytes, particularly metabolites. For example, whereas DNA markers are believed to be quite stable over minute to days, blood glucose concentration decreases within minutes when the blood is left at room temperature [117], owing to metabolic activities of white and red blood cells. The same must also apply to other metabolites that are detected by metabolomics. Gene expression assessed from messenger RNA transcript levels is also subject to rapid degradation and special care must be applied to blood destined to transcriptomic analyses [118]. These effects are minimized by rapid separation of the liquid and cellular components of whole blood by centrifugation immediately after blood draw, refrigeration (immediate storage of samples on wet ice, 4ºC), and subsequently freezing biological samples in a timely fashion.

Below we discuss specific molecular biological age indicators that have been subject of considerable research in relation to stress and psychopathology. Although exciting new findings from proteomic [69, 70] and metabolomic [71, 72] signatures of aging are beginning to arise, they have not been examined in relation to psychological factors. In this section, we focus our discussion on DNAm, TL, and mtDNAcn.

DNA methylation and epigenetic age

To date, several epigenetic age estimators have been developed from e.g. whole blood [64, 119, 120], neonatal cord blood and blood spots [121], and skin and blood cells [122]. Many potential confounders may cause technical variation in DNAm studies, of which population stratification and genetic ancestry are major contributors [123–125]. Therefore, there is also reason to assume that genetic variation impacts epigenetic age estimates, particularly considering recent studies that report strong genetic links [126]. Other potential confounders include smoking [127], sex, and prenatal factors [128]. Technically, DNAm arrays can show large variations between individual arrays and batches, and methods have been designed to statistically correct for these prior to analyses [129, 130].

While the validity of different methylation-based predictors is questioned, applications of the original pan-tissue Horvath clock [63] have been successful across tissue types and proven accurate even in embryonic brain samples [131]. The latest developed skin and blood predictor seems to be even more

robust across tissue types [122]. More recently, methods using as little as 3-10 CpG sites from blood samples also accurately predict age [120, 132] and mortality risk scores [133].

Whether statistical adjustment for cell type composition should be uniformly applied to whole blood-derived DNA to achieve optimal age prediction is the subject of ongoing debate. Whereas it has been argued that the Horvath method incorporates the estimation of cell type composition from blood and may not require cell type adjustment, some have shown that intrinsic (without adjustment for cell type composition) and extrinsic (with adjustment for cell type composition) age estimates may have a different biological meaning [134]. Also important to mention here is that other DNAm-based indicators of aging have been developed that are trained on phenotypic markers of age (DNAm PhenoAge) rather than chronological age, leading to improved predicted risk of mortality [119]. Briefly, phenotypic age is a combination of chronological age and nine disease-related biomarkers selected based on their association with mortality. The sensitivity of epigenetic predictors to psychosocial stress and psychopathology remains a gap of knowledge.

Telomere length

Issues related to TL assessments have been presented elsewhere [53] and will not be discussed in detail here. In general, we will note that multiple different assays exist, which vary in their cost, required volume, applicability on frozen samples, and throughput. Technically, these are important considerations that impact the feasibility of clinical and epidemiological studies. Moreover, because of the relatively inexpensive and high-throughput capacity of qPCR-based methods, TL has frequently been measured on total DNA extracted from cell mixtures, which can be derived from a variety of sources such as buccal swabs (which include both epithelial cells and leukocytes) and whole blood (which include a variety of leukocytes). For these measurements, and as for mtDNA measurements below, the limitations presented in Section 2.1 are particularly important.

mtDNA copy number and circulating cell-free mtDNA

Counting the number of mtDNA molecules per cell, or mtDNAcn, can indirectly provide an indication of the bioenergetic state of the cell. The mtDNAcn measurements are based on either qPCR (e.g., [135] or derived from whole exome or genome sequencing data (e.g., [136] where “counts” of both the mitochondrial and nuclear genomes are estimated. The ratio of mtDNA and nuclear DNA (nDNA) is then multiplied by 2 to account for the diploid nature of the nuclear genome and taken as mtDNAcn [137]. Here, given that cells with

different metabolic demand can differ by as much as an order of magnitude in their content of mtDNA, cell type differences may have a particularly profound effect on this measure. When applied to a homogenous cell population, mtDNAcn can provide valuable information. However, most reported studies with mtDNAcn have relied on whole blood DNA, which is confounded by cell type heterogeneity, and by the presence of platelets. Platelets do not have nDNA, but have mtDNA, which artificially inflates mtDNA copy number in whole blood preparations [138, 139]. In tissues with less heterogeneity than blood, such as skeletal muscle, some have observed no difference in mtDNAcn between young and old individuals [140]. The rate of decline in mtDNAcn per year also varies widely between studies, possibly as a result of differences in methodology and tissue source.

Similarly, measures of ccf-mtDNA are sensitive to cellular contamination and biological sample used. Serum (post-coagulation fraction of whole blood) may contain substantially more ccf-mtDNA than plasma (liquid fraction collected with an anticoagulant) [141]. Sufficient centrifugation speed and time are required to successfully eliminate cells, particularly platelets, that could artificially inflate serum or plasma ccf-mtDNA [142]. In studies where blood samples were not centrifuged at sufficient speeds, ccf-mtDNA levels are reportedly higher, making platelet contamination the most likely contributor to measured mtDNA levels and thus complicating interpretation of these results.

Associations among biological age indicators

While there is preliminary evidence for some cross-correlations among the different biological aging indicators, few have been examined in relation to other indicators. This highlights the need for examining multiple markers in an integrative study, as e.g. [143] recently showed low agreement between eleven quantifications of biological aging, with only modest associations to e.g. physical functioning and cognitive decline. Other studies suggest that TL is correlated to mtDNAcn [144], but the direction of stress and psychopathology effects with mtDNAcn or mitochondrial content (citrate synthase) and TL may vary [40, 42, 135, 136]. TL is not correlated with epigenetic age [58, 145, 146], although cell type composition adjustments may reveal a modest association [134]. Both epigenetic age and TL seem uncorrelated to brain age, and no associations were found between brain predicted age difference (brain-PAD) and epigenetic predicted age difference [39]. The correlations between the Hannum and Horvath clocks vary from relatively strong (r=0.76) to low (r=0.37) in independent studies [66, 143], and both clocks showed modest correlations (0.10-0.33) to the transcriptomic age indicator by [67]. The microRNA age

indicator of [68] was modestly correlated to epigenetic age (r=0.3) and microRNA expression (r=0.2). Cross-correlations between metabolomic/ proteomic aging and other biological aging indicators remain to be explored.

Do psychological stress and psychopathology influence biological aging?

Nearly 15 years have elapsed since Epel and colleagues first described the association of psychosocial stress with short leukocyte telomeres in a sample of healthy premenopausal mothers of a chronically ill child and mothers of healthy children [147]. The association of shortened telomeres with stress exposure has since been replicated in a wide variety of studies, and this observation stimulated several related lines of research examining the relationship between various forms of stress exposure or perceived stress and TL across the lifespan. Several excellent qualitative reviews describe and critically review this literature [10, 148–151] and meta-analyses now quantify the magnitude of these associations and identify potential moderators of effects (e.g., [47, 152–156]. More recently, an appreciation of the role of mitochondria in the acute stress response and chronic allostatic load [115, 157, 158] has led to investigations of the association of psychological states, stress exposure in relation to mitochondrial functions and mtDNA [40, 135, 136, 159]. Rather than being a comprehensive review, this section provides a brief overview of this field, emphasizing recent developments.

What is (psychological) stress?

Broadly defined, “stress” is the condition of being subjected to a stimulus (i.e., stressor) that invokes a response requiring the use of resources to adapt or cope [148, 160]. “Stress” may refer to particular life events (e.g., job loss, death of a loved one, assault), contexts that are experienced as stressful or contain numerous stressors (e.g., poverty, neighborhood violence, famine) or the psychological or biological response to such an event or exposure (i.e., stress response, perceived stress) [161]. Characteristics of the stressor(s) and the stress response may be important determinants of the biological response or adaptation and account for heterogeneity in the literature on stress and aging.

While some stressors occur in isolation, it is important to recognize that stress-inducing contexts, exposures, and perceptions of stress often covary, for example when families living in poverty experience neighborhood violence and feel unsafe. In addition, the level of perceived stress may vary

substantially within a group exposed to the same stressor. Determinants of psychological and biological stress responses include the nature of the stressor in terms of type, scope, severity, chronicity, and how predictable and controllable the stressor is. Individual and social characteristics influencing the level of perceived stress include social, financial, cognitive, emotional, and behavioral resources for coping with, controlling, avoiding, and compensating for stressors [161].

Stress and biological aging: Evidence for a stress-aging axis involving telomeres and mitochondria

The literature on the association of stressors and perceived stress on TL is now sufficiently large that a number of meta-analyses have been conducted on the topic. Meta-analyses of the association between TL and childhood psychosocial stressors document significant effects that vary from small to medium in size [152, 153, 162]. Moderator analyses suggest larger effects for studies that examine more severe exposures [152] and those that include wide range of adversity types [153]. In addition to cross-sectional investigations, a longitudinal study [163] found that exposure to violence over a 5-year period in childhood predicted greater TL attrition, suggesting the possibility of a causal relationship. The biological mechanisms whereby adverse or positive experiences exert their lasting health effects remain mostly unknown. Effects on the germline and stem cells reserves, metabolic reprogramming, and rewiring of neural networks and brain circuitry are among many areas that deserve further research.

Turning to stressors that occur in adulthood, a significant association between perceived stress and shorter TL has been documented in meta-analyses, though this effect ranged from very small [156] to modest [164] in size. Several studies have also shown associations of shorter TL with measures of severe or cumulative stress exposure in adulthood (for reviews see [10, 149, 165]). Although some studies suggest that childhood adversity may account for associations between TL and adult stressors [166, 167], there is also evidence that stressors experienced in adulthood prospectively predict telomere attrition [168, 169].

Given the growing literature demonstrating the central role that mitochondria play in the stress response and the aging process, recent studies have examined the association of early life stress with measures of mitochondrial function or mtDNAcn. Although mtDNAcn is not a measure of mitochondrial function and is impossible to interpret on its own, it is easily measured from stored DNA and has been measured in different studies. For example, childhood trauma or adversity, as well as adult psychopathology have been linked to higher mtDNAcn [135, 136]. In a small study of postpartum women, early life adversity was associated with greater cellular respiration reflecting increased cellular energy demand, which in turn was positively correlated with levels of pro-inflammatory cytokines and childhood maltreatment [159]. In a study of caregiving stress, caregivers were found to have reductions in a functional index of mitochondrial health (MHI) in blood leukocytes. Mitochondrial health was operationalized as a multivariate index designed to reflect functional capacity on a “per mitochondrion” basis. In this first study of MHI in mixed human leukocytes, the index included biochemical enzymatic activities for three mitochondrial enzymes and mtDNAcn. Using this composite index as an outcome, this study found that positive mood was associated with higher MHI and was a mediator of the association between caregiving and MHI [40]. Another study found that suicide attempters have significantly higher plasma levels of ccf-mtDNA [80] and another study found elevated ccf-mtDNA levels in individuals with major depressive disorder (MDD) [81].

A limitation of this body of research is that certain behavioral, psychiatric and medical conditions frequently co-occur with stress exposures and covary with TL and other biological processes central to aging, and thus may have confounding effects. These include smoking, obesity, dietary influences, anxiety, depressed mood, post-traumatic stress disorder (PTSD), medications, and cardiometabolic conditions, among others. These influences are not uniformly assessed, excluded, or statistically controlled. A meta-analysis on the association of early adversity and TL identified that the magnitude of the effect was smaller in studies that included participants with medical or psychiatric conditions, and participants on medications [153]. This finding suggests that the relationship between these conditions and shortened telomeres might obscure the effect of stress exposure, or, alternatively, that the psychiatric and medical conditions may be primarily responsible for some of the telomere effect [10]. Thus, the complex inter-relationships among these exposures, behavioral factors, and health conditions should be carefully considered when designing studies and analyzing and interpreting results.

Psychopathology and biological aging

Psychiatric disorders are associated with increased risk of aging-related medical conditions, including cardiovascular disease, stroke, dementia, diabetes, and obesity [7, 170], and early mortality [11]. While part of the association may be explained by differences in health behaviors, because individuals with psychiatric disorders are more likely to smoke, drink alcohol, eat poorly, and

exercise less than others [171], associations between psychiatric disorder status and medical morbidity remain significant after adjusting for these factors. This has led to the hypothesis that psychiatric conditions may induce or result from accelerated or premature biological aging. As reviewed in this review, there are multiple biological age indicators, including a range of cellular and molecular measures such as TL, mitochondrial dysfunction, oxidative stress, gene expression, and others [15]. Notably, inflammation is also a widely-used indicator of biological aging [172], coined as "inflammaging" by [21]. However, because of its elaborate discussion elsewhere [173], as well as in respect to mental health [174], we do not discuss it here. Overall, the following section will review the most frequently studied biological age indicators in epidemiological psychiatric research, including TL, epigenetic age, brain age, and to a lesser extent pro-inflammatory cytokines.

Associations of psychiatric disorders and biological age indicators

Simon and colleagues [175] were the first to report a relationship between psychiatric disorders and shorter telomeres in a sample that included MDD, bipolar disorder (BD) and anxiety disorder patients. Since then, a large number of studies have been conducted in an assortment of psychiatric disorders. MDD is among the most frequently studied disorders in this context, possibly as a consequence of its relatively well-documented associations with dysregulated physical health [7]. Several meta-analyses, the largest one containing >34,000 subjects from 38 studies, summarized the results and provided consistent evidence of an inverse association between TL and depression, generally with small to medium effect sizes [47, 155, 176]. Similar meta-analytic results were found for anxiety disorders (N>19,000) [48], and PTSD (N>3,800) [50]. BD, schizophrenia and other psychotic disorders have been less extensively examined. A meta-analysis including 1,100 subjects from 7 studies found no difference in TL between BD cases and controls [177]. Two meta-analyses on schizophrenia of 1,200 and 1,600 subjects, respectively, found small effects for TL differences [49, 178].

The epigenetic age indicator is most frequently examined in individuals with PTSD. A meta-analysis using data from 9 cohorts (combined N=2,186) found significant, albeit small, associations of greater epigenetic age with traumatic stress, but not with PTSD diagnosis [55]. Other studies also found such relations using the Horvath predictor [56, 179, 180], consistent with enrichment for glucocorticoid response elements [56]. Two recent studies for the first time showed "older" epigenetic age in MDD patients versus controls [58, 59], while this is not seen in schizophrenia [181, 182].

Furthermore, chronic, low-grade inflammation that increases on average with age is captured in the term "inflammaging" - a pro-inflammatory state proposed to contribute to the pathogenesis of age-related diseases [183]. An increase in the inflammatory response, together with microglial activation, in turn, can contribute to psychiatric diseases, such as MDD, schizophrenia, BD, and autism [184–186]. The mechanisms responsible for increased inflammation in mood disorders remains poorly understood. Recent evidence suggests that ccf-mtDNA could contribute to this pro-inflammatory state, although evidence is mixed [80, 81, 187]. Related to the bacterial origin of mitochondria, the mtDNA is immunogenic. Released mtDNA molecules thereby act as damage associated molecular patterns (DAMPs) recognized by toll-like receptors on immune cells and trigger immune cell activation [188]. Inflammaging could in part be due to increased ccf-mtDNA in older individuals [83].

Increased plasma levels of ccf-mtDNA have been reported in suicidal and depressed patients [80]. Worse response to an antidepressant was associated with increasing ccf-mtDNA levels over the treatment course, and ccf-mtDNA was correlated with antioxidant enzyme glutathione peroxidase, possibly as a result of a compensatory response to cellular oxidative stress [81]. An experimental study using psychological stress induction in healthy middle-aged individuals also demonstrated that an acute bout of psychological stress may be sufficient to elicit a 2-3 fold increase in serum ccf-mtDNA within 30 minutes, suggesting that ccf-mtDNA is dynamically regulated [189]. Consistent with previous findings linking ccf-mtDNA levels to cortisol levels following a dexamethasone suppression test [80], glucocorticoid stimulation of human cells (fibroblasts) induced the release of mtDNA by mitochondria within minutes [189]. Thus, the causes of elevated ccf-mtDNA in certain psychiatric conditions remain unknown, although clinical and cellular studies suggest that canonical neuroendocrine stress mediators, including but not limited to glucocorticoids, may be implicated.

In addition, associations have been reported between "older" brain age and psychiatric disorders such as borderline personality disorder [36], schizophrenia [33, 35, 36], and first-episode psychosis [34], as compared to younger in BD [36]. One relatively small study by [35] showed a higher brain-PAD of +4.0 years in MDD (N=104). However, a preliminary study by [190] finds increased brain age in schizophrenia (Cohen's d=0.55) and BD (d=0.30), but not MDD (N=211, d=0.10). In addition, these authors suggest that the brain age gap is a genetically modulated trait that is heritable and overlaps with polygenic architecture observed in common brain disorders. There is also preliminary evidence of an association between psychiatric pathology and glycomic-based biological age indicators, which represent sugar-based

modifications of proteins, RNA, and DNA molecules. Two studies have shown altered protein N-glycosylation profiles in female patients with MDD [73] and in PTSD [75], indicative of advanced aging at the glycomic level. Associations with other omics-based indicators (transcriptomics, proteomics, metabolomics) and their interrelation remain to be explored.

Biological aging and psychopathology: The chicken or the egg?

While robust cross-sectional associations between psychiatric disorders and biological aging have been documented - at least for TL - the nature and direction of these associations remain unclear. Longitudinal studies have found mixed effects (see, e.g. [179, 191–194]. It is currently unknown whether 1) psychopathology-associated physiological disturbances accelerate biological aging, 2) premature biological aging antedates and is a vulnerability factor that causes psychopathology, or alternatively, 3) psychopathology and biological aging processes share underlying etiological roots, such as shared genetic risks, and happen to be correlated without a causal link between them. Recent studies using genomic and causal inference tools are being developed to overcome this limitation of observational studies [195]. This challenge, among others, as well as recommendations to move the field towards a predictive science are discussed in details in Section 5.

Clinical implications

Disorder-specific or transdiagnostic phenomenon?

In a large meta-analysis considering multiple psychiatric disorders and TL, including depressive and anxiety disorders, PTSD, bipolar and psychotic disorder, no difference in effect sizes between disorders was found [176]. This indicates that different DSM-based diagnoses may not be associated with meaningful differences in biological aging [9]. Furthermore, several studies showed that short TL is associated with the same physiological dysregulations that are found in some but not all persons with psychiatric disorders. These include increased inflammation, oxidative stress markers, dysregulated HPA-axis, and metabolic dysregulations [196–199]. While the degree to which telomeres are causally related to these mechanisms is unknown, this is suggestive of pathways through which telomere shortening and psychiatric disorders are interrelated, that are not limited to one diagnostic category. The current evidence suggests that short TL may be a non-specific biological marker for conditions in which people experience chronic psychological or physiological stress, rather than being a marker of a specific psychiatric condition. Similarly, the downstream biological ramifications of different disorders also overlap, with alterations at the organs and systems level (e.g. brain aging patterns, [20]. This evidence leaves us to consider that these indicators are not disease-specific nor suitable as diagnostic tools, but rather general indicators of psychopathology or abnormal mental states.

Biological age indicators as predictors of treatment outcome

Only a small number of studies have investigated whether biological aging indicators predict antidepressant treatment response. The first study suggesting such a link showed in a small sample of previously unmedicated MDD subjects that low baseline telomerase activity, and a greater increase in telomerase activity during eight weeks of selective serotonin reuptake inhibitors (SSRI) treatment were associated to superior clinical outcome [200]. However, this study lacked a control condition, leaving open the possibility that naturally different clinical trajectories contributed to these effects. Nevertheless, these findings suggested that depressed patients with relatively low baseline telomerase activity may most benefit from therapies that may secondarily induce telomerase activation, and that telomerase activation may represent a mechanism of antidepressant action, consistent with several animal studies (reviewed in [201].

Subsequently, one human study found that shorter leukocyte TL predicted worse antidepressant response to an SSRI [202]. To the extent that accelerated biological aging is associated with antidepressant response, this effect does not seem likely to be confined to a specific treatment modality or drug. Shorter TL may also predict worse antidepressant response to pioglitazone, which also has antidiabetic effects [203]. Turning to other disorders, in a group of bipolar and schizophrenia/psychosis patients, lithium non-responders had shorter telomeres than responders [204], and similar associations were found linking short TL to poor treatment response [205, 206]. No studies have investigated the association between TL and response to psychotherapy (e.g., cognitive behavioral therapy), although preliminary evidence suggests a positive correlation between mindfulness/meditation practices and telomere biology, including increased telomerase activity [154, 207]. Furthermore, an increased inflammatory response may hamper the responsiveness to mood disorder treatments, and higher baseline inflammation may lead to treatment resistance [186, 208]. Changes in ccf-mtDNA levels were also found to be associated with SSRI treatment response, with the non-responders showing an increase in ccf-mtDNA and responders not changing [81]. A shared genetic disposition for inflammation, psychopathology, and treatment responsiveness

has also been suggested [209]. Furthermore, inflammatory indicators may potentially offer personalized antidepressant recommendations, and could eventually guide the development of novel antidepressant treatments [210]. Overall, the link between biological age and treatment responsiveness is a young field requiring further research.

Can biological aging be reversed with treatment?

One of the most relevant clinical questions in the field of biological aging and psychiatry is whether accelerated biological aging is a permanent imprint or a reversible process. While this may differ between indicators of biological aging, reversibility is at least possible to some extent for some indicators. For TL, the main mechanism of restoration is likely telomerase activation. Animal and in-vitro research provided evidence that telomerase-associated recovery of TL is to some extent possible [211, 212]. Several intervention studies have attempted to influence TL in humans (see [8] for an overview). For example, recent small controlled studies have shown elongation of telomeres in response to highly controlled aerobic exercise verified with actigraphy [213], losing and maintaining a weight loss of 10% or greater [214], and meditation-based interventions [207]. As mentioned above, for the field of psychiatry telomerase activation may be a mechanism of antidepressant action. Although no strong conclusions can be drawn due to a lack of well-powered clinical studies, there are several potential mechanisms by which psychiatric medications might modulate telomerase activity or TERT expression, including via increased brain-derived neurotrophic factor (BDNF) expression [201]. Increased telomerase activity may, in turn, induce clinical effects by promoting cellular survival and/or functioning. It should however be noted that increased telomerase activity can prevent cell senescence, an anti-cancer mechanism, and thus excessive telomerase activity is also associated with increased risk of cancer. Thus, while telomerase activation may hold the promise of reducing risk of aging-related disease, the risk of less common but very serious cancer outcomes must be carefully weighed [215].

Cross-sectional studies show that certain behaviors may provide some protection against brain aging. Higher physical activity levels are associated with lower brain age [216], and "younger brains" are seen in those that learn to play an instrument [217] and those who have practiced meditation for long periods [105]. It remains to be elucidated whether brain age is responsive to intervention, but a randomized controlled trial (RCT) showed that ibuprofen temporarily reduced brain-PAD by 1.1 years in healthy individuals, likely due to its acute anti-inflammatory effects [218]. Physical activity may slow age-related

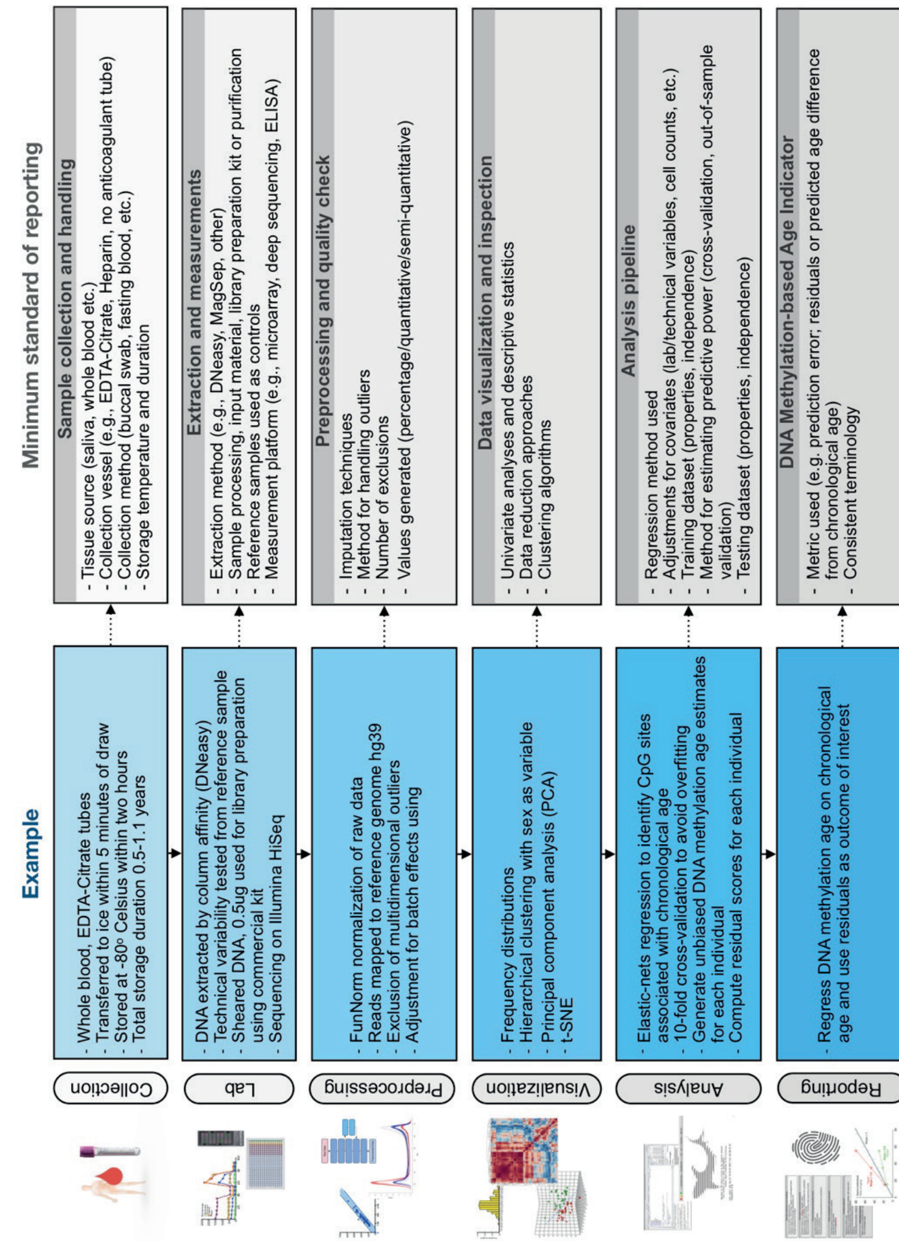


Figure 4. Overview of the biological age indicator prediction process and recommended minimum reporting guidelines. (**Left**) Example workflow for calculating a epigenetic age indicator and age acceleration. (**Right**) Recommended workflow that can inform study design, execution, analysis, and preparation of methods section in the resulting reports. Adherence to such standards for reporting results would facilitate harmonization of datasets across laboratories and cohort studies.

DNAm changes in humans [219, 220]. Longitudinal data shows that increasing BMI is associated with increasing epigenetic age [221], but in another report, epigenetic age from the liver was not “decelerated” after successful weight loss over a 9-month period through bariatric surgery [222]. [221] also found that consumption of fish, fruits, and vegetables, as well as effects of moderate alcohol, education, and income and exercise induced anti-aging effects based on epigenetic age (Hannum clock). However, these findings are cross-sectional observations rather than longitudinal effects from RCTs. Nonetheless, a recent RCT suggests that vitamin D supplementation may decrease epigenetic aging based on the Horvath, but not Hannum epigenetic clock [223].

More controlled intervention studies are needed to determine whether biological aging indicators are truly modifiable in response to exercise, nutritional and/or pharmacological interventions. A common problem to observational studies is that behaviors tend to correlate, making it difficult to evaluate the specific influence of a given intervention or behavior in isolation. Individuals who exercise more tend to practice meditation more frequently, eat more plant-based and vegetarian diets, consume less illicit substances, etc. An additional problem with observational studies is that it is impossible to establish the direction of effects; while exercise may attenuate indicators of aging, biologically younger individuals may be more able and inclined to exercise.

Key challenges and priorities for future research

There are key challenges to accurately measure and interpret biological age indicators and to further our understanding of stress and biological aging. A partial list of six major challenges related to priority areas for the field, as well as recommendations to overcome them is presented in this section. We also summarize essential steps and propose a minimum standard for the design, collection, processing, analysis, and reporting of data involving biological age indicators (**Figure 4**).

Challenge 1. Correlation is not causation. It is hazardous to infer causality from cross-sectional correlational data [224]. For example, in the case of TL, it is possible that telomere shortening reflects states of stress, or responds to somatic or psychiatric illness, or at least to biochemical abnormalities associated with these states. It is also possible, however, that telomere shortening precedes somatic or psychiatric illness [225] or even underlies biological changes that causes these conditions. Biological age indicators could be entirely independent from directly assessing biological age mechanisms

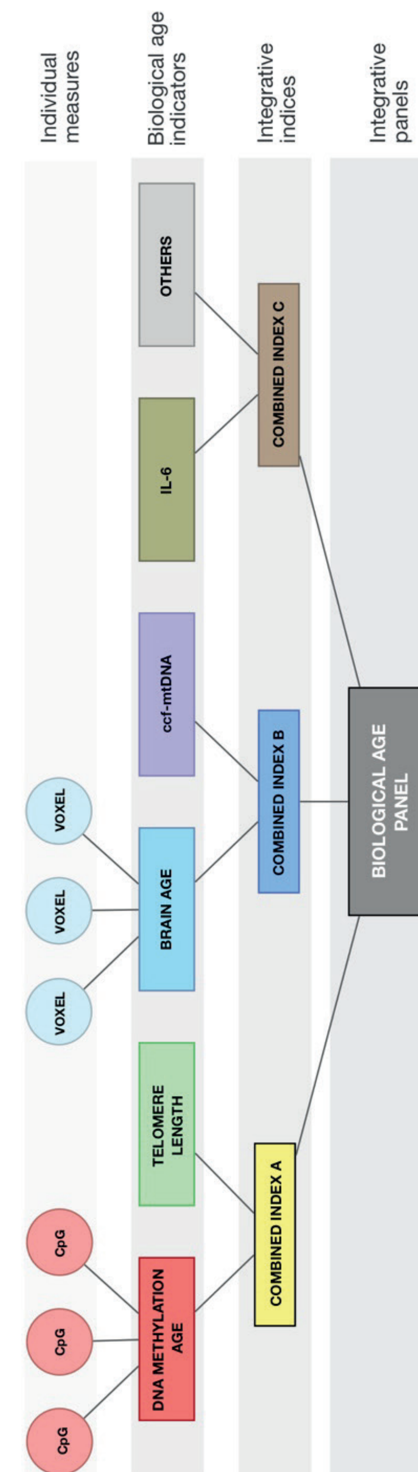


Figure 5. Topology of biological age indicators, upstream measures, and downstream integrative indices and panels. Biologically-informed and functionally relevant composite indices integrating two or more indicators can be derived from individual indicators. Their added predictive power should be validated by either in-sample cross-validation or preferably out-of-sample validation. Integrative biological age panels may eventually outperform single biological age indicators and indices due to the strength of their association, their construct stability, and/or their greater generalizability across individuals and independent samples.

that drive the aging process. Changes in biological age indicators could be the “canary in the coal mine” [226], representing factors *associated* with aging rather than aging itself.

Recommendation 1: Collect data longitudinally, consider experimentation, and choose prediction over explanation. This is a three-part recommendation. First, future studies should include longitudinal designs to increase the reliability and accuracy of measuring aging [227]. Longitudinal measurements will also be important in determining which variables are critical for the maintenance of successful aging throughout the lifespan (e.g. absolute levels, change, variability), the “recipe” of which may vary between individuals [228]. Moreover, it will be critical to determine the optimal intervals of time between repeated assessments that are needed to detect meaningful changes in specific biological age indicators (see **Figure 3**). This information about the timing and spacing of repeated measures, and real-life constraints, should then be used to inform the design and choice of outcome measures when evaluating the effectiveness of interventions aiming to influence biological aging.

Second, experimental approaches utilizing cellular (or animal) models allow the direct manipulation of a specific (set of) variable(s). Thus, if we assume that a given stressor or predictor can be modeled accurately *in vitro*, experimental designs can provide direct causal evidence that a given factor is necessary and sufficient to produce a given outcome of interest. For this approach to empirically support a biological interaction between stressors and biological aging, the biological age indicator also needs to be detectable and meaningful in vitro, such as epigenetic age (e.g., [122]. In cases where experimental demonstration is not possible, statistical methods such as causal inference [229] and genomic methods including Mendelian randomization [195, 230] can substantially reinforce our confidence regarding the direction of effects.

Third, in some cases, the number of predictors one wishes to consider is very large, either because there is no prior knowledge of their relative importance, or because the problem is truly complex – such as human biological aging. In such cases, the number of predictors can be large relative to the number of individuals, providing insufficient power for traditional inference-based statistics. In such cases, machine learning-based predictive modeling may be advisable to discover and validate predictive relationships between variables. Whereas statistics draw population inferences from a sample, machine learning finds generalizable predictive patterns [231]. Using predictive modeling approaches that identify and validate combinations of predictors in relation to a particular

health outcome can increase the likelihood that the identified predictors of biological age are robust, specific, and generalizable. An excellent article on the value of prediction over explanation in the psychological sciences is [232]. Regardless of the analytical approach taken, we should emphasize the value of converging evidence collected using different methods, measuring multiple (related and unrelated) predictors in parallel, and assessing multiple outcomes [233].

Challenge 2. Single biological age indicators are not correlated and may be better integrated. Not all measurements of biological aging are equally useful or inter-related, and it remains to be elucidated if and how different indicators relate to one another and which biological determinants are consistent across measures. As previously noted [3], no single biological age indicator can currently fully capture the complexity of the aging process, nor predict future health outcomes or lifespan with sufficient accuracy. There is therefore a need for combined indices that logically integrate multiple indicators (**Figure 5**), hopefully resulting in accurate integrative panels that outperform single measurements of biological aging, also previously suggested by [2]. Nevertheless, integration of indicators cannot compensate for inadequately powered studies, which require large sample sizes to ensure high generalizability.

Recommendation 2: Combine machine learning and other artificial intelligence techniques to create composite indices and panels of biological age indicators relevant to mental health. To date, several efforts to develop combined indices have been presented. For example, [145] showed an additive effect of combining TL and epigenetic age in explaining the proportion of age variance of their model. Similarly, [39] explained significantly more variance in the prediction of mortality by combining brain-PAD and the Horvath epigenetic predicted age difference, than either indicator alone. Other examples include multivariate indicators of aging incorporating multiple physiological and functional measures [234] and indices integrating multiple enzymatic and molecular measures of mitochondrial content and function in blood leukocytes as the MHI in association to psychological states (i.e., positive mood) [40]. These constitute early attempts to reverse our reductionist inclinations and to move towards integrative metrics that will hopefully lead to improved prediction.

Challenge 3. Biological aging may be tissue- and cell-type specific. Related to the above, biological age indicators derived from specific cell or tissue types may not generalize to other cells or tissues. Biomarkers assessed in blood, for example, represents the average of multiple heterogeneous cell types. But cellular or organismal health may be more closely related to or reflected by indicators within individual cell types or those with the most extreme values (e.g., Flow-FISH, [53]. Furthermore, TL differs across leukocyte cell types, such as naïve vs mature T cells [235, 236], and differs in different regions of the brain [92]. Moreover, TL of particular cell types can be differentially vulnerable to attrition or affected by stress-related pathology [95].

Recommendation 3: Purify cell types using established molecular markers. Purification of cell types can be accomplished by a variety of methods (flow cytometry, magnetic-activated cell sorting), yielding living cells amenable to downstream molecular and cellular analyses [96]. Under certain conditions where it is not possible to isolate specific cell subtypes, it may be difficult to interpret certain indicators that exhibit large cell type-specific values (such as mtDNAcn in blood). In some limited cases where a lot of information is available for adjustment, such as for DNAm measured on bead chips (100,000's of data points), it is possible to use statistical approaches, i.e. reference-based [237] or reference-free [238], to infer underlying cell type proportions [239] and adjust results accordingly. Another interesting application is demonstrated in a preliminary study by [240] that uses deconvolution approaches to show novel MDD-methylation associations in individual sub-populations of neurons/glia from bulk brain, as well as in granulocytes/T-cells/B-cells/monocytes from bulk blood data. In addition, creative ways to harvest other cell types from tissues other than blood could yield increasingly meaningful biological age indicators. For example, the DNAm-based skin & blood clock by [122] is robust across tissues (e.g. fibroblasts, buccal, endothelial, saliva samples) and can, therefore, be applied to many organs, as well as ex vivo. Thus, this approach provides extended information on synchronized biological aging, independent of sampling source.

Challenge 4. Within-group variance may be larger than between-group variance. Although group differences in biological age indicators have been reported in several psychiatric illnesses [9, 176], these reflect average group differences. However, there is often considerable within-groups variability and considerable overlap between groups, making it very difficult to use most biological age indicators as diagnostic aids. In addition, even specific psychopathological diagnoses (e.g., MDD or schizophrenia) often include individuals that vary widely in their symptoms and presentation, making the search for predictors of mental illnesses defined diagnostically somewhat elusive [241]. As [242] suggest, the complex, highly polygenic and multifaceted causes of severe mental disorders may only be fully understood by mapping patients' individual signatures, rather than studying the average patient. Population-based normal ranges have yet to be reliably determined, so biological age indicators may be more useful in detecting within-subject changes over time rather than in comparing individuals or in establishing actuarial norms. Nonetheless, a recent study utilizing flow-FISH assay techniques reported reproducible and definable upper and lower normal boundaries for TL in a hospital population [243], indicating that standardization of these measures may be achievable. Within-group variance is often due to measurable individual differences in behavior, health condition, and lifestyle.

Recommendation 4: Visualize your data, carefully assess known influences, move beyond group-based analyses, and use within-person modeling approaches. To visualize data where there are multiple measures over time per individual, we advocate for spaghetti plots (example with epigenetic age and TL can be found in [145], and with cortisol trajectories in [244]. Standard measures of variance can also be used to model population variability at different timepoints and to compare sub-groups, and more sophisticated mathematical approaches can be useful to generate individualized phenotypes [245]. Data reduction approaches such as principal component analysis (PCA), partial least square discriminant analysis (PLS-DA), and t-distributed stochastic neighbor embedding (t-SNE) are also useful to visualize high-dimensional data in two or three dimensions and to assess whether subsets of individuals in the sample naturally cluster together [246, 247]. This kind of approach can provide evidence of shared phenotypes or trajectories that would otherwise remain undetectable by standard uni- or multivariate analyses. Other statistical approaches to identify different subgroups exhibiting different trajectories in biological aging or in clinical course include growth mixture modeling [244], and random coefficients linear regression models to examine within-person changes over time [248].

Challenge 5. Large sample sizes are needed to detect small effect sizes. Related to the point above, group differences between individuals with certain psychiatric illnesses vs. healthy individuals, even if statistically significant, often have small effect sizes that require large sample sizes to be demonstrated [58, 59, 176, 249]. Similarly, even using predictive modeling and machine learning approaches, small samples sizes are more susceptible

to overfitting [232]. It would be informative to pool databases across studies and to check for consistency and predictive accuracy across studies, albeit at the cost of increasing heterogeneity of the samples studied and of the laboratory methods used. [250] argue that larger sample sizes will have more generalization power. This is important for creating robust "canonical" publicly available prediction models (e.g. Horvath's epigenetic clock) that can be readily applied to smaller studies that cannot permit partitioning their data into a training and validation set.

Recommendation 5: Collaborate and harmonize data collection and analysis protocols to facilitate data pooling worldwide. Certain types of data may be available through crowdsourcing, a data collection process with remarkable scalability that may enable identification of robust small effect size effects [251]. Large-scale collaborative initiatives like the Enhancing NeuroImaging Genetics through Meta-Analysis consortium (ENIGMA) for imaging and genetics data may be useful examples [252]. When not possible to replicate findings on sufficiently large independent datasets, use cross-validation methods to avoid overfitting [232].

Challenge 6. Non-uniform laboratory assays and storage conditions. Different assay methodologies can yield relatively different results (e.g. qPCR vs. Southern blot for TL assessment, [52], and most assays such as Flow-FISH assays and qPCR ascertain relative TL rather than absolute length. These issues have been discussed in detail elsewhere [53], and the relative merits of the different assays have been compared. A more mundane but important and under-appreciated caveat is that methodological differences can yield spurious results (e.g., length of time a specimen was kept in a freezer, freezer temperature, whether whole blood or intact cells vs. cellular lysate were frozen, specific batch of reagents used, assay technique, method of DNA extraction [for TL] [253–255]. An important issue that has not yet been experimentally assessed in multi-year human studies is the relative merit of assaying batches of samples at short intervals (e.g., every year) to minimize freezer time, compared to keeping all samples frozen until the end of the entire multi-year study so that all samples can be assayed simultaneously using identical procedures and reagents.

Recommendation 6: Harmonize measurements and storage conditions - colder is better. Follow guidelines, where available, to ensure that samples are collected and measured with the highest standards. Efforts are currently underway to systematically compare methods available to measure TL and

will hopefully produce specific guidelines that can be implemented at a large scale. **Figure 4** summarizes some measures that can be considered in the design, collection, pre-processing, analysis, and reporting of data to give researchers the ability to critically evaluate published results and hopefully harmonize methods and datasets.

Summary

Much progress has been made in the last decade towards developing objective biological age indicators but several key challenges and opportunities remain. Multiple new indicators spanning physiological and functional capacity, brain function and structure, and cellular and molecular levels of analysis have recently emerged, particularly under the force of 'omics' technologies. Most indicators show good to excellent correlations with chronological age, and some have the ability to predict age-related outcomes such as mortality with moderate accuracy (see **Table 1**). However, some have not yet been prospectively studied in large cohorts so their predictive power in relation to health outcomes remains unknown. In fact, many proposed biological age indicators still require replication and validation in larger independent datasets. As a whole, the development of multi-systemic biological age indicators has demonstrated that aging occurs not at a single level in a specific cell type, but rather manifests somewhat differently in various organs and tissues, cell types, and across a number of levels (see **Figure 1**). Rapidly developing methods show that biological age indicators gain in precision and prediction accuracy by leveraging biologically-informed approaches to integrate individual indicators into more powerful indices and panels of measures.

In relation to psychological stress and mental health, much work also remains to establish to what extent and how psychological states influence the aging process. Equally important to mapping these psycho-biological processes is to understand the "reverse" causal link whereby accelerated biological aging may impact physiological vulnerability and resilience to life stressors and psychopathology. A shared objective for our field is to disentangle these associations and identify the causal pathways that drive aging and mental health trajectories. To do so, we need to address number of key challenges that, if adequately met, will yield exciting opportunities to advance our understanding of human health.

To achieve this objective, it will also be essential for clinicians, psychologists, epidemiologists, and behavioral scientists to enter in a dialogue with experimental biologists. Such dialogue immediately opens new questions that would not otherwise arise within the silos of individual disciplines, departments,

and laboratories. For example, the joining of ideas around psychosocial stress and cellular aging [147], age-related elevation in circulating DNA [256], and of psychoneuroimmunology and the pro-inflammatory effects of psychological stress [257] has led to the idea that acute psychological stress may rapidly trigger immunogenic mtDNA release into the circulation [189]. If met with sufficient enthusiasm and resources, these jointly-created interdisciplinary questions can subsequently push the development of new laboratory methods, statistical and analytical tools, and new theoretical models. The interdisciplinary field of human psychobiology is replete with opportunities for innovation and discoveries.

Overall, we believe that developing, validating, and studying predictive biological age indicators – and defining how psychological states or psychiatric illnesses influence them – will fill important knowledge gaps linking stress and mental illness to aging. Achieving this goal will have at least three main positive consequences for the biomedical sciences generally. *First*, it will enable the stratification of individuals (and group of individuals) based on their current health state and future disease risk, including symptoms, disorders. This notion converges with the precision medicine agenda that aims to identify individualized predictors of future mental health and disease states [258]. Achieving this goal could benefit clinical practice by providing clear and objective guidelines to direct health-promoting interventions and treatment delivery in a personalized way, specifically one that is most aligned with the individual's needs [259]. *Second*, achieving this goal will provide objective and sensitive methods to evaluate the effectiveness of health-promoting interventions. The efficacy of interventions aimed at decreasing or even reversing advanced or premature aging, as well as interventions aimed at enhancing well-being and other health outcomes would be more effectively assessed using precise and sensitive biological age indicators. *Finally*, mapping stress-sensitive biological age indicators will also generate knowledge about human aging that can be taken back to the laboratory bench to orient basic biological science. Specifically, understanding the basis of normal and abnormal aging processes may identify new physiological targets for therapeutic intervention. Thus, an unintended consequence of improving existing biological age indicators and developing new end points for clinical and epidemiological human research may, in the end, create leaps in understanding about the fundamental biological mechanisms that explain why we age in the first place.

REFERENCES

1. Fernandes M, Wan C, Tacutu R, Barardo D, Rajput A, Wang J, et al. Systematic analysis of the gerontome reveals links between aging and age-related diseases. Hum Mol Genet. 2016;25:4804–4818.
2. Xia X, Chen W, McDermott J, Han J-DJ. Molecular and phenotypic biomarkers of aging. F1000Res. 2017;6:860.
3. Cole JH, Marioni RE, Harris SE, Deary IJ, Cole JH. Brain age and other bodily ' ages ' : implications for neuropsychiatry. Mol Psychiatry. 2018. 2018. https://doi.org/10.1038/s41380-018-0098-1.
4. de Magalhães JP. Programmatic features of aging originating in development: aging mechanisms beyond molecular damage? FASEB J. 2012;26:4821–4826.
5. de Magalhães JP, Passos JF. Stress, cell senescence and organismal ageing. Mech Ageing Dev. 2018;170:2–9.
6. Jylhava J, Pedersen NL, Hagg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.
7. Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 2013;11:129.
8. Verhoeven JE, Révész D, Wolkowitz OM, Penninx BWJH. Cellular aging in depression: Permanent imprint or reversible process?: An overview of the current evidence, mechanistic pathways, and targets for interventions. Bioessays. 2014:1–11.
9. Lindqvist D, Epel ES, Mellon SH, Penninx BW, Révész D, Verhoeven JE, et al. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neurosci Biobehav Rev. 2015;55:333–364.
10. Epel ES, Prather AA. Stress, Telomeres, and Psychopathology: Toward a Deeper Understanding of a Triad of Early Aging. Annu Rev Clin Psychol. 2018;14:371–397.
11. Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA Psychiatry. 2015;72:334–341.
12. Lohr JB, Palmer BW, Eidt CA, Aailaboyina S, Mausbach BT, Wolkowitz OM, et al. Is Post-Traumatic Stress Disorder Associated with Premature Senescence? A Review of the Literature. Am J Geriatr Psychiatry. 2015;23:709–725.
13. Abi-Dargham A, Horga G. The search for imaging biomarkers in psychiatric disorders. Nat Med. 2016;22:1248–1255.
14. Engelfriet PM, Jansen EHJM, Picavet HSJ, Dollé MET. Biochemical markers of aging for longitudinal studies in humans. Epidemiol Rev. 2013;35:132–151.
15. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–1217.
16. Wagner KH, Cameron-Smith D, Wessner B, Franzke B. Biomarkers of aging: From function to molecular biology. Nutrients. 2016;8:8–10.
17. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. 2018;19:371–384.
18. Stephan Y, Sutin AR, Terracciano A. Subjective Age and Mortality in Three Longitudinal Samples. Psychosom Med. 2018;80:659–664.
19. Levy BR, Slade MD, Kunkel SR, Kasl SV. Longevity increased by positive self-perceptions of aging. J Pers Soc Psychol. 2002;83:261–270.
20. Cole JH. Neuroimaging Studies Illustrate the Commonalities Between Ageing and Brain Diseases. Bioessays. 2018;1700221:1700221.

21. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244–254.
22. Lemke MR, Wendorff T, Mieth B, Buhl K, Linnemann M. Spatiotemporal gait patterns during over ground locomotion in major depression compared with healthy controls. J Psychiatr Res. 2000;34:277–283.
23. Michalak J, Troje NF, Fischer J, Vollmar P, Heidenreich T, Schulte D. Embodiment of Sadness and Depression—Gait Patterns Associated With Dysphoric Mood. Psychosom Med. 2009;71:580.
24. Cooper R, Kuh D, Hardy R, Mortality Review Group, FALCon and HALCyon Study Teams. Objectively measured physical capability levels and mortality: systematic review and meta-analysis. BMJ. 2010;341:c4467.
25. Yates T, Zaccardi F, Dhalwani NN, Davies MJ, Bakrania K, Celis-Morales CA, et al. Association of walking pace and handgrip strength with all-cause, cardiovascular, and cancer mortality: a UK Biobank observational study. Eur Heart J. 2017;38:3232–3240.
26. Ortega FB, Silventoinen K, Tynelius P, Rasmussen F. Muscular strength in male adolescents and premature death: cohort study of one million participants. BMJ. 2012;345:e7279.
27. Bohannon RW. Hand-grip dynamometry predicts future outcomes in aging adults. J Geriatr Phys Ther. 2008;31:3–10.
28. Lee M-R, Jung SM, Bang H, Kim HS, Kim YB. The association between muscular strength and depression in Korean adults: a cross-sectional analysis of the sixth Korea National Health and Nutrition Examination Survey (KNHANES VI) 2014. BMC Public Health. 2018;18:1123.
29. Lever-van Milligen BA, Lamers F, Smit JH, Penninx BW. Six-year trajectory of objective physical function in persons with depressive and anxiety disorders. Depress Anxiety. 2017;34:188–197.
30. Sasaki H, Kasagi F, Yamada M, Fujita S. Grip strength predicts cause-specific mortality in middle-aged and elderly persons. Am J Med. 2007;120:337–342.
31. Sayer AA, Kirkwood TBL. Grip strength and mortality: a biomarker of ageing? Lancet. 2015;386:226–227.
32. Stavem K, Aaser E, Sandvik L, Bjørnholt JV, Erikssen G, Thaulow E, et al. Lung function, smoking and mortality in a 26-year follow-up of healthy middle-aged males. Eur Respir J. 2005;25:618–625.
33. Schnack HG, van Haren NEM, Nieuwenhuis M, Hulshoff Pol HE, Cahn W, Kahn RS. Accelerated Brain Aging in Schizophrenia: A Longitudinal Pattern Recognition Study. Am J Psychiatry. 2016;173:607–616.
34. Kolenic M, Franke K, Hlinka J, Matejka M, Capkova J, Pausova Z, et al. Obesity, dyslipidemia and brain age in first-episode psychosis. J Psychiatr Res. 2018;99:151–158.
35. Koutsouleris N, Davatzikos C, Borgwardt S, Gaser C, Bottlender R, Frodl T, et al. Accelerated brain aging in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. Schizophr Bull. 2014;40:1140–1153.
36. Nenadic I, Dietzek M, Langbein K, Sauer H, Gaser C. BrainAGE score indicates accelerated brain aging in schizophrenia, but not bipolar disorder. Psychiatry Research: Neuroimaging. 2017. 2017. https://doi.org/10.1016/j.pscychresns.2017.05.006.
37. Hajek T, Franke K, Kolenic M, Capkova J, Matejka M, Propper L, et al. Brain Age in Early Stages of Bipolar Disorders or Schizophrenia. Schizophr Bull. 2017. December 20, 2017. https://doi.org/10.1093/schbul/sbx172.
38. Hatton SN, Franz CE, Elman JA, Panizzon MS, Hagler DJ Jr, Fennema-Notestine C, et al. Negative fateful life events in midlife and advanced predicted brain aging. Neurobiol Aging. 2018;67:1–9.
39. Cole JH, Ritchie SJ, Bastin ME, Valdés Hernández MC, Muñoz Maniega S, Royle N, et al. Brain age predicts mortality. Mol Psychiatry. 2017:1–8.

40. Picard M, Prather AA, Puterman E, Cuillerier A, Coccia M, Aschbacher K, et al. A Mitochondrial Health Index Sensitive to Mood and Caregiving Stress. Biol Psychiatry. 2018;84:9–17.
41. Boeck C, Gumpp AM, Calzia E, Radermacher P, Waller C, Karabatsiakis A, et al. The association between cortisol, oxytocin, and immune cell mitochondrial oxygen consumption in postpartum women with childhood maltreatment. Psychoneuroendocrinology. 2018;96:69–77.
42. Boeck C, Salinas-Manrique J, Calzia E, Radermacher P, von Arnim CAF, Dietrich DE, et al. Targeting the association between telomere length and immuno-cellular bioenergetics in female patients with Major Depressive Disorder. Sci Rep. 2018;8:9419.
43. Karabatsiakis A, Böck C, Salinas-Manrique J, Kolassa S, Calzia E, Dietrich DE, et al. Mitochondrial respiration in peripheral blood mononuclear cells correlates with depressive subsymptoms and severity of major depression. Transl Psychiatry. 2014;4:e397.
44. Tyrrell DJ, Bharadwaj MS, Van Horn CG, Kritchevsky SB, Nicklas BJ, Molina AJA. Respirometric Profiling of Muscle Mitochondria and Blood Cells Are Associated With Differences in Gait Speed Among Community-Dwelling Older Adults. J Gerontol A Biol Sci Med Sci. 2015;70:1394–1399.
45. Gonzalez-Freire M, Scalzo P, D'Agostino J, Moore ZA, Diaz-Ruiz A, Fabbri E, et al. Skeletal muscle ex vivo mitochondrial respiration parallels decline in vivo oxidative capacity, cardiorespiratory fitness, and muscle strength: The Baltimore Longitudinal Study of Aging. Aging Cell. 2018;17.
46. Greco M, Villani G, Mazzucchelli F, Bresolin N, Papa S, Attardi G. Marked aging-related decline in efficiency of oxidative phosphorylation in human skin fibroblasts. FASEB J. 2003;17:1706–1708.
47. Schutte NS, Malouff JM. The association between depression and leukocyte telomere length: A meta-analysis. Depress Anxiety. 2015;32:229–238.
48. Malouff JM, Schutte NS. A meta-analysis of the relationship between anxiety and telomere length. Anxiety Stress Coping. 2017;30:264–272.
49. Polho GB, De-Paula VJ, Cardillo G, dos Santos B, Kerr DS. Leukocyte telomere length in patients with schizophrenia: A meta-analysis. Schizophr Res. 2015;165:195–200.
50. Li X, Wang J, Zhou J, Huang P, Li J. The association between post-traumatic stress disorder and shorter telomere length: A systematic review and meta-analysis. J Affect Disord. 2017;218:322–326.
51. Cawthon RM. Telomere measurement by quantitative PCR. 2002;30:1–6.
52. Aviv A, Hunt SC, Lin J, Cao X, Kimura M, Blackburn E. Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. Nucleic Acids Res. 2011;39:e134.
53. Aubert G, Hills M, Lansdorp PM. Telomere length measurement-caveats and a critical assessment of the available technologies and tools. Mutat Res. 2012;730:59–67.
54. Wang Q, Zhan Y, Pedersen NL, Fang F, Hägg S. Telomere Length and All-Cause Mortality: A Meta-analysis. Ageing Res Rev. 2018;48:11–20.
55. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A, et al. Traumatic stress and accelerated DNA methylation age: A meta-analysis. Psychoneuroendocrinology. 2018;92:123–134.
56. Zannas AS, Arloth J, Carrillo-Roa T, Iurato S, Röh S, Ressler KJ, et al. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. Genome Biol. 2015;16:266.

57. Fries GR, Bauer IE, Scaini G, Wu MJ, Kazimi IF, Valvassori SS, et al. Accelerated epigenetic aging and mitochondrial DNA copy number in bipolar disorder. Transl Psychiatry. 2017;7.
58. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic Aging in Major Depressive Disorder. Am J Psychiatry. 2018:appi.ajp.2018.1.
59. Whalley HC, Gibson J, Marioni R, Walker RM, Clarke T-K, Howard DM, et al. Accelerated epigenetic ageing in depression. 2017. 2017.
60. McKinney BC, Lin H, Ding Y, Lewis DA, Sweet RA. DNA methylation age is not accelerated in brain or blood of subjects with schizophrenia. Schizophr Res. 2017. 2017. https://doi.org/10.1016/j.schres.2017.09.025.
61. Christiansen L, Lenart A, Tan Q, Vaupel JW, Aviv A, Mcgue M, et al. DNA methylation age is associated with mortality in a longitudinal Danish twin study. Aging Cell. 2016;15:149–154.
62. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16:25.
63. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
64. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol Cell. 2013;49:359–367.
65. Vetter VM, Antje M, Karbasiyan M, Steinhagen-Thiessen E, Hopfenmüller W, Demuth I. Epigenetic clock and relative telomere length represent largely different aspects of aging in the Berlin Aging Study II (BASE-II). J Gerontol A Biol Sci Med Sci. 2018. August 18, 2018. https://doi.org/10.1093/gerona/gly184.
66. Chen BH, Marioni RE, Colicino E, Peters MJ, Ward CK, Tsai PC, et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging . 2016;8:1844–1859.
67. Peters MJ, Joehanes R, Pilling LC, Schurmann C, Conneely KN, Powell J, et al. The transcriptional landscape of age in human peripheral blood. Nat Commun. 2015;6:8570.
68. Huan T, Chen G, Liu C, Bhattacharya A, Rong J, Chen BH, et al. Age-associated micro RNA expression in human peripheral blood is associated with all-cause mortality and age-related traits. Aging Cell. 2018;17:e12687.
69. Menni C, Kiddle SJ, Mangino M, Viñuela A, Psatha M, Steves C, et al. Circulating Proteomic Signatures of Chronological Age. J Gerontol A Biol Sci Med Sci. 2015;70:809–816.
70. Tanaka T, Biancotto A, Moaddel R, Moore AZ, Gonzalez-Freire M, Aon MA, et al. Plasma proteomic signature of age in healthy humans. Aging Cell. 2018:e12799.
71. Hertel J, Friedrich N, Wittfeld K, Pietzner M, Budde K, Van der Auwera S, et al. Measuring Biological Age via Metabonomics: The Metabolic Age Score. J Proteome Res. 2016;15:400–410.
72. Chaleckis R, Murakami I, Takada J, Kondoh H, Yanagida M. Individual variability in human blood metabolites identifies age-related differences. Proc Natl Acad Sci U S A. 2016;113:4252–4259.
73. Boeck C, Pfister S, Bürkle A, Vanhooren V, Libert C, Salinas-Manrique J, et al. Alterations of the serum N-glycan profile in female patients with Major Depressive Disorder. J Affect Disord. 2018;234:139–147.
74. Park DI, Štambuk J, Razdorov G, Pučić-Baković M, Martins-de-Souza D, Lauc G, et al. Blood plasma/IgG N-glycome biosignatures associated with major depressive disorder symptom severity and the antidepressant response. Sci Rep. 2018;8:179.

75. Moreno-Villanueva M, Morath J, Vanhooren V, Elbert T, Kolassa S, Libert C, et al. N-glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder. Transl Psychiatry. 2013;3:e320.
76. Krištić J, Vučković F, Menni C, Klarić L, Keser T, Beceheli I, et al. Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci. 2014;69:779–789.
77. Peng W, Zhao J, Dong X, Banazadeh A, Huang Y, Hussien A, et al. Clinical application of quantitative glycomics. Expert Rev Proteomics. 2018;15:1007–1031.
78. Yu X, Wang Y, Kristic J, Dong J, Chu X, Ge S, et al. Profiling IgG N-glycans as potential biomarker of chronological and biological ages: A community-based study in a Han Chinese population. Medicine . 2016;95:e4112.
79. Vanhooren V, Laroy W, Libert C, Chen C. N-glycan profiling in the study of human aging. Biogerontology. 2008;9:351–356.
80. Lindqvist D, Fernström J, Grudet C, Ljunggren L, Träskman-Bendz L, Ohlsson L, et al. Increased plasma levels of circulating cell-free mitochondrial DNA in suicide attempters: associations with HPA-axis hyperactivity. Transl Psychiatry. 2016;6:e971.
81. Lindqvist D, Wolkowitz OM, Picard M, Ohlsson L, Bersani FS, Fernström J, et al. Circulating cell-free mitochondrial DNA, but not leukocyte mitochondrial DNA copy number, is elevated in major depressive disorder. Neuropsychopharmacology. 2018;43:1557–1564.
82. Hummel EM, Hessas E, Müller S, Beiter T, Fisch M, Eibl A, et al. Cell-free DNA release under psychosocial and physical stress conditions. Transl Psychiatry. 2018;8:236.
83. Pinti M, Cevenini E, Nasi M, De Biasi S, Salvioli S, Monti D, et al. Circulating mitochondrial DNA increases with age and is a familiar trait: Implications for “inflamm-aging.” Eur J Immunol. 2014;44:1552–1562.
84. Mengel-From J, Thinggaard M, Dalgård C, Kyvik KO, Christensen K, Christiansen L. Mitochondrial DNA copy number in peripheral blood cells declines with age and is associated with general health among elderly. Hum Genet. 2014;133:1149–1159.
85. Verhoeven JE, Révész D, Picard M, Epel EE, Wolkowitz OM, Matthews KA, et al. Depression, telomeres and mitochondrial DNA: Between- and within-person associations from a 10-year longitudinal study. Mol Psychiatry. 2018;23:850–857.
86. Zhang R, Wang Y, Ye K, Picard M, Gu Z. Independent impacts of aging on mitochondrial DNA quantity and quality in humans. BMC Genomics. 2017;18:890.
87. Horvath S, Mah V, Lu AT, Woo JS, Choi O-W, Jasinska AJ, et al. The cerebellum ages slowly according to the epigenetic clock. Aging . 2015;7:294–306.
88. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, et al. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Genet. 2006;38:515–517.
89. Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nat Genet. 1992;2:324–329.
90. Picard M, Hirano M. Disentangling (Epi) Genetic and Environmental Contributions to the Mitochondrial 3243A> G Mutation Phenotype: Phenotypic Destiny in Mitochondrial Disease? JAMA Neurol. 2016. 2016.
91. Vincent AE, Rosa HS, Pabis K, Lawless C, Chen C, Grünewald A, et al. Subcellular origin of mitochondrial DNA deletions in human skeletal muscle. Ann Neurol. 2018. July 16, 2018. https://doi.org/10.1002/ana.25288.

92. Mamdani F, Rollins B, Morgan L, Myers RM, Barchas JD, Schatzberg a. F, et al. Variable telomere length across post-mortem human brain regions and specific reduction in the hippocampus of major depressive disorder. Transl Psychiatry. 2015;5:e636.
93. Yeung SSY, Reijnierse EM, Trappenburg MC, Hogrel J-Y, McPhee JS, Piasecki M, et al. Handgrip Strength Cannot Be Assumed a Proxy for Overall Muscle Strength. J Am Med Dir Assoc. 2018;19:703–709.
94. Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlén S-E, Greco D, et al. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. PLoS One. 2012;7:e41361.
95. Boeck C, Krause S, Karabatsiakis A, Schury K, Gündel H, Waller C, et al. History of child maltreatment and telomere length in immune cell subsets: Associations with stress- and attachment-related hormones. Dev Psychopathol. 2018;30:539–551.
96. Lin J, Epel E, Cheon J, Kroenke C, Sinclair E, Bigos M, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B cells: insights for epidemiology of telomere maintenance. J Immunol Methods. 2010;352:71–80.
97. Chacko BK, Kramer PA, Ravi S, Johnson MS, Hardy RW, Ballinger SW, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. Lab Invest. 2013;93:690–700.
98. Rantanen T, Harris T, Leveille SG, Visser M, Foley D, Masaki K, et al. Muscle strength and body mass index as long-term predictors of mortality in initially healthy men. J Gerontol A Biol Sci Med Sci. 2000;55:M168-73.
99. Chung S, Wang X, Lui YW. Influence of T1-Weighted Signal Intensity on FSL Voxel-Based Morphometry and FreeSurfer Cortical Thickness. AJNR Am J Neuroradiol. 2017;38:726–728.
100. Clarkson MJ, Cardoso MJ, Ridgway GR, Modat M, Leung KK, Rohrer JD, et al. A comparison of voxel and surface based cortical thickness estimation methods. Neuroimage. 2011;57:856–865.
101. Tardif CL, Gauthier CJ, Steele CJ, Bazin P-L, Schäfer A, Schaefer A, et al. Advanced MRI techniques to improve our understanding of experience-induced neuroplasticity. Neuroimage. 2016;131:55–72.
102. Geerligs L, Renken RJ, Saliasi E, Maurits NM, Lorist MM. A Brain-Wide Study of Age-Related Changes in Functional Connectivity. Cereb Cortex. 2015;25:1987–1999.
103. Garrett DD, Kovacevic N, McIntosh AR, Grady CL. Blood oxygen level-dependent signal variability is more than just noise. J Neurosci. 2010;30:4914–4921.
104. Griffanti L, Douaud G, Bijsterbosch J, Evangelisti S, Alfaro-Almagro F, Glasser MF, et al. Hand classification of fMRI ICA noise components. Neuroimage. 2017;154:188–205.
105. Luders E, Cherbuin N, Gaser C. Estimating brain age using high-resolution pattern recognition: Younger brains in long-term meditation practitioners. Neuroimage. 2016;134:508–513.
106. Cole JH, Franke K. Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. Trends Neurosci. 2017;40:681–690.
107. Gaser C, Franke K, Klöppel S, Koutsouleris N, Sauer H. BrainAGE in Mild Cognitive Impaired Patients: Predicting the Conversion to Alzheimer's Disease. PLoS One. 2013;8.
108. Aycheh HM, Seong J-K, Shin J-H, Na DL, Kang B, Seo SW, et al. Biological Brain Age Prediction Using Cortical Thickness Data: A Large Scale Cohort Study. Front Aging Neurosci. 2018;10:1385.

109. Liem F. Predicting brain-age from multimodal imaging data captures cognitive impairment. 2016. 2016.
110. Cole JH, Poudel RPK, Tsagkrasoulis D, Caan MWA, Steves C, Spector TD, et al. Predicting brain age with deep learning from raw imaging data results in a reliable and heritable biomarker. 2016. 2016.
111. Ball G, Adamson C, Beare R, Seal ML. Modelling neuroanatomical variation during childhood and adolescence with neighbourhood-preserving embedding. Sci Rep. 2017;7:17796.
112. Gutierrez Becker B, Klein T, Wachinger C. Gaussian process uncertainty in age estimation as a measure of brain abnormality. Neuroimage. 2018;175:246–258.
113. Franke K, Gaser C. Longitudinal Changes in Individual *BrainAGE* in Healthy Aging, Mild Cognitive Impairment, and Alzheimer's Disease 1Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni. GeroPsych . 2012;25:235–245.
114. Lawless C, Wang C, Jurk D, Merz A, Zglinicki T von, Passos JF. Quantitative assessment of markers for cell senescence. Exp Gerontol. 2010;45:772–778.
115. Picard M, McEwen BS. Psychological Stress and Mitochondria: A Systematic Review. Psychosom Med. 2018;80:141–153.
116. Ehinger JK, Morota S, Hansson MJ, Paul G, Elmér E. Mitochondrial dysfunction in blood cells from amyotrophic lateral sclerosis patients. J Neurol. 2015;262:1493–1503.
117. Chan AY, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem. 1989;35:315–317.
118. Kågedal B, Lindqvist M, Farnebäck M, Lenner L, Peterson C. Failure of the PAXgene Blood RNA System to maintain mRNA stability in whole blood. Clin Chem Lab Med. 2005;43:1190–1192.
119. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging . 2018;10:573–591.
120. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 2014;15:R24.
121. Knight AK, Conneely KN, Smith AK. Gestational age predicted by DNA methylation: potential clinical and research utility. Epigenomics. 2017. January 23, 2017. https://doi.org/10.2217/epi-2016-0157.
122. Horvath S, Oshima J, Martin GM, Lu AT, Quach A, Cohen H, et al. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. Aging . 2018;10:1758–1775.
123. Susser E, Verhulst S, Kark JD, Factor-Litvak PR, Keyes K, Magnus P, et al. Non-Dynamic Association of Depressive and Anxiety Disorders With Leukocyte Telomere Length? Am J Psychiatry. 2016;173:1147.
124. Heijmans BT, Mill J. Commentary: The seven plagues of epigenetic epidemiology. Int J Epidemiol. 2012;41:74–78.
125. Ratanatharathorn A, Boks MP, Maihofer AX, Aiello AE, Amstadter AB, Ashley-Koch AE, et al. Epigenome-wide association of PTSD from heterogeneous cohorts with a common multi-site analysis pipeline. Am J Med Genet B Neuropsychiatr Genet. 2017;174:619–630.
126. Lu AT, Xue L, Salfati EL, Chen BH, Ferrucci L, Levy D, et al. GWAS of epigenetic aging rates in blood reveals a critical role for TERT. Nat Commun. 2018;9:387.
127. Elliott HR, Tillin T, McArdle WL, Ho K, Duggirala A, Frayling TM, et al. Differences in smoking associated DNA methylation patterns in South Asians and Europeans. Clin Epigenetics. 2014;6:4.

128. Simpkin AJ, Hemani G, Suderman M, Gaunt TR, Lyttleton O, Mcardle WL, et al. Prenatal and early life influences on epigenetic age in children: a study of mother–offspring pairs from two cohort studies. Hum Mol Genet. 2016;25:191–201.
129. Price EM, Robinson WP. Adjusting for Batch Effects in DNA Methylation Microarray Data, a Lesson Learned. Front Genet. 2018;9:83.
130. Akulenko R, Merl M, Helms V. BEclear: Batch Effect Detection and Adjustment in DNA Methylation Data. PLoS One. 2016;11:e0159921.
131. Spiers H, Hannon E, Schalkwyk LC, Smith R, Wong CCY, O'Donovan MC, et al. Methylomic trajectories across human fetal brain development. Genome Res. 2015;25:338–352.
132. Li X, Li W, Xu Y. Human Age Prediction Based on DNA Methylation Using a Gradient Boosting Regressor. Genes . 2018;9.
133. Gao X, Zhang Y, Mons U, Brenner H. Leukocyte telomere length and epigenetic-based mortality risk score: associations with all-cause mortality among older adults. Epigenetics. 2018. August 28, 2018. https://doi.org/10.1080/15592294.2018.1514853.
134. Chen BH, Carty CL, Kimura M, Kark JD, Chen W, Li S, et al. Leukocyte telomere length, T cell composition and DNA methylation age. Aging . 2017;9:1983–1995.
135. Tyrka AR, Parade SH, Price LH, Kao H-T, Porton B, Philip NS, et al. Alterations of Mitochondrial DNA Copy Number and Telomere Length With Early Adversity and Psychopathology. Biol Psychiatry. 2016;79:78–86.
136. Cai N, Chang S, Li Y, Li Q, Hu J, Liang J, et al. Molecular signatures of major depression. Curr Biol. 2015;25:1146–1156.
137. Malik AN, Shahni R, Rodriguez-de-Ledesma A, Laftah A, Cunningham P. Mitochondrial DNA as a non-invasive biomarker: accurate quantification using real time quantitative PCR without co-amplification of pseudogenes and dilution bias. Biochem Biophys Res Commun. 2011;412:1–7.
138. Urata M, Koga-Wada Y, Kayamori Y, Kang D. Platelet contamination causes large variation as well as overestimation of mitochondrial DNA content of peripheral blood mononuclear cells. Ann Clin Biochem. 2008;45:513–514.
139. Hurtado-Roca Y, Ledesma M, Gonzalez-Lazaro M, Moreno-Loshuertos R, Fernandez-Silva P, Enriquez JA, et al. Adjusting MtDNA Quantification in Whole Blood for Peripheral Blood Platelet and Leukocyte Counts. PLoS One. 2016;11:e0163770.
140. Miller FJ, Rosenfeldt FL, Zhang C, Linnane AW, Nagley P. Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: lack of change of copy number with age. Nucleic Acids Res. 2003;31:e61–e61.
141. Xia P, Radpour R, Zachariah R, Fan AXC, Kohler C, Hahn S, et al. Simultaneous quantitative assessment of circulating cell-free mitochondrial and nuclear DNA by multiplex real-time PCR. Genet Mol Biol. 2009;32:20–24.
142. Nakahira K, Kyung S-Y, Rogers AJ, Gazourian L, Youn S, Massaro AF, et al. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. PLoS Med. 2013;10:e1001577; discussion e1001577.
143. Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, et al. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? Am J Epidemiol. 2018;187:1220–1230.
144. Tyrka AR, Carpenter LL, Kao H-T, Porton B, Philip NS, Ridout SJ, et al. Association of telomere length and mitochondrial DNA copy number in a community sample of healthy adults. Exp Gerontol. 2015;66:17–20.
145. Marioni RE, Harris SE, Shah S, Mcrae AF, Zglinicki TV, Martin-ruiz C, et al. The epigenetic clock and telomere length are independently associated with chronological age and mortality. Int J Epidemiol. 2016:1–9.
146. Breitling LP, Saum K-U, Perna L, Schöttker B, Holleczek B, Brenner H. Frailty is associated with the epigenetic clock but not with telomere length in a German cohort. Clin Epigenetics. 2016;8:21.
147. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, et al. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci U S A. 2004;101:17312–17315.
148. Shields GS, Slavich GM. Lifetime Stress Exposure and Health: A Review of Contemporary Assessment Methods and Biological Mechanisms. Soc Personal Psychol Compass. 2017;11.
149. Shalev I, Entringer S, Wadhwa PD, Wolkowitz OM, Puterman E, Lin J, et al. Stress and telomere biology: a lifespan perspective. Psychoneuroendocrinology. 2013;38:1835–1842.
150. Price LH, Kao H-T, Burgers DE, Carpenter LL, Tyrka AR. Telomeres and early-life stress: an overview. Biol Psychiatry. 2013;73:15–23.
151. Entringer S, de Punder K, Buss C, Wadhwa PD. The fetal programming of telomere biology hypothesis: an update. Philos Trans R Soc Lond B Biol Sci. 2018;373.
152. Hanssen LM, Schutte NS, Malouff JM, Epel ES. The Relationship Between Childhood Psychosocial Stressor Level and Telomere Length: A Meta-Analysis. Health Psychol Res. 2017;5:6378.
153. Ridout KK, Levandowski M, Ridout SJ, Gantz L, Goonan K, Palermo D, et al. Early life adversity and telomere length: a meta-analysis. Mol Psychiatry. 2018;23:858–871.
154. Schutte NS, Malouff JM. A meta-analytic review of the effects of mindfulness meditation on telomerase activity. Psychoneuroendocrinology. 2014;42:45–48.
155. Ridout KK, Ridout SJ, Price LH, Sen S, Tyrka AR. Depression and telomere length: A meta-analysis. J Affect Disord. 2016;191:237–247.
156. Mathur MB, Epel E, Kind S, Desai M, Parks CG, Sandler DP, et al. Perceived stress and telomere length: A systematic review, meta-analysis, and methodologic considerations for advancing the field. Brain Behav Immun. 2016;54:158–169.
157. Picard M, McEwen BS. Psychological Stress and Mitochondria: A Conceptual Framework. Psychosom Med. 2018;80:126–140.
158. Picard M, Juster R-P, McEwen BS. Mitochondrial allostatic load puts the "gluc" back in glucocorticoids. Nat Rev Endocrinol. 2014;10:303–310.
159. Boeck C, Koenig AM, Schury K, Geiger ML, Karabatsiakis A, Wilker S, et al. Inflammation in adult women with a history of child maltreatment: The involvement of mitochondrial alterations and oxidative stress. Mitochondrion. 2016;30:197–207.
160. Monroe SM. Modern approaches to conceptualizing and measuring human life stress. Annu Rev Clin Psychol. 2008;4:33–52.
161. Epel ES, Crosswell AD, Mayer SE, Prather AA, Slavich GM, Puterman E, et al. More than a feeling: A unified view of stress measurement for population science. Front Neuroendocrinol. 2018;49:146–169.
162. Li Z, He Y, Wang D, Tang J, Chen X. Association between childhood trauma and accelerated telomere erosion in adulthood: A meta-analytic study. J Psychiatr Res. 2017;93:64–71.

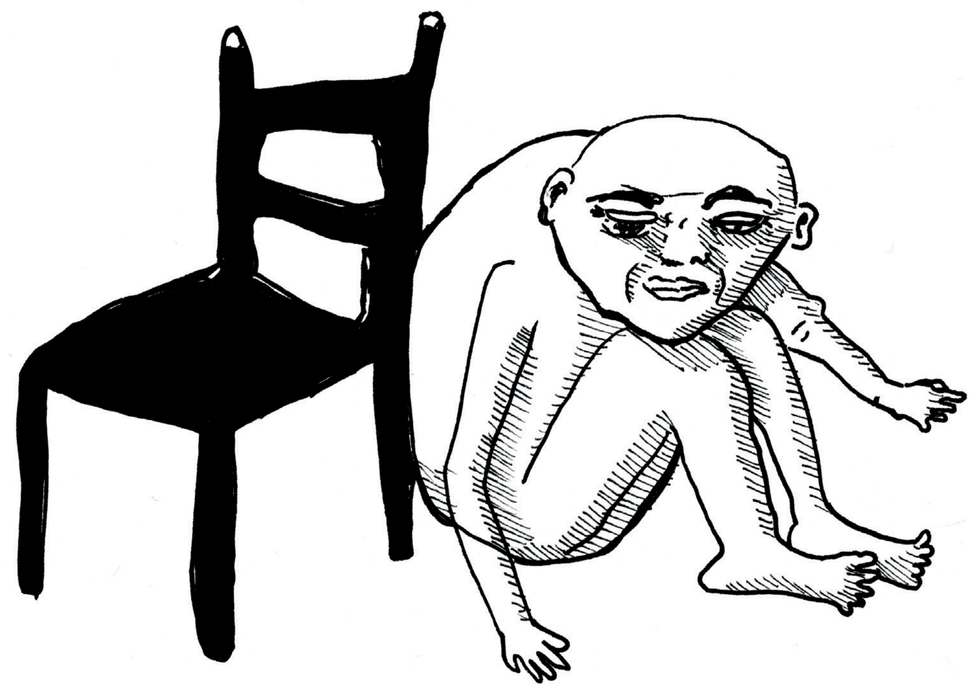
163. Shalev I, Moffitt TE, Sugden K, Williams B, Houts RM, Danese A, et al. Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age : a longitudinal study. Mol Psychiatry. 2012;18:576–581.
164. Schutte NS, Malouff JM. The relationship between perceived stress and telomere length: A meta-analysis. Stress Health. 2016;32:313–319.
165. Oliveira BS, Zunzunegui MV, Quinlan J, Fahmi H, Tu MT, Guerra RO. Systematic review of the association between chronic social stress and telomere length: A life course perspective. Ageing Res Rev. 2016;26:37–52.
166. Puterman E, Gemmill A, Karasek D, Weir D, Adler NE, Prather AA, et al. Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study. Proc Natl Acad Sci U S A. 2016;113:E6335–E6342.
167. Révész D, Milaneschi Y, Terpstra EM, Penninx BWJH. Baseline biopsychosocial determinants of telomere length and 6-year attrition rate. Psychoneuroendocrinology. 2016;67:153–162.
168. Puterman E, Lin J, Krauss J, Blackburn EH, Epel ES. Determinants of telomere attrition over 1 year in healthy older women: stress and health behaviors matter. Mol Psychiatry. 2015;20:529–535.
169. van Ockenburg SL, Bos EH, de Jonge P, van der Harst P, Gans ROB, Rosmalen JGM. Stressful life events and leukocyte telomere attrition in adulthood: a prospective population-based cohort study. Psychol Med. 2015;45:2975–2984.
170. Viron MJ, Stern TA. The impact of serious mental illness on health and healthcare. Psychosomatics. 2010;51:458–465.
171. van Gool CH, Kempen GIJM, Bosma H, van Boxtel MPJ, Jolles J, van Eijk JTM. Associations between lifestyle and depressed mood: longitudinal results from the Maastricht Aging Study. Am J Public Health. 2007;97:887–894.
172. Baylis D, Bartlett DB, Patel HP, Roberts HC. Understanding how we age: insights into inflammaging. Longev Healthspan. 2013;2:8.
173. Franceschi C, Zaikin A, Gordleeva S, Ivanchenko M, Bonifazi F, Storci G, et al. Inflammaging 2018: An update and a model. Semin Immunol. 2018. November 2, 2018. https://doi.org/10.1016/j.smim.2018.10.008.
174. Diniz BS, Vieira EM. Stress, Inflammation, and Aging: An Association Beyond Chance. Am J Geriatr Psychiatry. 2018;26:964–965.
175. Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, et al. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol Psychiatry. 2006;60:432–435.
176. Darrow SM, Verhoeven JE, Révész D, Lindqvist D, Penninx BWJH, Delucchi KL, et al. The Association Between Psychiatric Disorders and Telomere Length. Psychosom Med. 2016;78:776–787.
177. Colpo GD, Leffa DD, Kohler CA, Kapczinski F, Quevedo J, Carvalho AF. Is bipolar disorder associated with accelerating aging? A meta-analysis of telomere length studies. J Affect Disord. 2015;186:241–248.
178. Rao S, Kota LN, Li Z, Yao Y, Tang J, Mao C, et al. Accelerated leukocyte telomere erosion in schizophrenia: Evidence from the present study and a meta-analysis. J Psychiatr Res. 2016;79:50–56.
179. Boks MP, Mierlo HC van, Rutten BPF, Radstake TRDJ, De Witte L, Geuze E, et al. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. Psychoneuroendocrinology. 2015;51:506–512.
180. Mehta D, Bruenig D, Lawford B, Harvey W, Carrillo-Roa T, Morris CP, et al. Accelerated DNA methylation aging and increased resilience in veterans: The biological cost for soldiering on. Neurobiol Stress. 2018;8:112–119.

181. Voisey J, Lawford BR, Morris CP, Wockner LF, Noble EP, Young RM, et al. Epigenetic analysis confirms no accelerated brain aging in schizophrenia. Npj Schizophrenia. 2017;3:26.
182. McKinney BC, Lin H, Ding Y, Lewis DA, Sweet RA. DNA methylation evidence against the accelerated aging hypothesis of schizophrenia. Npj Schizophrenia. 2017;3:13.
183. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol. 2018. July 25, 2018. https://doi.org/10.1038/s41574-018-0059-4.
184. Martínez-Cengotitabengoa M, Carrascón L, O'Brien JT, Díaz-Gutiérrez M-J, Bermúdez-Ampudia C, Sanada K, et al. Peripheral Inflammatory Parameters in Late-Life Depression: A Systematic Review. Int J Mol Sci. 2016;17.
185. Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. Lancet Psychiatry. 2015;2:258–270.
186. Réus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, et al. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience. 2015;300:141–154.
187. Kageyama Y, Kasahara T, Kato M, Sakai S, Deguchi Y, Tani M, et al. The relationship between circulating mitochondrial DNA and inflammatory cytokines in patients with major depression. J Affect Disord. 2018;233:15–20.
188. West AP, Shadel GS. Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat Rev Immunol. 2017;17:363–375.
189. Trumpff C, Marsland L, Basualto-Alarcon C, Martin JL, Carroll JE, Sturm G, Vincent AE, Mosharov EV, Gu Z, Kaufman BA, Picard M. Acute Psychological Stress Triggers Circulating Cell-Free Mitochondrial DNA.
190. Kaufmann T, Meer DVD, Doan NT, Schwarz E, Martina J, Alnæs D, et al. Genetics of brain age suggest an overlap with common brain disorders. 2018. 2018.
191. Shalev I, Moffitt TE, Braithwaite AW, Danese A, Fleming NI, Goldman-Mellor S, et al. Internalizing disorders and leukocyte telomere erosion: a prospective study of depression, generalized anxiety disorder and post-traumatic stress disorder. Mol Psychiatry. 2014;19:1163–1170.
192. Verhoeven JE, van Oppen P, Révész D, Wolkowitz OM, Penninx BWJH. Depressive and Anxiety Disorders Showing Robust, but Non-Dynamic, 6-Year Longitudinal Association With Short Leukocyte Telomere Length. Am J Psychiatry. 2016;173:617–624.
193. Vance MC, Bui E, Hoeppner SS, Kovachy B, Prescott J, Mischoulon D, et al. Prospective association between major depressive disorder and leukocyte telomere length over two years. Psychoneuroendocrinology. 2018;90:157–164.
194. Maniates H, Stoop TB, Miller MW, Halberstadt L, Wolf EJ. Stress-Generative Effects of Posttraumatic Stress Disorder: Transactional Associations Between Posttraumatic Stress Disorder and Stressful Life Events in a Longitudinal Sample. J Trauma Stress. 2018;31:191–201.
195. Wium-Andersen MK, Ørsted DD, Rode L, Bojesen SE, Nordestgaard BG. Telomere length and depression: prospective cohort study and Mendelian randomisation study in 67 306 individuals. Br J Psychiatry. 2017;210:31–38.
196. Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, Samy RP, et al. Ageing and the telomere connection: An intimate relationship with inflammation. Ageing Res Rev. 2016;25:55–69.

197. Wikgren M, Maripuu M, Karlsson T, Nordfjll K, Bergdahl J, Hultdin J, et al. Short telomeres in depression and the general population are associated with a hypocortisolemic state. BiolPsychiatry. 2012;71:294–300.
198. Lee M, Martin H, Firpo MA, Demerath EW. Inverse association between adiposity and telomere length: The Fels Longitudinal Study. Am J Hum Biol. 2011;23:100–106.
199. Monickaraj F, Gokulakrishnan K, Prabu P, Sathishkumar C, Anjana RM, Rajkumar JS, et al. Convergence of adipocyte hypertrophy, telomere shortening and hypoadiponectinemia in obese subjects and in patients with type 2 diabetes. Clin Biochem. 2012;45:1432–1438.
200. Wolkowitz OM, Mellon SH, Epel ES, Lin J, Reus VI, Rosser R, et al. Resting leukocyte telomerase activity is elevated in major depression and predicts treatment response. Mol Psychiatry. 2012;17:164–172.
201. Bersani FS, Lindqvist D, Mellon SH, Penninx BWJHBWJH, Verhoeven JE, R??v??sz D, et al. Telomerase activation as a possible mechanism of action of psychopharmacological interventions. Drug Discov Today. 2015;(accepted):1305–1309.
202. Hough CM, Bersani FS, Mellon SH, Epel ES, Reus VI, Lindqvist D, et al. Leukocyte telomere length predicts SSRI response in major depressive disorder: A preliminary report. Mol Neuropsychiatry. 2016;2:88–96.
203. Rasgon N, Lin KW, Lin J, Epel E, Blackburn E. Telomere length as a predictor of response to Pioglitazone in patients with unremitted depression: a preliminary study. Transl Psychiatry. 2016;6:e709.
204. Martinsson L, Wei Y, Xu D, Melas PA, Mathé AA, Schalling M, et al. Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres. Transl Psychiatry. 2013;3:e261.
205. Yu W-Y, Chang H-W, Lin C-H, Cho C-L. Short telomeres in patients with chronic schizophrenia who show a poor response to treatment. J Psychiatry Neurosci. 2008;33:244–247.
206. Li Z, Hu M, Zong X, He Y, Wang D, Dai L, et al. Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep. 2015;5:18553.
207. Conklin QA, Crosswell AD, Saron CD, Epel ES. Meditation, Stress Processes, and Telomere Biology. Current Opinion in Psychology. 2018. November 19, 2018. https://doi.org/10.1016/j.copsyc.2018.11.009.
208. Strawbridge R, Arnone D, Danese A, Papadopoulos A, Herane Vives A, Cleare AJ. Inflammation and clinical response to treatment in depression: A meta-analysis. Eur Neuropsychopharmacol. 2015;25:1532–1543.
209. Zwicker A, Fabbri C, Rietschel M, Hauser J, Mors O, Maier W, et al. Genetic disposition to inflammation and response to antidepressants in major depressive disorder. J Psychiatr Res. 2018;105:17–22.
210. Jha MK, Trivedi MH. Personalized Antidepressant Selection and Pathway to Novel Treatments: Clinical Utility of Targeting Inflammation. Int J Mol Sci. 2018;19.
211. Jaskelioff M, Muller FL, Paik J-HH, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011;469:102–106.
212. Batista LFZ, Pech MF, Zhong FL, Nguyen HN, Xie KT, Zaug AJ, et al. Telomere shortening and loss of self-renewal in dyskeratosis congenita induced pluripotent stem cells. Nature. 2011;474:399–402.

213. Puterman E, Weiss J, Lin J, Schilf S, Slusher AL, Johansen KL, et al. Aerobic exercise lengthens telomeres and reduces stress in family caregivers: A randomized controlled trial - Curt Richter Award Paper 2018. Psychoneuroendocrinology. 2018;98:245–252.
214. Mason AE, Hecht FM, Daubenmier JJ, Sbarra DA, Lin J, Moran PJ, et al. Weight Loss Maintenance and Cellular Aging in the Supporting Health Through Nutrition and Exercise Study. Psychosom Med. 2018;80:609–619.
215. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science. 2015;350:1193–1198.
216. Steffener J, Habeck C, O'Shea D, Razlighi Q, Bherer L, Stern Y. Differences between chronological and brain age are related to education and self-reported physical activity. Neurobiol Aging. 2016;40:138–144.
217. Rogenmoser L, Kernbach J, Schlaug G, Gaser C. Keeping brains young with making music. Brain Struct Funct. 2018;223:297–305.
218. Le TT, Kuplicki R, Yeh HW, Aupperle RL, Khalsa SS, Simmons WK, et al. Effect of Ibuprofen on BrainAGE: A Randomized, Placebo-Controlled, Dose-Response Exploratory Study. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2018:1–8.
219. Voisin S, Eynon N, Yan X, Bishop DJ. Exercise training and DNA methylation in humans. Acta Physiol . 2015;213:39–59.
220. Ren H, Collins V, Clarke SJ, Han J-S, Lam P, Clay F, et al. Epigenetic changes in response to tai chi practice: a pilot investigation of DNA methylation marks. Evid Based Complement Alternat Med. 2012;2012:841810.
221. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging . 2017;9:419–446.
222. Horvath S, Erhart W, Brosch M, Ammerpohl O, Schönfels W von, Ahrens M, et al. Obesity accelerates epigenetic aging of human liver. Proceedings of the National Academy of Sciences. 2014:201412759.
223. Chen L, Dong Y, Bhagatwala J, Raed A, Huang Y, Zhu H. Effects of Vitamin D3 supplementation on epigenetic aging in overweight and obese African Americans with suboptimal vitamin D status: a randomized clinical trial. J Gerontol A Biol Sci Med Sci. 2018. September 25, 2018. https://doi.org/10.1093/gerona/gly223.
224. Simons MJP. Questioning causal involvement of telomeres in aging. Ageing Res Rev. 2015;24:191–196.
225. Gotlib IH, LeMoult J, Colich NL, Foland-Ross LC, Hallmayer J, Joormann J, et al. Telomere length and cortisol reactivity in children of depressed mothers. Mol Psychiatry. 2014:1–6.
226. Effros RB. Kleemeier Award Lecture 2008—The Canary in the Coal Mine: Telomeres and Human Healthspan. J Gerontol A Biol Sci Med Sci. 2009;64A:511–515.
227. Moffitt TE, Belsky DW, Danese A, Poulton R, Caspi A. The Longitudinal Study of Aging in Human Young Adults: Knowledge Gaps and Research Agenda. J Gerontol A Biol Sci Med Sci. 2017;72:210–215.
228. Sanders JL, Boudreau RM, Newman AB, Newman AB, Newman AB. Understanding the Aging Process Using Epidemiologic Approaches. In: Newman AB, Cauley JA, editors. The Epidemiology of Aging, Dordrecht: Springer Netherlands; 2012. p. 187–214.
229. Bind M-A, VanderWeele TJ, Schwartz JD, Coull BA. Quantile causal mediation analysis allowing longitudinal data. Stat Med. 2017;36:4182–4195.
230. Burgess S, Butterworth A, Malarstig A, Thompson SG. Use of Mendelian randomisation to assess potential benefit of clinical intervention. BMJ. 2012;345:e7325.

231. Bzdok D, Altman N, Krzywinski M. Points of significance: statistics versus machine learning. Nat Methods. 2018:1–7.
232. Yarkoni T, Westfall J. Choosing Prediction Over Explanation in Psychology: Lessons From Machine Learning. Perspect Psychol Sci. 2017;12:1100–1122.
233. Munafò MR, Davey Smith G. Robust research needs many lines of evidence. Nature. 2018;553:399–401.
234. Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, et al. Quantification of biological aging in young adults. Proc Natl Acad Sci U S A. 2015;112:E4104-10.
235. Chou JP, Effros RB. T cell replicative senescence in human aging. Curr Pharm Des. 2013;19:1680–1698.
236. Lin J, Cheon J, Brown R, Coccia M, Puterman E, Aschbacher K, et al. Systematic and Cell Type-Specific Telomere Length Changes in Subsets of Lymphocytes. J Immunol Res. 2016;2016:5371050.
237. Hattab MW, Shabalin AA, Clark SL, Zhao M, Kumar G, Chan RF, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. Genome Biol. 2017;18.
238. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. Bioinformatics. 2014;30:1431–1439.
239. Titus AJ, Gallimore RM, Salas LA, Christensen BC. Cell-type deconvolution from DNA methylation: a review of recent applications. Hum Mol Genet. 2017;26:R216–R224.
240. Chan RF, Turecki G, Shabalin AA, Guintivano J, Zhao M, Xie LY, et al. Cell-type-specific methylome-wide association studies implicate neurodegenerative processes and neuroimmune communication in major depressive disorder. BioRxiv. 2018. 2018.
241. Cuthbert BN, Insel TR. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. BMC Med. 2013;11:126.
242. Wolfers T, Doan NT, Kaufmann T, Alnæs D, Moberget T, Agartz I, et al. Mapping the Heterogeneous Phenotype of Schizophrenia and Bipolar Disorder Using Normative Models. JAMA Psychiatry. 2018. October 10, 2018. https://doi.org/10.1001/jamapsychiatry.2018.2467.
243. Alder JK, Hanumanthu VS, Strong MA, DeZern AE, Stanley SE, Takemoto CM, et al. Diagnostic utility of telomere length testing in a hospital-based setting. Proc Natl Acad Sci U S A. 2018;115:E2358–E2365.
244. Ram N, Grimm KJ. Growth Mixture Modeling: A Method for Identifying Differences in Longitudinal Change Among Unobserved Groups. Int J Behav Dev. 2009;33:565–576.
245. Hertel J, Frenzel S, König J, Wittfeld K, Fuellen G, Holtfreter B, et al. The informative error: A framework for the construction of individualized phenotypes. Stat Methods Med Res. 2018:962280218759138.
246. Maaten L van der, Hinton G. Visualizing Data using t-SNE. J Mach Learn Res. 2008;9:2579–2605.
247. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0—making metabolomics more meaningful. Nucleic Acids Res. 2015;43:W251–W257.
248. Diaz KM, Thanataveerat A, Parsons FE, Yoon S, Cheung YK, Alcántara C, et al. The Influence of Daily Stress on Sedentary Behavior: Group and Person (N of 1) Level Results of a 1-Year Observational Study. Psychosom Med. 2018;80:620–627.
249. Verhoeven JE, Révész D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH, et al. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. MolPsychiatry. 2014;19:895–901.
250. Schnack HG, Kahn RS. Detecting neuroimaging biomarkers for psychiatric disorders: Sample size matters. Front Psychiatry. 2016;7.
251. Mohammadi D. ENIGMA: crowdsourcing meets neuroscience. Lancet Neurol. 2015;14:462–463.
252. Thompson PM, Stein JL, Medland SE, Hibar DP, Vasquez AA, Renteria ME, et al. The ENIGMA Consortium: large-scale collaborative analyses of neuroimaging and genetic data. Brain Imaging Behav. 2014;8:153–182.
253. Dagnall CL, Hicks B, Teshome K, Hutchinson AA, Gadalla SM, Khincha PP, et al. Effect of pre-analytic variables on the reproducibility of qPCR relative telomere length measurement. PLoS One. 2017;12:e0184098.
254. Khincha PP, Dagnall CL, Hicks B, Jones K, Aviv A, Kimura M, et al. Correlation of Leukocyte Telomere Length Measurement Methods in Patients with Dyskeratosis Congenita and in Their Unaffected Relatives. Int J Mol Sci. 2017;18.
255. Shiels PG. Improving precision in investigating aging: why telomeres can cause problems. J Gerontol A Biol Sci Med Sci. 2010;65:789–791.
256. Teo YV, Capri M, Morsiani C, Pizza G, Faria AMC, Franceschi C, et al. Cell-free DNA as a biomarker of aging. Aging Cell. 2019;18:e12890.
257. Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. Brain Behav Immun. 2017;64:208–219.
258. Insel TR, Cuthbert BN. Brain disorders? Precisely. Science. 2015;348:499–500.
259. Picard M, Juster R-P, Sabiston CM. Is the whole greater than the sum of the parts? Self-rated health and transdisciplinarity. Health . 2013;5:24.

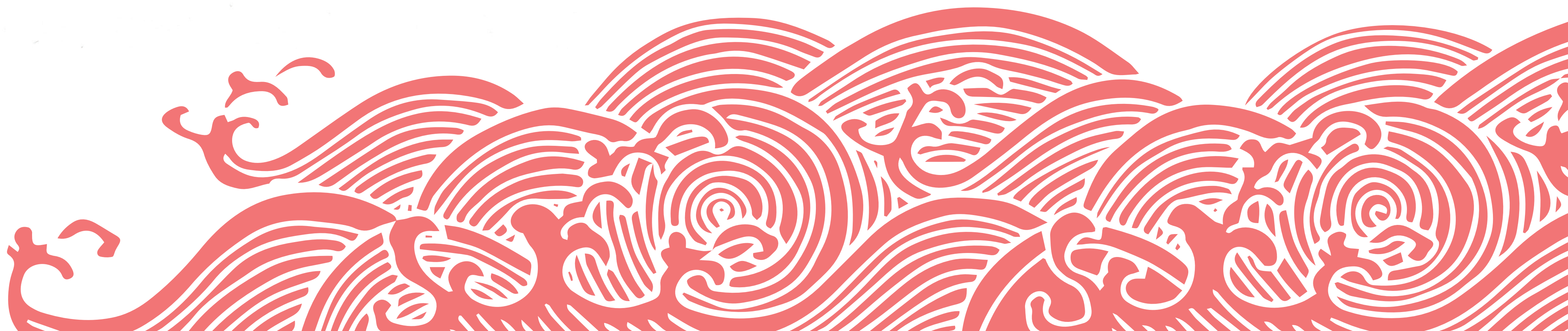


CHAPTER 3

Epigenetic Aging in Major Depressive Disorder

Published in *The American Journal of Psychiatry* 2018, 175(8), 774-782.

Laura K.M. Han
Moji Aghajani
Shaunna L. Clark
Robin F. Chan
Mohammad W. Hattab
Andrey A. Shabalin
Min Zhao
Gaurav Kumar
Lin Ying Xie
Rick Jansen
Yuri Milaneschi
Brian Dean
Karolina A. Aberg
Edwin J.C.G. van den Oord
Brenda W.J.H. Penninx



ABSTRACT

Major Depressive Disorder (MDD) is associated with increased risk of mortality and aging-related diseases. Here, it was examined whether MDD is associated with higher epigenetic aging (EA) in blood as measured by DNA methylation (DNAm) patterns, whether clinical characteristics of MDD further impact these patterns, and whether findings replicate in brain tissue. DNAmAge was estimated using all methylation sites in blood of 811 depressed patients and 319 controls from the Netherlands Study of Depression and Anxiety. The residuals of the DNAmAge estimates regressed on chronological age were calculated to indicate epigenetic aging (EA). MDD diagnosis and clinical characteristics were assessed with questionnaires and psychiatric interviews. Analyses were adjusted for socio demographics, lifestyle, and health status. Post-mortem brain samples of 74 depressed patients and 64 controls were used for replication. Pathway enrichment analysis was conducted using ConsensusPathDB to gain insight into the biological processes underlying EA in blood and brain. Higher EA was observed in MDD patients compared to controls (P=0.008; Cohen's d=0.18), with a dose-effect with increasing symptom severity in the overall sample (P=0.001). Within MDD patients, EA was positively associated with childhood trauma scores (P=0.02). The case-control difference was replicated in an independent dataset of post-mortem brain samples (P=0.03). Top significantly enriched Gene Ontology terms included neuronal processes. As compared to controls, MDD patients show higher epigenetic aging in blood and brain tissue, suggesting that they are biologically older than their corresponding chronological age. This effect was even more profound in the presence of childhood trauma.

INTRODUCTION

A growing body of literature suggests that Major Depressive Disorder (MDD) is associated with increased risk of mortality and aging-related phenotypes and diseases, including cardiovascular disease, diabetes, obesity [1], cancer [2], cognitive impairment [3], and frailty [4]. Given the associated negative impact on the patient's quality of life and health care costs [5], it is of interest to investigate if MDD patients are prone to accelerated aging.

Current literature provides evidence for advanced biological aging in MDD as indicated by shorter telomere length [6, 7] and advanced brain aging [8]. Recently, alternative markers of biological age derived from DNA methylation have been developed (also known as "epigenetic clocks"). Chronological age can be accurately predicted from methylation data and yields estimates of DNA methylation age (DNAmAge) [9, 10]. Further, DNAmAge can be studied as either "decelerated" or "accelerated" by regressing it on chronological age to get a measure of epigenetic aging (EA). Thus, this measure is a promising candidate for reliably investigating accelerated or premature aging in MDD.

Previous studies have shown EA in Down syndrome [11], HIV-positive patients [12], and obesity [13]. In addition, EA has been associated with poorer physical and cognitive fitness [14], increased smoking and alcohol use [15], cancer [16], Alzheimer disease [17], cardiovascular disease [16], and increased risk of mortality [18]. A few studies have investigated DNAmAge in relation to schizophrenia [19, 20], life stress [21], and post-traumatic stress disorder [22, 23], with mixed findings. However, studies examining epigenetic aging in relation to MDD are currently lacking.

In this study, we examined whether MDD is associated with higher EA in blood, using a large clinically well-phenotyped sample to further explore associations with clinical characteristics. Moreover, we aimed to replicate findings in post-mortem brain tissue. To examine these aims, we used a sequencing based approach [24, 25] that yields almost complete coverage of the CpG methylome which allowed us to obtain the most accurate DNAmAge estimates for our sample and to better explore the biological processes underlying epigenetic aging.

METHODS

The Netherlands Study of Depression and Anxiety

Participants were from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal, multi-center, cohort study designed to investigate the long-term course and consequences of depression and anxiety disorders [26]. Its 2981 participants (18-65 years) include patients with a current or lifetime diagnosis of depression and/or anxiety disorder and controls (without any lifetime depressive disorder and/or anxiety disorder). Participants were recruited from the general population, general practices, and mental health organizations in order to reflect various settings and the entire range of psychopathology. Presence of MDD was ascertained with the DSM-IV based Composite International Diagnostic Interview (CIDI version 2.1 [27]) assessed by trained research staff. Exclusion criteria were: a) clinically overt primary diagnosis of other psychiatric conditions, e.g. psychotic, obsessive compulsive, bipolar, or severe substance use disorder, and b) not being fluent in Dutch. The study was approved by the ethical committee of all participating centers, and participants provided written informed consent.

A total sample of N=1130 participants were selected that were divided into control (no lifetime psychiatric disorders and low depressive symptoms (Inventory of Depressive Symptomatology (IDS)<14), n=319) and current MDD (within the past 6 months, IDS>14, n=811) groups, leaving out those that did not meet criteria for either of the two groups. The sample selection was further based on good quality GWAS genotype information available from a previous investigation [28].

Assessed phenotypes

Sex, education (in years), and body mass index (BMI) data were collected during interviews. Alcohol was calculated as the mean number of drinks/week. Smoking behavior was represented by cotinine levels, an adequate marker for calculating recent tobacco exposure [29]. Physical activity was assessed using the International Physical Activity Questionnaire and indicated by Metabolic Equivalent Total-minutes per week. Health status was assessed as the number of chronic diseases for which participants received medical treatment.

In all subjects, depression severity was measured with the 30-item IDS self-report version [30]. Childhood trauma was assessed using the NEMESIS childhood trauma interview with personal history questions including a

structured inventory of trauma exposure during childhood. Finally, frequent use of antidepressants was assessed through container inspection and categorized using World Health Organization Anatomical Therapeutic Chemical classifications: tricyclic antidepressants, selective serotonin reuptake inhibitors, and other antidepressants.

In those with MDD, depression duration was measured by the Life Chart interview, utilizing a calendar method to assess the percentage of time in which symptoms were present during the past four years [31]. Also, current comorbid anxiety (panic disorder, generalized anxiety disorder, agoraphobia, social phobia) and alcohol disorder, as well as age of onset of depression were assessed with the CIDI. A more detailed description of all phenotypes can be found in the supplement.

DNA methylation measurements

To assay the methylation status of the approximately 28 million common CpG sites in the human genome, we used an optimized protocol for MBD-seq [25]. With this approach, genomic DNA is fragmented and the methylated fragments are then bound to the MBD2 protein that has high affinity for methylated DNA. The non-methylated fraction is washed away and only the methylation-enriched fraction is sequenced (for more detail, see Supplement). This optimized protocol assesses about 94% of the CpGs in the methylome [25]. The sequenced reads were aligned to the reference genome (build hg19/GRCh37) with Bowtie2 [32] using local and gapped alignment. Aligned reads were further processed using the RaMWAS Bioconductor package [33] to perform quality control and calculate methylation scores for each CpG.

DNAmAge estimation

While existing algorithms [9, 10] have gone through demonstrations of utility and reliability, estimating DNAmAge with those prediction models will be suboptimal for the current study. These algorithms were derived using methylation data from a different platform in study populations with different characteristics (e.g., age distribution). Commonly used methods for assaying DNA methylation depend on the Illumina arrays, platforms that generate variables representing percentage methylated (ranging from 0 to 1). The current study used MBD-seq, generating methylation data that is semi quantitative (scores may range from 0-20) [24]. As the weights assigned to individual CpGs when making age predictions directly depend on the platform and study population, they will not optimally capture the effects of CpGs on

age in the current study. Therefore, we "re-calibrated" the DNAmAge estimate in a way that is optimal for the current study. It is important to stress that we aimed to obtain the best possible DNAmAge estimates for MBD-seq data in our sample. We have not developed a new clock to be generalized to data from platforms other than MBD-seq. Furthermore, as we already collected MBD-seq data, we did not attempt to reduce the predictor set to the smallest number of CpGs as pruning sites may reduce the precision of the DNAmAge estimates.

Our approach for estimating DNAmAge is similar to the one taken by Horvath [10]. Specifically, we used elastic-nets, a variable selection method that is particularly useful when the number of predictors is much larger than the number of observations [34]. Parameter alpha was set to zero (i.e. ridge regression, retaining all sites in the model) where chronological age was used as outcome and methylation sites as predictors. To estimate predictive power and obtain DNAmAge estimates for each subject, k fold cross validation was used with k = 10. Thus, the sample was randomly partitioned into 10 equal sized subsamples. Of the 10 subsamples, 9 were used as training data and the remaining subsample as validation data. This ensures that in samples with the same properties and platform, our results would "replicate" and provide unbiased estimates of DNAmAge. In the RaMWAS implementation, a cycle of MWAS, marker selection, and estimation via ridge regression was repeated in each training dataset with the resulting model applied to the test data to obtain unbiased estimates of DNAmAge for each of the k = 10 iterations.

Validation of the use of MBD-seq data to estimate DNAmAge

Several analyses were performed to validate the model. First, the model used to estimate DNAmAge contained 80,000 CpGs (Supplementary **Table S1, available online**). 10-fold cross-validation showed that chronological age could be predicted very well with a correlation of 0.95 (P<0.001, **Figure 1**). Second, when analyzing assessed phenotypes in NESDA with DNAmAge, we confirmed some similar determinants of DNAmAge found in prior studies validating our outcome measure (Supplementary **Table S3**). Male sex [35], and higher BMI [13, 36, 37] were associated with higher EA. Third, to validate calculation of DNAmAge, we used both ridge regression (current study), as well as the lasso method (used by Horvath). The additional elastic-net model with parameter alpha set to 0.5, resulted in a quite comparable correlation of 0.93 between chronological age and predicted DNAmAge in our dataset (vs. 0.95 with ridge regression and alpha=0), indicating that parameter set point did not largely impact our outcome measure. Finally, to ensure no systematic

bias was introduced by training the model on both cases and controls, we also trained the prediction model in controls only. This resulted in a slightly lower correlation between DNAmAge estimates and chronological age (r=0.93) since the controls represented only a third of our total sample (i.e., lower statistical power). However, the correlation between the DNAmAge estimates obtained in the full sample and those obtained using controls-only was high (r=0.98, P<0.001), indicating that psychiatric status did not impact the estimation of DNAmAge.

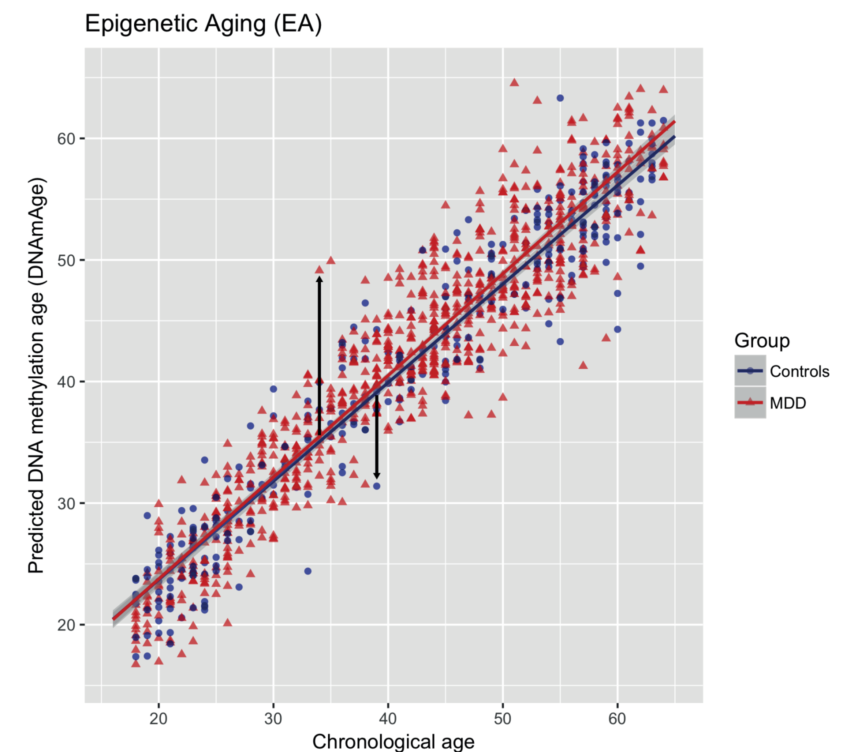


Figure 1. DNA Methylation Age Prediction Using Methyl-CpG Binding Domain Protein-Enriched Genome Sequencing (MBD-seq) in the Netherlands Study of Depression and Anxiety. The plot shows the prediction of DNA methylation (DNAm) age using MBD-seq across groups in blood. Each circle or triangle represents an individual subject (N=1,130), and the lines indicate regression lines (control group [N=319]: r=0.94, p<0.001; major depression group [N=811]: r=0.96, p<0.001). The arrows indicate the outcome variable epigenetic aging, representing higher epigenetic aging if the individual's estimated DNAm age exceeds chronological age (upward arrow), whereas negative epigenetic aging indicates lower epigenetic aging (downward arrow).

Post-mortem brain samples

We pooled data of five brain sample collections from four different brain banks (Victorian Brain Bank Network, Australia; Harvard Brain Bank; the Netherlands Brain Bank; Stanley Medical Institute), including a total of 141 brain samples from BA10 and BA25 brain regions. Presence of MDD (n=74) was determined by at least one psychiatrist by using information obtained from a family member who is well acquainted with the deceased. Controls (n=67) had no history of psychiatric disorders. Post-mortem intervals (hours) and pH were recorded in brain collections, with the exception of the Harvard Brain Bank.

To further test the reliability and validity of our methods, we used the same approach to predict DNAmAge in these samples with MBD-seq methylation data generated from the SOLiD 5500 W platform (Life Technologies). The model used to predict DNAmAge contained 100,000 CpGs (supplementary **Table S2, available online**), obtaining a correlation of 0.69 between predicted DNAmAge and chronological age. The lower correlation with age is likely because the methylation data was generated with an older platform with lower quality (e.g. lower alignment of reads) from a more heterogeneous dataset. More details about the samples and methods can be found in the supplement.

Statistical analyses for discovery

To investigate case-control differences in EA, we conducted linear regression models with EA as the outcome and all covariates as predictors. To correct for the relative abundance of cell types that may be differentially associated with MDD an additional model included cell-type proportions as covariates [38]. Other linear regression models were used to examine the relationship between EA and IDS-score across groups and clinical characteristics within MDD patients. All analyses were corrected for all sociodemographic, lifestyle and health covariates, using two-tailed tests considering $P<0.05$ significant.

Statistical analyses for replication

Within post-mortem brain samples, we constructed a linear mixed model in R using the nlme package to account for the heterogeneity of EA across brain collections. Thus, brain collection was entered as random effect and sex as fixed effect. The P-value was derived by a likelihood ratio test, hypothesis-driven one-sided tested, and considered significant at $P<0.05$.

Bioinformatics analyses

To perform enrichment tests of top MWAS findings in brain and blood, we used the shiftR R-package with 1 million permutations for each test and used three thresholds (0.5, 1% and 5%) to define "top findings". To account for this "multiple testing", shiftR uses the same thresholds in the permutations where the test statistic distribution under the null hypothesis is generated from the most significant (combination of) thresholds. A more in-depth description is provided in the supplement. To gain insight into the overlapping biological pathways affecting EA in blood and brain, we used ConsensusPathDB [39] to test whether genes harboring EA-associated CpGs were enriched for level-5 Gene Ontology (GO) terms. Methylation sites with P<1×10-5 were selected and had to be within gene boundaries. At least four genes had to be present in the GO term to be considered. Finally, we also evaluated the overlap between chronological age-associated and EA-associated CpG sites to examine whether similar biological processes were involved in chronological and biological aging.

RESULTS

Higher epigenetic aging and MDD in NESDA

The mean age of the NESDA sample was 41.5 years (s.d.=13.0 years, range 18-64 years) with 64.5% of females (**Table 1**). The groups did not differ in age (P=0.67), but the MDD group was more often female (P=0.02) and less educated (P<0.001). As anticipated, MDD patients reported higher levels of depression severity and use of antidepressants (all P's<0.001). Childhood trauma scores were also higher in the depressed group (P<0.001).

EA showed (by design) a mean of zero (s.d.=3.58 years), ranging from -13.26 to 15.00. MDD patients had significantly higher EA compared to controls (b=0.64, t=2.65, P=0.008) (effect size, Cohen's d=0.18) indicating patients were estimated to be 0.64 years (or 7.68 months) older than controls after full adjustment for covariates (**Table 2**). Additional analyses correcting for cell type proportions did not change results and produced a Cohen's d of 0.14 (Supplement). Consistent with a dose-response effect, a fully-adjusted linear regression showed that greater EA was significantly associated with higher IDS-score in the overall sample (β=0.10, P=0.001). As expected from the high correlation between the DNAmAge estimates generated by both models (r=0.98), above mentioned results remained unchanged when performing the

same analyses with the DNAmAge estimates from the controls-only model (Supplement).

Table 1. Participant characteristics of the Netherlands Study of Depression and Anxiety

Characteristic	Controls (N=319)		MDD (N=811)	
	Mean	SD	Mean	SD
Sociodemographic				
Age (years)	41.6	14.63	41.5	12.26
Education (years)	13.1	3.15	11.5	3.20
	N	%	N	%
Female Sex	188	58.9	541	66.7
	Mean	SD	Mean	SD
Lifestyle and health				
Body Mass Index	25.2	4.50	25.9	5.31
Cotinine levels (ng/ml)	70.9	200.8	103.3	183.9
Alcohol intake (mean number of drinks/week)	7.10	7.11	6.32	9.12
Physical activity (1000 MET-minutes/week)	3.91	2.86	3.49	3.17
Number of chronic diseases	0.46	0.74	0.69	0.92
	Mean	SD	Mean	SD
Clinical characteristics				
Severity (Inventory of Depressive Symptoms score)	5.02	3.54	33.8	10.9
Childhood trauma index score	0.30	0.69	1.23	1.24
Age of onset (years)	NA	NA	27.0	12.5
Symptom duration (% time in the past 4 years)	NA	NA	0.39	0.30
	N	%	N	%
Comorbid anxiety disorder	NA	NA	538	66.4
Comorbid alcohol disorder	NA	NA	270	33.3
Antidepressant use				
Tricyclic antidepressants	0	0.0	38	4.7
Selective serotonin reuptake inhibitor	1	0.3	243	30.0
Other antidepressants	0	0.0	90	11.2
	Mean	SD	Mean	SD
Epigenetic Aging				
EA	-0.45	3.37	0.18	3.65

Abbreviations: MDD, major depressive disorder; MET-minutes, metabolic equivalent of number of calories spent per minute; NA, not applicable; Epigenetic Aging (EA), unstandardized residuals of DNAmAge regressed on chronological age.

Exploratory analyses of epigenetic aging and clinical characteristics

Within MDD cases, we found EA to be positively associated with childhood trauma scores (β=0.09, P=0.02, see **Table 3**). The association between EA and IDS-score in the overall sample did not remain significant when analyzed only within MDD patients (β=0.05, P=0.21), likely due to reduced variation in symptom severity. No further significant associations with clinical characteristics were found.

Table 2. Estimated marginal means of epigenetic aging by major depressive disorder status and association with depression severity in the overall sample in basic- and fully adjusted analyses

Model	Controls (N=319)			MDD (N=811)			MDD versus Controls		IDS-score	
	Mean	SE	95% CI	Mean	SE	95% CI	*P*-value	Cohen's *d*	*β*	*P*-value
Basic adjusted[a]	-0.51	0.20	-0.90, -0.11	0.20	0.13	-0.05, 0.45	0.004	0.20	0.12	<0.001
Fully adjusted[b]	-0.46	0.20	-0.86, -0.06	0.18	0.13	-0.07, 0.43	0.008	0.18	0.10	0.001

Abbreviations: SE. standard error; CI, confidence intervals; MDD, major depressive disorder; IDS, Inventory of Depressive Symptoms. [a]Adjusted for sex and education. [b]Adjusted for sex, education, body mass index, cotinine levels, alcohol use, physical activity, and number of chronic diseases.

Table 3. Associations between epigenetic aging and clinical characteristics in major depressive disorder patients (N=811)

Variable	*β*	*P*[a]
Severity (IDS score)	0.05	0.21
Duration	-0.02	0.58
Age of Onset	0.03	0.42
Comorbid anxiety disorder	-0.02	0.53
Comorbid alcohol dependence disorder	0.05	0.21
Childhood trauma index score	0.09	0.02
Antidepressant Use		
Tricyclic antidepressant	0.02	0.67
SSRI	-0.04	0.31
Other antidepressant	-0.04	0.29

Abbreviations: Epigenetic aging, the residuals of DNA methylation age regressed on chronological age; IDS, Inventory of Depressive Symptoms; SSRI, selective serotonin reuptake inhibitor. [a]Analyses are adjusted for sex, education, body mass index, cotinine levels, alcohol use, physical activity, and number of chronic diseases.

Further analyses revealed that MDD patients with childhood trauma showed the highest EA compared to controls without childhood trauma (P=0.001, Cohen's d=0.29), highlighting that this MDD and childhood trauma subgroup is associated with the highest EA (Supplementary **Figure S1**). Important to note, more severe symptomatology of chronic MDD was correlated with childhood trauma (r=0.39, P<0.001), making it difficult to discern which of these two factors drives increased EA. Linear regression indeed showed that both childhood trauma (β=0.08, P=0.01) and depression severity scores (β=0.07, P=0.03) were significant predictors of EA when analyzed in the same model.

Replication in post-mortem brain samples

The mean age of the post-mortem brain samples was 55.2 years (s.d.=19.3, age range 20-100), with 45.4% of females. Groups were matched on age and sex. Mean post-mortem interval was 35.1 hours (s.d.=21.1) and mean pH was 6.51 (s.d.=0.25) across samples. EA was uncorrelated to pH or Post-mortem interval (both P's>0.05). **Table 4** shows an overview of the descriptive characteristics by brain collection. Only the control (n=67) and MDD (n=74) samples from the same brain collection were included in analyses (see supplement for more detail).

Our replication findings in independent brain samples supported our findings in NESDA and again showed that the EA was higher in MDD cases than in controls (b=1.11, χ^2=3.41, P=0.03). The beta indicates that the MDD group was estimated to be on average 1.11 years older than controls. The phenotype information available from the post-mortem samples was limited, and therefore we were unable to attempt any replication of the exploratory clinical associations observed for e.g., childhood trauma in NESDA.

Enrichment testing and gene ontology analyses

When evaluating the overlap between both epigenetic aging indicators, we found that after correcting for multiple testing the top 1% findings from the EA MWAS in blood were significantly enriched for CpGs in the top 0.5% of the EA MWAS from brain (odds ratio=1.19, P<0.001). To examine possible processes underlying epigenetic aging in both tissues, we performed pathway analyses on the 1084 overlapping CpGs associated with EA, leading to 330 genes (90.7%) that were present in at least one GO category. Subsequently, this resulted in 53 significantly enriched GO terms (Supplementary **Table S4, available online**). Top GO terms included neurogenesis (P-value=9.79×10-9), neuron differentiation (P-value=5.34×10-8) and regulation of neuron death

Table 4. Descriptive characteristics of the post-mortem brain samples

Characteristic	Controls (N=67)		MDD (N=74)	
Brain collection 1*	*N*=30		*N*=30	
	Mean	SD	Mean	SD
Age (years)	51.63	12.94	51.93	18.61
	N	%	N	%
Female sex	17	56.7	18	60.0
Post-mortem interval (hours)	47.04	14.63	41.74	15.74
pH	6.32	0.21	6.50	0.28
Brain collection 2	*N*=4		*N*=3	
	Mean	SD	Mean	SD
Age (years)	77.00	12.25	83.00	13.00
	N	%	N	%
Female sex	1	25	1	33.3
PMI (hours)	NA	NA	NA	NA
pH	NA	NA	NA	NA
Brain collection 3	*N*=9		*N*=3	
	Mean	SD	Mean	SD
Age (years)	85.67	8.57	86.44	8.37
	N	%	N	%
Female sex	6	66.7	2	66.7
Post-mortem interval (hours)	5.28	0.78	6.59	1.93
pH	6.56	0.15	6.49	0.23
Brain collection 4	*N*=11		*N*=22	
	Mean	SD	Mean	SD
Age (years)	48.00	11.95	42.32	11.15
	N	%	N	%
Female sex	4	36.4	10	45.5
Post-mortem interval (hours)	26.72	9.79	30.14	12.84
pH	6.64	0.19	6.65	0.13
Brain collection 5	*N*=13		*N*=10	
	Mean	SD	Mean	SD
Age (years)	51.15	8.35	48.80	8.35
	N	%	N	%
Female sex	0	0	1	10
Post-mortem interval (hours)	24.00	15.00	49.00	49.50
pH	6.69	0.18	6.64	0.23

Note: *N* is number of samples left after quality control. Abbreviations: MDD, major depressive disorder; NA, not applicable. *Brain collection 1 contains tissue dissected from BA25, all other collections contain tissue from BA10.

(P-value=4.67×10-5), indicating that several MDD-relevant pathways were enriched in the cross-tissue EA indicators.

DISCUSSION

To the best of our knowledge, this is the first time higher epigenetic aging in MDD patients compared to controls is shown. Exploratory analyses suggested even more pronounced epigenetic aging in MDD patients with more childhood trauma. The case-control difference in blood was replicated in post-mortem brain tissue. Finally, analyses showed significantly enriched neuronal pathways associated with the overlap between EA-associated CpGs from blood and brain tissue.

Replication of our main finding in post-mortem brain tissue bolstered confidence in the observed higher epigenetic aging in MDD. Moreover, the significantly enriched overlap suggests that at least some processes affecting epigenetic aging are at play in both blood and brain. There is some evidence that blood and brain show concordance in methylation [40] and epigenetic aging [10]. However, considering the interactions between stress, central and peripheral immune processes, and neurobiology [41], it is plausible and likely EA in MDD is also dictated by many systemic processes. Nonetheless, more work is needed to confirm higher epigenetic aging findings and better characterize advanced aging associated genes and their implications in MDD.

DNAmAge is just one of the several available markers of biological aging [35]. The current study confirms advanced or premature biological aging in MDD with a novel platform and is consistent with literature regarding telomere length as a biological marker of aging in MDD [7, 42]. Also, in line with other studies [43, 44], post-hoc analyses between telomere length and EA showed non-significant relationships, suggesting that both measures likely independently track different aspects of biological aging. Similarly, other post-hoc analyses showed that telomere length did not alter this study's findings when accounted for, providing further evidence that EA captures significant aging signal different from telomere length (supplementary results).

We found that childhood trauma was positively associated with higher epigenetic aging in MDD patients. It seems conceivable that MDD and accumulated stress throughout the lifetime due to childhood trauma may alter the epigenetic landscape and influence genomic regulation and function [45]. However, this study did not further identify additional relationships between higher epigenetic aging and more cumulative clinical characteristics such as

earlier onset age or longer duration of MDD. Rather, our findings suggest that higher epigenetic aging in MDD may be largely driven by severity of disease.

Alternatively, childhood trauma may produce long-lasting epigenetic “scars” that impact MDD and advanced or premature aging processes later in life. Individuals with childhood trauma and depressive disorders have earlier onset age, higher symptom severity, more comorbidities, increased suicidality, and poorer treatment response than patients without childhood trauma [46, 47]. As Teicher & Samson (2013) suggest, presence of childhood trauma is associated with a clinically and neurobiologically distinct subtype of depression.

Strikingly, three out of ten top GO categories enriched across tissues included neuronal pathways. Epigenetic mechanisms are critical in early brain development, adult neurogenesis, and late-stage brain maturation [48]. Being processes that all seem markedly aberrant in MDD [49], the implicated pathways suggest EA in MDD directly contributes to disease symptomatology. Additionally, the degree of overlap between the top 1% findings of EA from blood/brain and top 0.5% findings of chronological age in NESDA (odds ratio: 85.31, $P<0.001$/odds ratio: 1.64, $P<0.001$) was highly significant, suggesting that biological aging is overlapping with the same epigenetic processes underlying chronological aging.

Although effect sizes such as those observed in the current study are common in MDD research (e.g. oxidative stress, brain-derived neurotrophic factor, and cortisol yield effect sizes ranging from 0.15-0.31 [50–52]), it is possible that this is an underestimate. The reason is that DNAmAge is estimated from the residuals of the regression of methylation data on chronological age. This residual variance comprises two components: i) true unique variance associated with DNAmAge and ii) measurement errors. However, because the residual variance is very small (the correlation between chronological age and methylation data was 0.95), even small measurement errors can have a large negative effect on the reliability of the DNAmAge that is defined as: reliability = Var(DNAmAge)/[Var(DNAmAge)+VAR(errors of measurement)]. As a less than perfect reliability will attenuate the correlation of DNAmAge and MDD status, the real effect size may have been underestimated.

Strengths of this study are the replication in post-mortem brain tissue and inclusion of a large clinically well-characterized and representative sample including many potential confounders that did not explain our findings. Given the full methylome coverage, we were also able to examine which biological pathways seemingly underlie epigenetic aging. However, the findings of our study should also be considered against some limitations. A direct comparison of our MBD-seq-based epigenetic clock and existing Illumina array-based

clocks was not possible and beyond the current scope. However, a side-by-side comparison is an interesting endeavor for a future methodological paper. Furthermore, with the current cross-sectional data we were not able to disentangle whether greater EA in MDD truly reflects aging acceleration over time or if subjects have increased EA from birth or before adulthood that continues to be stable thereafter [53]. Future studies with longitudinal designs are needed to distinguish the two possibilities.

In conclusion, our findings show that DNAmAge from both the blood and brain of MDD patients is higher than their corresponding chronological age, which may contribute to their increased risk for mortality and aging-related diseases. Further, higher childhood trauma scores correlated with higher epigenetic aging in MDD patients. Taken together, our findings suggest that higher methylation aging in MDD is present in both blood and brain, and that higher epigenetic aging largely overlaps with the same underpinnings associated with chronological aging. Further research is needed to investigate the causal relationships between age-associated alterations in DNA methylation and MDD.

REFERENCES

1. Penninx BWJH. Depression and cardiovascular disease: Epidemiological evidence on their linking mechanisms. Neurosci Biobehav Rev. 2016. 2016. https://doi.org/10.1016/j.neubiorev.2016.07.003.
2. Spiegel D, Giese-Davis J. Depression and cancer: Mechanisms and disease progression. Biological Psychiatry. 2003;54:269–282.
3. McDermott LM, Ebmeier KP. A meta-analysis of depression severity and cognitive function. J Affect Disord. 2009;119:1–8.
4. Buigues C, Padilla-Sanchez C, Garrido JF, Navarro-Martinez R, Ruiz-Ros V, Cauli O. The relationship between depression and frailty syndrome: a systematic review. Aging Ment Health. 2015;19:762–772.
5. Ben-Avraham D, Muzumdar RH, Atzmon G. Epigenetic genom-wide association methylation in aging and longevity. Ep. 2012;4:503–509.
6. Schutte NS, Malouff JM. The association between depression and leukocyte telomere length: A meta-analysis. Depress Anxiety. 2015;32:229–238.
7. Ridout KK, Ridout SJ, Price LH, Sen S, Tyrka AR. Depression and telomere length: A meta-analysis. J Affect Disord. 2016;191:237–247.
8. Koutsouleris N, Davatzikos C, Borgwardt S, Gaser C, Bottlender R, Frodl T, et al. Accelerated brain aging in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. Schizophr Bull. 2014;40:1140–1153.
9. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol Cell. 2013;49:359–367.
10. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
11. Horvath S, Garagnani P, Bacalini MG, Pirazzini C, Salvioli S, Gentilini D, et al. Accelerated epigenetic aging in Down syndrome. Aging Cell. 2015;14:491–495.
12. Levine AJ, Quach A, Moore DJ, Achim CL, Soontornniyomkij V, Masliah E, et al. Accelerated epigenetic aging in brain is associated with pre-mortem HIV-associated neurocognitive disorders. J Neurovirol. 2015:366–375.
13. Horvath S, Erhart W, Brosch M, Ammerpohl O, Schönfels W von, Ahrens M, et al. Obesity accelerates epigenetic aging of human liver. Proceedings of the National Academy of Sciences. 2014:201412759.
14. Marioni RE, Shah S, McRae AF, Ritchie SJ, Muniz-Terrera G, Harris SE, et al. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. Int J Epidemiol. 2015;44:1388–1396.
15. Beach SRH, Dogan MV, Lei MK, Cutrona CE, Gerrard M, Gibbons FX, et al. Methylomic Aging as a Window onto the Influence of Lifestyle: Tobacco and Alcohol Use Alter the Rate of Biological Aging. J Am Geriatr Soc. 2015;63:2519–2525.
16. Perna L, Zhang Y, Mons U, Holleczek B, Saum K-U, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenetics. 2016;8:64.
17. Levine ME, Lu AT, Bennett DA, Horvath S. Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. Aging . 2015;7:1198–1211.
18. Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai P-C, et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging . 2016;8:1844–1865.

19. Voisey J, Lawford BR, Morris CP, Wockner LF, Noble EP, Young RM, et al. Epigenetic analysis confirms no accelerated brain aging in schizophrenia. Npj Schizophrenia. 2017;3:26.
20. McKinney BC, Lin H, Ding Y, Lewis DA, Sweet RA. DNA methylation age is not accelerated in brain or blood of subjects with schizophrenia. Schizophr Res. 2017. 2017. https://doi.org/10.1016/j.schres.2017.09.025.
21. Zannas AS, Arloth J, Carrillo-Roa T, Iurato S, Röh S, Ressler KJ, et al. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. Genome Biol. 2015;16:266.
22. Wolf EJ, Logue MW, Hayes JP, Sadeh N, Schichman SA, Stone A, et al. Accelerated DNA methylation age: Associations with PTSD and neural integrity. Psychoneuroendocrinology. 2016;63:155–162.
23. Boks MP, Mierlo HC van, Rutten BPF, Radstake TRDJ, De Witte L, Geuze E, et al. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. Psychoneuroendocrinology. 2015;51:506–512.
24. Aberg KA, Mcclay JL, Nerella S, Xie LY, Clark SL, Hudson AD, et al. MBD-seq as a cost-effective approach for methylome-wide assocciation studies: demonstration in 1500 case-control samples. Epigenomics. 2012;4:605–621.
25. Chan RF, Shabalin AA, Xie LY, Adkins DE, Zhao M, Turecki G, et al. Enrichment methods provide a feasible approach to comprehensive and adequately powered investigations of the brain methylome. Nucleic Acids Res. 2017;45:e97.
26. Penninx BWJH, Beekman ATF, Smit JH, Zitman FG, Nolen WA, Spinhoven P, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale , objectives and methods. 2008;17:121–140.
27. Wittchen HU. Reliability and validity studies of the WHO-Composite International Diagnostic Interview (CIDI): A critical review. J Psychiatr Res. 1994;28:57–84.
28. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. Psychol Med. 1996;26:477–486.
29. Boomsma DI, Willemsen G, Sullivan PF, Heutink P, Meijer P, Sondervan D, et al. Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. Eur J Hum Genet. 2008;16:335–342.
30. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol. 2009;169:236–248.
31. Lyketsos CG, Nestadt G, Cwi J, Heithoff K, Et al. The Life Chart Interview: A standardized method to describe the course of psychopathology. Int J Methods Psychiatr Res. 1994;4:143–155.
32. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat. 940. Methods. 2012;9:541.
33. Shabalin A, Clark S, Hattab M, Aberg K, van den Oord E. ramwas: Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms. R package version 1.0.0, https://bioconductor.org/packages/ramwas/. 2017.
34. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw. 2010;33.
35. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.

36. Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 2014;15:R24.
37. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging . 2017;9:419–446.
38. Hattab MW, Shabalin AA, Clark SL, Zhao M, Kumar G, Chan RF, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. Genome Biol. 2017;18.
39. Kamburov A, Stelzl U, Lehrach H, Herwig R. The ConsensusPathDB interaction database: 2013 Update. Nucleic Acids Res. 2013;41:793–800.
40. Tylee DS, Kawaguchi DM, Glatt SJ. On the outside, looking in: A review and evaluation of the comparability of blood and brain '-omes'. Am J Med Genet B Neuropsychiatr Genet. 2013;162:595–603.
41. Wohleb ES, Franklin T, Iwata M, Duman RS. Integrating neuroimmune systems in the neurobiology of depression. Nat Rev Neurosci. 2016;17:497–511.
42. Henje Blom E, Han LKM, Connolly CG, Ho TC, Lin J, LeWinn KZ, et al. Peripheral telomere length and hippocampal volume in adolescents with major depressive disorder. Transl Psychiatry. 2015;5:e676.
43. Breitling LP, Saum K-U, Perna L, Schöttker B, Holleczek B, Brenner H. Frailty is associated with the epigenetic clock but not with telomere length in a German cohort. Clin Epigenetics. 2016;8:21.
44. Marioni RE, Harris SE, Shah S, Mcrae AF, Zglinicki TV, Martin-ruiz C, et al. The epigenetic clock and telomere length are independently associated with chronological age and mortality. Int J Epidemiol. 2016:1–9.
45. Gassen NC, Chrousos GP, Binder EB, Zannas AS. Life stress, glucocorticoid signaling, and the aging epigenome: implications for aging-related diseases. Neurosci Biobehav Rev. 2016. 2016. https://doi.org/10.1016/j.neubiorev.2016.06.003.
46. Nanni V, Uher R, Danese A. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: A meta-analysis. Am J Psychiatry. 2012;169:141–151.
47. Teicher MH, Samson JA. Childhood maltreatment and psychopathology: A case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. Am J Psychiatry. 2013;170:1114–1133.
48. Matt S, Roth ED, Roth TL. Role of epigenetics in the brain. Epigenetics in Psychiatry, Elsevier; 2014. p. 79–99.
49. Balu DT, Lucki I. Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci Biobehav Rev. 2009;33:232–252.
50. Black CN, Bot M, Scheffer PG, Cuijpers P, Penninx BWJH. Is depression associated with increased oxidative stress? A systematic review and meta-analysis. Psychoneuroendocrinology. 2015;51:164–175.
51. Vreeburg SA, Hoogendijk WJG, van Pelt J, Derijk RH, Verhagen JCM, van Dyck R, et al. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. Arch Gen Psychiatry. 2009;66:617–626.
52. Molendijk ML, Bus BAA, Spinhoven P, Penninx BWJH, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state–trait issues, clinical features and pharmacological treatment. Mol Psychiatry. 2011;16:1088–1095.
53. Kananen L, Marttila S, Nevalainen T, Kummola L, Junttila I, Mononen N, et al. The trajectory of the blood DNA methylome ageing rate is largely set before adulthood: evidence from two longitudinal studies. Age . 2016;38:65.

SUPPLEMENTARY MATERIALS

Epigenetic Aging in Major Depressive Disorder

SUPPLEMENTARY METHODS

Assessed phenotypes

Body Mass Index (BMI) was calculated as weight (kilograms) divided by squared length (meters). Smoking behavior was represented by cotinine levels, an adequate marker for calculating recent tobacco exposure and discriminating smokers from non-smokers [1]. Physical activity was assessed using the International Physical Activity Questionnaire and indicated by overall energy expenditure in Metabolic Equivalent Total-minutes per week. Health status was assessed as number of chronic diseases (heart disease, epilepsy, diabetes, osteoarthritis, cancer, stroke, intestinal disorders, ulcers, and lung-, liver-, and thyroid disease) for which participants received medical treatment. In line with earlier work, a cumulative childhood trauma index (CTI) was created that reported the sum of the categories that were scored from 0 to 2 (0: never happened, 1: sometimes, 2: happened regularly), resulting in an index score ranging from 0-8 [2]. Finally, frequent use of antidepressants was assessed through container inspection and categorized using World Health Organization Anatomical Therapeutic Chemical (ATC) classifications: antidepressants (tricyclic antidepressants (ATC code N06AA), selective serotonin reuptake inhibitors (ATC code N06AB), and other antidepressants (ATC codes N06AF, N06AG, N06AX). Childhood trauma was assessed using the NEMESIS childhood trauma interview with personal history questions including a structured inventory of trauma exposure during childhood (emotional neglect, psychological abuse, physical abuse, sexual abuse, and important life-events in early life).

Quality control of MBD-seq data in NESDA

Of the 1200 samples from participants eligible for methylome-wide sequencing, 34 samples were excluded because of failed methylation enrichment (*n*=16) or failed library construction (*n*=18) where we did not have sufficient DNA available for a repeated assay. Reads aligning to loci without CpGs (non-CpGs) represent "noise" caused by, for example, imperfect enrichment leading to non-methylated fragments being sequenced. We used a threshold of 0.05 for

"non-CpG/CpG coverage ratio" to remove samples with high "noise" levels (*n*=10), leaving an average ratio of 0.010 (SD=0.005) in the remaining samples. For 10 samples, sequence variants called from the methylation data did not match the genotype information, indicating that a sample swap or sample contamination may have occurred. As it is not possible to determine whether the sample handling errors occurred in the GWAS or in the MWAS data, we conservatively excluded all 10 samples from further analysis. We used the R function 'pcout' in the 'mvoutliers' package (with the upper boundary for outlier detection set to 15, the scaling constant set to 0.5, and the boundary for final outliers set to 0.2) to identify multidimensional outliers using principal components of the methylation data as input. Fourteen samples were multidimensional outliers and omitted. Finally, two samples were removed after DNAm age residual estimation because there were determined to be extreme outliers as they were both more than seven standard deviations away from the mean.

This left a sample of 1130 subjects. The mean number of reads for these samples was 59,954,723. The average alignment rate was 99.2%. We performed quality control (QC) for multi- and duplicate-reads. Although reads often map to multiple genomic locations, in most cases, a single alignment can be selected because it is clearly better than other alignments. In the case of multi-reads, multiple alignments are about equally good (in terms of alignment score). When Bowtie2 encounters a set of equally good alignments, it uses a pseudo-random number to select one primary alignment. Duplicate-reads are reads that start at the same nucleotide positions. When sequencing a whole genome, duplicate-reads often arise from artifacts in template preparation or amplification. However, in the context of sequencing an enriched genomic fraction such as methyl-CG binding domain sequencing (MBD-seq), duplicate-reads are increasingly likely to occur because reads originate from a smaller fraction of the genome. We therefore allow for three reads to occur at the same location but for instances where more than 3 (duplicate) reads start at the same position, we reset the read count to 1 implicitly assuming excess reads are tagging a single clonal fragment. This left an average of 48,653,227 reads per sample (=81.9% of all reads).

To identify CpGs, we combined reference genome sequence (hg19/GRCh37) with common SNPs calculated on the European superpopulation from 1000 Genomes (Phase 3). To avoid including sites that are CpGs in only a very small proportion of subjects, we excluded CpGs created by SNPs with minor allele frequency <1%. This resulted in 27,916,990 CpGs. CpGs in loci prone to alignment errors, e.g., in repetitive regions, were eliminated prior to the analysis. To identify these CpGs, we used RaMWAS to perform the

in-silico alignment experiment outlined elsewhere.[3] In this experiment, the vast majority of CpGs (89.3%) were located in regions that showed perfect alignment coverage and only 1.3% (365,223 CpGs) showed evidence of alignment problems defined as 15% or more reads from this locus not aligning properly. These CpGs with alignment problems were removed from further analyses. Finally, we eliminated 5,682,206 CpGs with average coverage less than 0.3 or having zero coverage in over 70% of the samples. This resembles filtering GWAS SNPs on minor allele frequency and avoids statistical problems associated with analyzing sparse data that is the result of sites that are not methylated in almost any subject in the study. This left a total of 21,869,561 CpGs.

Cell type measurements

To estimate cell type proportions, we used reference methylomes[4, 5] requirements for cell processing, and timely cell analysis. In a diverse array of diseases and following numerous immune-toxic exposures, leukocyte composition will critically inform the underlying immuno-biology to most chronic medical conditions. Emerging research demonstrates that DNA methylation is responsible for cellular differentiation, and when measured in whole peripheral blood, serves to distinguish cancer cases from controls.\\n\\nRESULTS: Here we present a method, similar to regression calibration, for inferring changes in the distribution of white blood cells between different subpopulations (e.g. cases and controls. Whole blood samples of six subjects were used to isolated cells with 5 clusters of differentiation (CD3, CD19, CD20, CD14, and CD15) that capture the most common cell types in blood (T-cells, B-cells, monocytes, and granulocytes). Cell populations were isolated by positive selection using EasySep™ kits (Stemcell technologies) that apply magnetic nanoparticles coated with antibodies against a particular surface antigen (CD molecules). All reference methylomes were generated using MBD-seq. In a previous paper we showed that the estimated cell type proportions effectively controlled for cell type heterogeneity in methylome-wide association studies [6].

We further validated our cell type proportion estimates by correlating them with automated counts from 337 subjects obtained with the Abbott Sapphire system that uses optical scatter and impedance. We only had automated cell counts for these subjects 2-3 and 5-6 years after the methylation measurement and the cross-year correlation was 0.589. This correlation is attenuated by the fact that cell counts will have changed over the 2 to 3-year period but serves as a comparison. The correlation between MBD-seq estimates at baseline

and automated counts 2-3 years later was 0.534 and only slightly lower. This suggests that the reliability of MBD-seq estimates of cell type proportions was comparable to that of automated counts as typically used in clinical settings.

Post-mortem brain samples for replication

Here, we summarize the post-mortem brain samples used in the replication analysis. Diagnosing disease in subjects providing post-mortem brain samples can be challenging [7]neurochemistry, and molecular pathways of genes associated with bipolar disorder (BPD. In most cases one or two psychiatrists determine the diagnosis by using information obtained from a family member who is well acquainted with the deceased. This technique has been validated for axis I and II diagnoses [8, 9]social support and suicidal behavior among individuals who attempted suicide. Subjects were 80 psychiatric inpatients (aged 50-91 yrs and has shown to have high inter-rater agreement [10]. We pooled the data of six brain collections from four different brain banks that are described in detail below.

The first subsample included post-mortem brain tissue from 30 MDD cases and 30 matched controls, obtained from the Victorian Brain Bank Network, Australia [11]. For MDD cases, DSM-IV diagnoses were confirmed post-mortem by two psychiatrists, using clinical case histories and the Diagnostic Instrument for Brain Studies (DIBS)[12]. The controls had no history of psychiatric symptoms or substance abuse (as determined by both information from relatives and medical records) and were age/sex matched to the cases. The tissue samples were dissected from the cerebral cortex (BA25) for each subject.

The second subsample included 3 cases and 4 controls from the Harvard Brain Bank [13]. Family members initially reported diagnoses at the time of death and next of kin were asked to complete a questionnaire/participate in a phone interview to provide further details. A staff psychiatrist then reviewed the clinical records and family questionnaires to confirm or correct the psychiatric diagnosis.

The third subsample included 9 cases and 9 controls from the Netherlands Brain Bank [14]each with its own promoter providing a mechanism for tissue-specific fine-tuning of GR levels. Recently epigenetic methylation of these GR promoters was shown to modulate hippocampal GR levels. Here we investigate in post-mortem brain tissues whether in MDD HPA axis hyperactivity may be due to epigenetic modulation of GR transcript variants.Levels of GRα, GRβ and GR-P transcripts were homogeneous throughout the limbic system, with GRα being the most abundant (83%. Reports by family members of a lifetime diagnosis of MDD was confirmed post-mortem by a certified psychiatrist on

the basis of the medical records following DSM-IV criteria. Controls never received any psychiatric diagnosis or long-term psychotropic medication.

The fourth and fifth independent replication samples included BA10 samples from the Stanley Medical Research Institute (SMRI)[15]. The first consisted of 22 MDD cases (with or without psychosis) and 11 controls. The second collection comprised 10 (non-psychotic) cases and 13 controls. The SMRI uses DSM-IV diagnoses made by two senior psychiatrists on the basis of medical records and, when necessary, telephone interviews with family members. Diagnoses of unaffected controls are based on structured interviews by a senior psychiatrist with family member(s) to rule out Axis I diagnoses. In addition to the balanced MDD case-control collections, we also used methylome data from the same brain bank and brain region BA10 from an additional 78 individuals (25 schizophrenia cases, 18 bipolar cases and 25 controls) that were solely used to train the DNAmAge prediction model, but were excluded from all replication analyses.

MBD-seq data from post-mortem brain samples

To study the CpG methylome of the post-mortem brain samples we again used the MBD-seq approach. All brain samples were assayed using the same MBD-seq protocol [16]. In short, we used ultrasonicaton to shear genomic DNA into an average of 150 bp fragments. Next, we performed enrichment with MethylMiner™ (Invitrogen), following the same procedure as was described for the blood samples, to capture the methylated fraction of the genome. Barcoded sequencing libraries were manually created for each methylation capture, were pooled in equal molarities, sequenced on a SOLiD5500 wildfire instrument (Life technologies) with 50bp reads and were aligned with Cushaw3[17]. As for the MBD-seq data from blood, the aligned MBD-seq data from brain was processed and analyzed using RaMWAS (RaMWAS: Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms. https://bioconductor.org/packages/ramwas).

Quality control of MBD-seq data from post-mortem brain samples

Quality control for the post-mortem brain tissues largely followed the procedures used for the blood samples (see above). A summary of the samples available after QC is reported in **Table 2**. The mean number of reads after sample quality control was 57.5 million (SD = 18.6 million). The average alignment rate was 77.9%. After removing multi- and duplicate-reads, an average of 27.7 million reads (SD = 10.2 million reads) per sample remained. As previously described

[3], to identify regions showing alignment problems, we conducted an *in silico* alignment experiment using the appropriate settings (50bp reads aligned with CUSHAW3 [17]). With these settings 7.9% of the CpGs were removed from further analyses. After also excluding sporadically methylated CpGs (average coverage <0.3), 18.9 million CpGs remained for statistical analysis.

DNA methylation prediction model in post-mortem brain samples

To maximize the sample size of the data used for age prediction, we pooled all methylation data from post-mortem brain (regions BA10 and BA25) samples (N=211) available that had been generated using the same MBD-seq protocol and sequenced on the SOLiD system. Using the same 10-fold resampling approach as was described for the blood samples, chronological age could be predicted with a correlation of 0.69 ($P<0.001$) using 100,000 CpGs (Supplementary **Table S2, available online**). The brain data included a subset of MDD patients (n=74) and matched controls (n=67) without psychiatric diagnosis used to examine case-control differences. We again regressed chronological age on DNAmAge, and used the unstandardized residuals as outcome measure.

Enrichment testing

To perform enrichment tests analysis of top MWAS findings in brain and blood, we used the R package shiftR. ShiftR cross-classifies CpGs as being in the top or bottom of the two MWAS. Using the resulting 2 by 2 tables as input, shiftR tests the null hypothesis that the enrichment odds ratio equals one. To perform these tests, it uses circular permutations [18]due to the large number of tests, standard analysis techniques impose highly stringent significance thresholds, leaving potentially associated SNPs undetected, and much of the trait genetic variation unexplained. Pathway- and network-based methodologies applied to GWAS aim to detect associations missed by standard single-marker approaches. The complex and non-random architecture of the genome makes it a challenge to derive an appropriate testing framework for such methodologies. We developed a rapid and simple permutation approach that uses GWAS SNP association results to establish the significance of pathway associations while accounting for the linkage disequilibrium structure of SNPs and the clustering of functionally related elements in the genome. All SNPs used in the GWAS are placed in a \”circular genome\” according to their location. Then the complete set of SNP association P values are permuted by rotation with respect to the genomic locations of the SNPs. Once these \”simulated\” P values are

assigned, the joint gene P values are calculated using Fisher's combination test, and the association of pathways is tested using the hypergeometric test. The circular genomic permutation approach was applied to a human genome-wide association dataset. The data consists of 719 individuals from the ORCADES study genotyped for ~300,000 SNPs and measured for 51 traits ranging from physical to biochemical measurements. KEGG pathways (n = 225 that destroy possible association signals while preserving the correlational structure between adjacent CpGs. Thus, it generates an empirical test statistic distribution under the null hypothesis that takes into account the dependency between CpGs. We used 1 million permutations for each enrichment test. Three thresholds of 0.5, 1 and 5% were specified to define the "top" MWAS findings. To account for this "multiple testing", the same thresholds were used in the permutations where the test statistic distribution under the null hypothesis is generated from most significant (combination of) thresholds. Of the overlapping sites in each comparison, only those below a combined *q*-value<0.1 were considered for pathway analysis.

SUPPLEMENTARY RESULTS

Accelerated aging and covariates

In addition, to further validate our main outcome measure, we examined the impact of all selected covariates on epigenetic aging (EA, unstandardized residuals of DNAmAge regressed on chronological age) with one multiple linear regression model (Supplementary **Table S3**). As expected based on earlier results [19–21] higher EA was associated with male sex (β=-0.11, P<0.001). In addition, higher EA was associated with increased body mass index (β=0.08, P=0.007) and low physical activity (β=-0.07, P=0.03). There was no relationship between EA and education (in years), cotinine levels, alcohol consumption or the number of chronic diseases under treatment (all *P*s>0.05).

DNA methylation prediction model trained in controls-only

We repeated the between-group comparison of EA that was based on the prediction model trained in controls-only, rather than the full sample. This "controls-only model" included 10,000 CpGs, compared to the 80,000 CpG sites used in the "full sample model". 10-fold cross-validation showed that chronological age could be predicted less precise, with a less slightly reliable correlation of 0.93 (P<0.001).

EA (from "controls only model") showed a normal distribution with, by design, a mean of zero (s.d.=3.92 years), ranging from -13.21 to 15.17. Depressed patients had significantly higher EA compared to controls (mean EA ± s.e. (CI) MDD: 0.21 ± 0.14 (-0.07, 0.48), controls: -0.52 ± 0.22 (-0.96, -0.08); $F_{(1,1121)}$=7.46, P=0.006, effect size (Cohen's d)=0.18) after full adjustment for covariates. Consistent with a dose-response association, a fully-adjusted linear regression showed that higher EA was significantly associated with higher IDS-score in the overall sample (β=0.10, P=0.002). Thus, although the "controls only model" resulted in a less accurate prediction of chronological age, likely due to the fact that it is trained in only 1/3 of the sample, results remained unchanged.

SUPPLEMENTARY TABLE S3. The relationship between all selected covariates and epigenetic aging

	β	*P-value*
Sex (M/F)	-0.11	<0.001
Education (in years)	0.01	0.72
BMI	0.08	0.007
Cotinine levels (ng/ml)	0.04	0.15
Alcohol consumption (mean number of drinks/week)	-0.03	0.30
Physical activity (MET-minutes/week)	-0.07	0.03
Number of chronic diseases	0.001	0.98

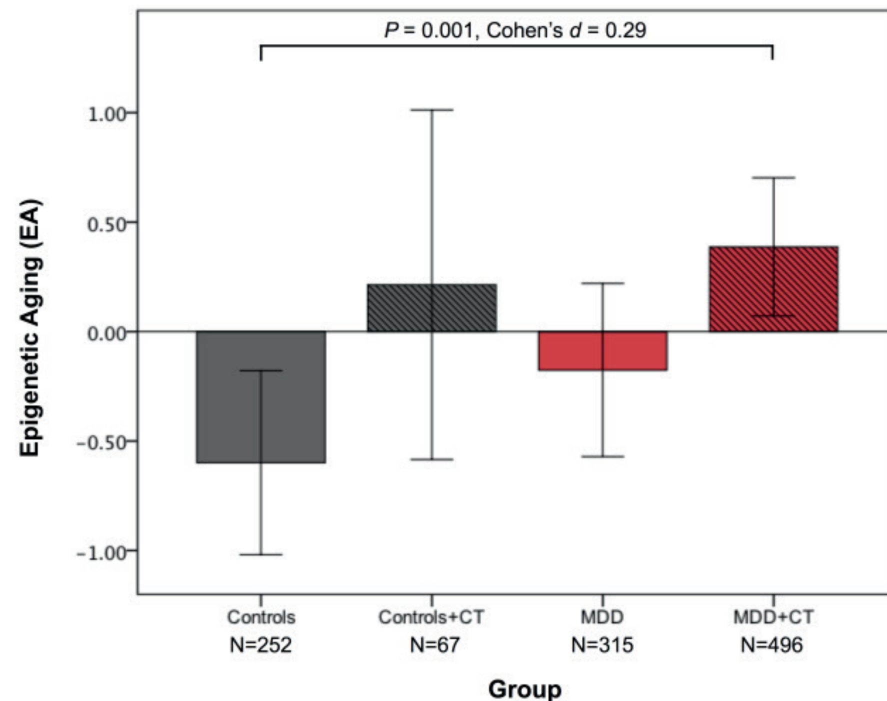
Abbreviations: epigenetic aging, unstandardized residuals of DNAmAge regressed on chronological age; M, Male; F, female; BMI, Body Mass Index; MET, Metabolic Equivalent Total (MET level * minutes of activity * events per week).

Cell type proportion correction

To examine whether the between-group difference in EA was not confounded by differences in blood cell composition, we performed additional analyses including cell type proportions (CD03, CD14, CD15) as covariates. Given that these cell type proportion estimates were highly dependent on some lab technical covariates, we also included those variables as covariates in the model. The analysis of covariance model showed that the between-group difference in EA remained significant ($F_{(1,1102)}$=3.92, P=0.048, effect size (Cohen's d)=0.14). Overall, these results indicate that MDD disease status explained the difference in EA independent from blood cell composition.

Accelerated aging and childhood trauma

To gain further insight into the association of EA with childhood trauma, we conducted a one-way analysis of variance of fully-adjusted EA against groups of controls and MDD patients with and without childhood trauma (CT, yes≥1 and no=0) Bonferroni-corrected for multiple testing. This revealed that MDD patients with CT showed the highest EA compared to controls without CT (*P*=0.001, Cohen's *d*=0.29), highlighting that this MDD+CT subgroup is associated with the highest EA (**Supplementary Figure S1**).



Supplementary Figure S1. Epigenetic aging (EA) in major depressive disorder (MDD) and childhood trauma (CT). Mean EA by control and MDD group, with and without presence of CT. The means are adjusted for sex, education, body mass index, smoking, drinking, physical activity, and somatic diseases. The error bars represent 95% confidence intervals.

Post-hoc analyses with telomere length

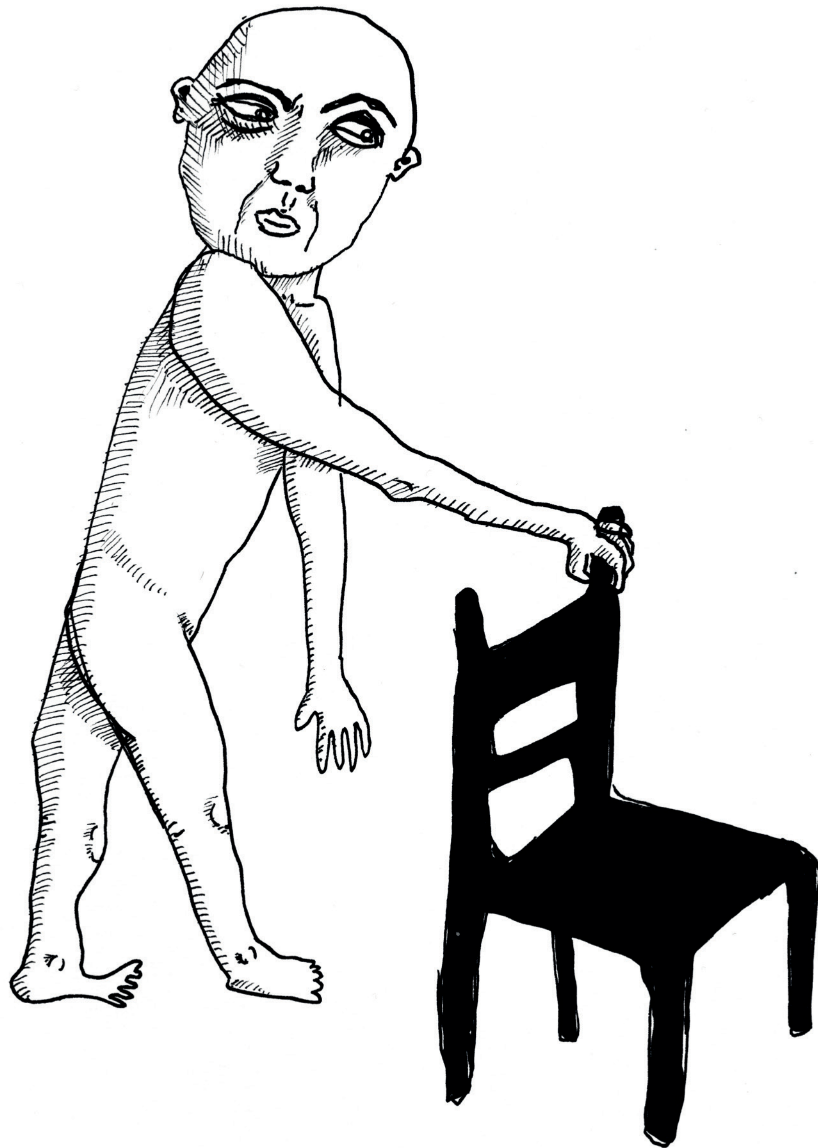
To investigate the relationship of our main outcome measure with another biological aging marker available in our dataset (for detailed methods and telomere assays, see [22]diabetes, obesity and cancer. This suggests mechanisms of accelerated biological aging among the depressed, which can be indicated by a shorter length of telomeres. We examine whether MDD is associated with accelerated biological aging, and whether depression characteristics such as severity, duration, and psychoactive medication do further impact on biological aging. Data are from the Netherlands Study of Depression and Anxiety, including 1095 current MDD patients, 802 remitted MDD patients and 510 control subjects. Telomere length (TL), we performed post-hoc age-corrected partial and non-adjusted bivariate correlations between telomere length (TL) and EA. The results showed non-significant relationships of r=-0.03, P=0.34 and r=-0.03, P=0.37, respectively. Thus, EA and TL were not significantly correlated in our study.

Additional post-hoc analyses showed that TL did not change any of the study's findings when added as a covariate to the analysis of covariance model. Significantly higher EA was still uniquely observed in the MDD group (mean EA ± s.e. MDD: 0.18 ± 0.13, controls: -0.46 ± 0.20; $F_{(1,1120)}$=7.00, P=0.008). There were also significant main effects of sex, BMI, and physical activity (all Ps<0.05). However, there was no effect of TL on EA ($F_{(1,1120)}$=0.07, P=0.79).

To further investigate whether TL was associated with sociodemographic and lifestyle parameters, we performed a similar multiple linear regression model as performed with EA. As was expected from a previous study[22] diabetes, obesity and cancer. This suggests mechanisms of accelerated biological aging among the depressed, which can be indicated by a shorter length of telomeres. We examine whether MDD is associated with accelerated biological aging, and whether depression characteristics such as severity, duration, and psychoactive medication do further impact on biological aging. Data are from the Netherlands Study of Depression and Anxiety, including 1095 current MDD patients, 802 remitted MDD patients and 510 control subjects. Telomere length (TL, shorter TL was significantly associated to male sex (β=0.08, P=0.009), BMI (β=-0.11, P=0.001), and increased alcohol intake (β=-0.08, P=0.01). TL was not associated to education, cotinine levels, physical activity or number of chronic diseases under treatment (all Ps>0.05).

REFERENCES

1. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol. 2009;169:236–248.
2. Hovens JGFM, Wiersma JE, Giltay EJ, Van Oppen P, Spinhoven P, Penninx BWJH, et al. Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. Acta Psychiatr Scand. 2010;122:66–74.
3. Aberg KA, Mcclay JL, Nerella S, Xie LY, Clark SL, Hudson AD, et al. MBD-seq as a cost-effective approach for methylome-wide assocciation studies: demonstration in 1500 case-control samples. Epigenomics. 2012;4:605–621.
4. Houseman E, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics. 2012;13:86.
5. Koestler DC, Christensen BC, Karagas MR, Marsit CJ, Langevin SM, Kelsey KT, et al. Blood-based profiles of DNA methylation predict the underlying distribution of cell types: A validation analysis. Epigenetics. 2013;8:816–826.
6. Hattab MW, Shabalin AA, Clark SL, Zhao M, Kumar G, Chan RF, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. Genome Biol. 2017;18.
7. Deep-Soboslay A, Iglesias B, Hyde TM, Bigelow LB, Imamovic V, Herman MM, et al. Evaluation of tissue collection for postmortem studies of bipolar disorder. Bipolar Disord. 2008;10:822–828.
8. Conner KR, Conwell Y, Duberstein PR. The validity of proxy-based data in suicide research: a study of patients 50 years of age and older who attempted suicide. II. Life events, social support and suicidal behavior. Acta Psychiatr Scand. 2001;104:452–457.
9. Kelly TM, Mann JJ. Validity of DSM-III-R diagnosis by psychological autopsy: a comparison with clinician ante-mortem diagnosis. Acta Psychiatr Scand. 1996;94:337–343.
10. Dumais A, Lesage AD, Alda M, Rouleau G, Dumont M, Chawky N, et al. Risk factors for suicide completion in major depression: A case-control study of impulsive and aggressive behaviors in men. Am J Psychiatry. 2005;162:2116–2124.
11. Gibbons AS, Brooks L, Scarr E, Dean B. AMPA receptor expression is increased post-mortem samples of the anterior cingulate from subjects with major depressive disorder. J Affect Disord. 2012;136:1232–1237.
12. Keks N et al. Diagnostic instrument for brain studies (DIBS). 1997;24.
13. Deep-Soboslay A, Benes FM, Haroutunian V, Ellis JK, Kleinman JE, Hyde TM. Psychiatric brain banking: Three perspectives on current trends and future directions. Biol Psychiatry. 2011;69:104–112.
14. Alt SR, Turner JD, Klok MD, Meijer OC, Lakke EAJF, DeRijk RH, et al. Differential expression of glucocorticoid receptor transcripts in major depressive disorder is not epigenetically programmed. Psychoneuroendocrinology. 2010;35:544–556.
15. Torrey EF, Webster M, Knable M, Johnston N, Yolken RH. The Stanley Foundation brain collection and Neuropathology Consortium. Schizophr Res. 2000;44:151–155.
16. Van Den Oord EJCG, Clark SL, Xie LY, Shabalin AA, Dozmorov MG, Kumar G, et al. A whole methylome CpG-SNP association study of psychosis in blood and brain tissue. Schizophr Bull. 2016;42:1018–1026.
17. Liu Y, Popp B, Schmidt B. CUSHAW3: Sensitive and accurate base-space and color-space short-read alignment with hybrid seeding. PLoS One. 2014;9.
18. Cabrera CP, Navarro P, Huffman JE, Wright AF, Hayward C, Campbell H, et al. Uncovering Networks from Genome-Wide Association Studies via Circular Genomic Permutation. G3: Genes|Genomes|Genetics. 2012;2:1067–1075.
19. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol Cell. 2013;49:359–367.
20. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
21. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16:25.
22. Verhoeven JE, Révész D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. MolPsychiatry. 2014;19:895–901.



CHAPTER 4

Do Psychiatric Problems, Lifestyle Variables and Adversities Accelerate Epigenetic Aging?

Submitted for publication

Laura K.M. Han
Moji Aghajani
Brenda W.J.H. Penninx
William E. Copeland
Karolina A. Aberg
Edwin J.C.G. van den Oord

ABSTRACT

Cross-sectional studies have identified a variety of correlates of epigenetic aging. However, it is unclear whether these correlates make epigenetic clocks "tick faster" (i.e., accelerate biological aging). In this longitudinal study, we examined whether a change in psychiatric problems, lifestyle variables or adversities at a particular time point (wave) was accompanied by a change in epigenetic aging. Such covariance is a necessary, but not sufficient, condition for a causal link between correlates and epigenetic aging. Our data consisted of 539 individuals (9-35 years old) and 1,029 measurements. Results showed that changes in correlates at a wave were often unaccompanied by a change in epigenetic aging at that wave. We therefore caution against interpreting associations with epigenetic aging in cross-sectional studies as being causal effects of the correlate.

INTRODUCTION

Recent years have shown a rapid increase in the number of articles examining the so-called "biological age". Biological age is different from chronological age because it reflects the individual's biological state, rather than the years that have passed since birth. The most promising and accurate indicators of biological age are based on DNA methylation levels[1]. These so-called "epigenetic clocks" track the aging process with correlations between DNA methylation predicted age (**DNAm age**) and chronological age of typically over 0.90 [2–4]. Importantly, by contrasting the DNAm age to chronological age, it can be studied whether individuals are biologically younger or older than expected on the basis of chronological age. From here on we refer to this difference as "**epigenetic aging**".

Studies have shown associations between epigenetic aging and age-related morbidities and, most importantly, mortality [5, 6]. Correlations of epigenetic aging have also been found with traumatic stress including childhood trauma [7], depression [8, 9], bipolar disorder [10], alcohol use disorder, metabolic syndrome components [11, 12], body mass index (BMI), and psychosocial factors [13]. However, as the vast majority of studies are cross sectional, it remains to be elucidated if these **epigenetic correlates** make epigenetic clocks "tick faster" (i.e., accelerate biological aging). Alternatively, the direction of effects may be the other way around or associations may be caused by "third" variables that affect both the correlate and epigenetic aging. For example, individuals who are more prone to health problems may also be predisposed to age faster biologically due to genetic or environmental factors.

Longitudinal studies have the potential to shed light on the causal status of epigenetic correlates. However, existing longitudinal studies have mainly focused on epigenetic aging trajectories over time rather than studying causality [14, 15]. Studying potential causal contributors to epigenetic aging is critical and may ultimately inform prevention and treatment regimens, especially for younger populations when age-related comorbid conditions have not manifested yet [16].

The current study thus examines whether cross-sectionally identified correlates such as psychiatric problems, lifestyle variables, and adversities have the potential to play a causative role in epigenetic aging. This may provide valuable insight as to whether epigenetic aging drives the identified correlates, or whether epigenetic aging is a consequence of these correlates [17]. In this article we address this knowledge gap by examining DNAm age estimated from blood in individuals aged 9 to 35 years old. Importantly, we collected longitudinal information for both DNAm age and a variety of

variables previously identified as cross-sectional correlates of epigenetic aging [18]. We tested whether a change in these correlates at a particular wave was accompanied by a change in epigenetic aging. Such covariance is a necessary, but not sufficient, condition for causality.

METHODS

The Great Smoky Mountains Study

The Great Smoky Mountain Study (GSMS) is a longitudinal study of 1,420 participants from the southeast United States [19]. Of these individuals, 539 are included in the current study. GSMS started in 1993 when participants were children aged 9 to 13. Clinical data and blood spots were collected annually until age 16, and then again around ages 19, 21, 25 and 30. Subset sampling of the current study included enrichment for childhood trauma exposure. Although conducted separately, interviews were completed by both a parent figure and the participant until the age of 16. After 16 years, interviews were conducted with the participant only. Both parents and participants signed informed consent or assent forms. The study was approved by Institutional Review Boards at Duke University and Virginia Commonwealth University.

Measures

We examined linked patterns between the epigenetic aging metric and a selection of correlates. Some correlate assessments temporally coincided with the methylation assessments, while others showed variable time lags. Time lags are explicitly reported in the specific sections of the investigated correlates.

Physical development

Data on height (cm) and weight (kg) were measured during interviews. BMI was assessed as kg/m^2, where kg was a person's weight in kilograms and m^2 their height in meters squared. There was no time lag between these and the methylation assessments.

Psychiatric problems and trauma

Depression and anxiety symptoms were assessed as follows: before age 16, both the child and parent completed a structured clinical interview using the Child and Adolescent Psychiatric Assessment (CAPA)[20]. After age 16 the Young Adult Psychiatric Assessment (YAPA)[21], the upward extension of the CAPA, was used. Depression and anxiety symptoms were reported with a 3-month recency, introducing a time lag ranging anywhere between 0 to 3 months compared to the methylation assessment. Cumulative childhood trauma exposure was assessed by taking the sum of events concerning exposure to violence, sexual trauma, and other injury or trauma. Impairments were assessed as a cumulative score of the total number of functional impairments measured by summarizing dichotomous indicators across 17 areas of disrupted functioning in areas such as relationship with parents, teachers, peers, ability to complete chores at home, and disrupted schoolwork [22]. As the cumulative scores reflect sum scores of all previous wave assessments, potential temporal differences between the cumulative score and corresponding methylation assessments were limited.

Social environment

Being impoverished was positive if one met the poverty guidelines updated periodically in the Federal Register by the U.S. Department of Health and Human Services under the authority of 42 U.S.C. 9902(2).

Substance use

Smoking tobacco, drinking alcohol, and cannabis use was also assessed. Either of the substance use categories were positive if reported through self- (adult) or parent (childhood) reports. Smoking was coded as one if the participant regularly smoked in the past three months. Alcohol and cannabis were coded as one when daily/weekly use or a use disorder for the substance was reported in the past three months. The potential temporal time lag compared to the methylation assessment was thus between 0-3 months.

Covariates

Information on age, sex, Tanner pubertal stage, and race/ethnicity was collected during interviews. Children completed a self-report measure of Tanner staging [23]. Adult observations were coded as Tanner stage 5.

DNA Methylation

Nearly all 28 million CpG sites in the methylome were assayed with an optimized protocol [8, 24] for methyl-CG binding domain sequencing (MBD-seq). Elsewhere we summarized key features of optimized MBD-seq using empirical data[25]. We quality controlled reads, samples, and methylation sites. Data was processed and analyzed using the RaMWAS Bioconductor package [26]. The

distribution of blood cell types for CD3 (T-lymphocytes), CD14 (monocytes), CD15 (granulocytes), and CD19 (B-lymphocytes) were estimated from the methylation data [27] using reference methylomes specifically generated for this purpose [28]. For more details on the methylation assay, see **Supplement**.

DNA methylation age

Following standard methods[8], we used elastic nets to predict chronological age (in years) from all blood methylation with parameter alpha set to zero (i.e. ridge regression)[29]. To estimate predictive power and to obtain unbiased estimates for each subject, k-fold cross-validation was used, with k=10. Importantly, all data points from the same subject were either included in the training or test dataset to ensure independence and thereby resulting in unbiased DNAm age estimates. Of the k subsets, k – 1 were used as a "training set" to fit the elastic net and obtain regression coefficients. The regression coefficients were then used to estimate chronological age for participants in the "test set". By repeating this cycle of training and testing for each subset, age estimates are obtained for all participants. To evaluate model performance, we used the calculated: a) mean absolute error (MAE), b) Pearson correlation coefficient between predicted DNAm age and chronological age, c) variance explained by the model (R2 "traditional formula" function implemented in the caret package accounting for systematic over- and underestimations)[30]. To avoid analyzing all methylation sites, of which the majority will not be associated with outcome and only add "noise" to the model, we increased the number of sites included in the elastic net in steps (100, 1000, 2500, 5000, 10,000, 25,000, 50,000, 75,000) until the explained variance of age did not improve anymore (Supplementary Figure S1). We previously performed tests where the number of sites/genes was included in the loop over the k-folds. However, as it produced very similar results but is much more computer intensive [31], this latter approach was not used. Of note, we also tested whether the previously built model in the Netherlands Study of Depression and Anxiety (NESDA) would generalize to the current dataset, even though the sample properties (e.g. adult age range of 18-64 years) were different, but this resulted in poorer prediction accuracy (results are provided in the Supplement).

Statistical Approach

All statistical analyses were performed using R version 3.5.3 (R Core Team, 2019), the *nlme* package was used to specify the bivariate mixed models. Our data further consists of longitudinal information for both epigenetic aging

and correlates. This allowed us to test whether a change in correlate at a particular wave was accompanied by a change in epigenetic aging at that wave. Such a covariance is a necessary, but not sufficient, condition for a causal link between correlates and epigenetic aging. Technically these tests are conducted by fitting a bivariate mixed model (see Chapter 14 in [32]) that decomposes the covariance between epigenetic aging and the correlate into a **subject-level contribution** and a **wave-level contribution.** Intuitively speaking, the **subject-level contribution** captures the correlation between the mean subject specific epigenetic aging across all waves and the mean correlate values across all waves. The **wave-level contribution** captures the correlation between epigenetic aging and correlate values at each wave after considering the subject specific epigenetic aging and correlate means across all waves. For modeling details, we refer to the **Supplement.**

To assess whether changes in a specific correlate were correlated with changes in epigenetic aging, we fitted eleven separate bivariate mixed models, one for each correlate. To assess significance and obtain P-values for the subject- and wave-level contributions, we ran 10,000 iterations of the model estimation procedure described above but with the order of responses (epigenetic aging and correlate) randomly permuted in each iteration, while respecting the subject-level dependent structure (i.e. multiple responses from the same subject were randomly sampled within random subjects with the same number of responses). To assess the robustness of effects, we also averaged the eleven observed (i.e., all subject- and wave level contributions) and permutation results (10,000 random iterations) from the above bivariate mixed models to calculate whether an overall change in correlates was correlated with an overall change in epigenetic aging (i.e., the overall test).

Continuous variables with values >3×SD away from the mean were winsorized. All models were corrected for linear and quadratic age terms, sex, Tanner pubertal stage, race/ethnicity, estimated cell counts, and lab technical covariates. As we were interested in contributing factors to accelerated aging, analyses were tested one-sided and considered significant at $p<0.05$. Model specifications and R code for analysis can be found on GitHub**.**

RESULTS

Participant Characteristics

Demographics and assessed phenotypes of the current study sample can be found in **Table 1**. Briefly, participants had between 1 and 3 DNAm age measurements, resulting in N=1,029 measurements from N=539 participants (mean number of measurements per individual was 1.9). Out of the total number of measurements, collected from 18 different timepoints (waves), 5.9% were conducted at childhood (<12 years), 52.1% at adolescence (12-18 years) and 42.0% in adulthood (>18 years). **Figure 1** shows the chronological age distributions of individuals with 1 (N=539, mean=17.54 years, SD=5.20 years, range=9.47-31.66 years), 2 (N=296, mean=18.78 years, SD=5.71 years, range=9.07-33.31 years), or 3 measurements (N=97, mean=17.15 years, SD=6.92, range=9.01-34.55 years). The mean follow-up time for those with two measurements was 9.84 years (SD=4.00 years). For those with three measurements the mean follow-up time was 1.98 years (SD=1.69 years) between the first and second, 12.22 years (SD=4.77 years) between the second and the third, and 14.20 years (SD=4.45 years) between the first and the third measurement.

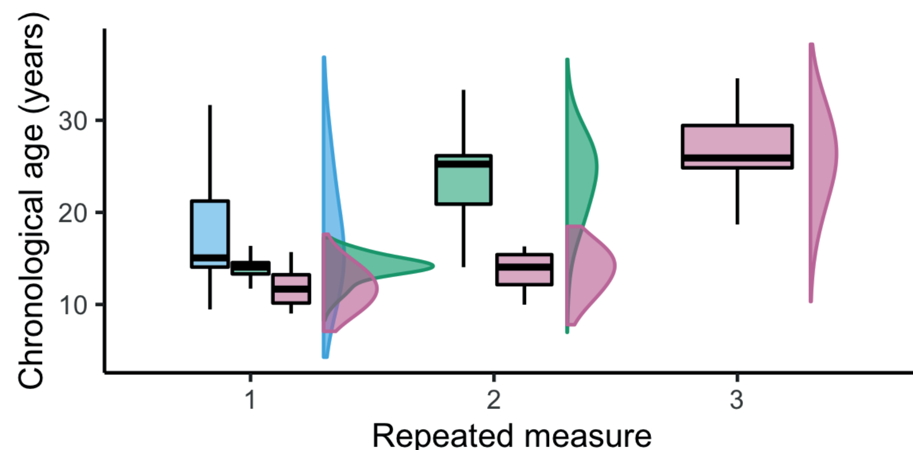


Figure 1. Chronological age distributions. The plot shows individuals with one (blue, N=539), two (green, N=296), or three (pink, N=97) temporally coinciding epigenetic aging and correlate measurements.

Table 1. Participant Characteristics, Number of individuals and Measurements.

Characteristic	N Individuals	N Measurements	Mean ± SD (range)/N (%)
Demographics			
Chronological age (years)	539	1,029	18.14 ± 6.05 (9.02-34.55)
Sex (female)	539	1,029	521 (51%)
Physical development			
Weight (kg)	537	971	69.26 ± 24.23 (20.00-144.89)
Height (cm)	538	979	163.79 ± 12.86 (125.21-202.40)
Body Mass Index (kg/m²)	537	968	25.31 ± 6.95 (12.73-47.25)
Psychiatric problems & Trauma			
Depression	539	1,029	0.85 ± 1.11 (0.00-5.00)
Anxiety	539	1,029	0.95 ± 1.69 (0.00-7.00)
Childhood trauma	539	1,029	1.03 ± 1.07 (0.00-4.00)
Impairments	539	1,029	1.00 ± 2.22 (0.00-10.00)
Social environment			
Poverty (yes)	518	959	279 (29%)
Substance use			
Smoking (yes)	539	1,029	361 (35%)
Cannabis (yes)	539	1,029	180 (17%)
Alcohol (yes)	539	1,029	188 (18%)

Statistic presented: mean across measurements ± SD (minimum-maximum); n (%).

Estimating DNA methylation age

DNAm age was estimated using elastic nets. Of note, all data points from the same subject were either included in the training *or* test dataset to ensure independence and thereby resulting in unbiased DNAm age estimates. **Supplementary Figure S1** illustrates the explained variance of chronological age by the elastic net as a function of the number of methylation sites included as predictors. We used the methylation prediction model that was based on 25,000 sites as the model fit did not further improve by adding more methylation sites. Moreover, the model showed that, as we found before using similar sequencing data[8], chronological age could be accurately predicted with a correlation of r=0.93, R^2=0.85, mean absolute error of 1.85 years (**Figure 2**).

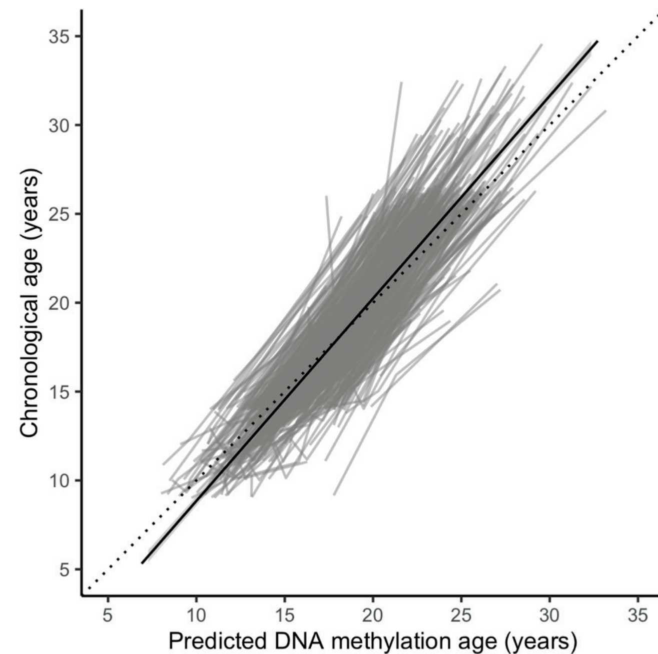


Figure 2. DNA methylation age. Correlation between predicted DNA methylation age and chronological age (r=0.93, P<0.0001). Diagonal dashed line reflects the line of identity (x=y). Each gray line represents one individual.

Bivariate mixed model

To examine whether changes in correlates at a particular wave were accompanied by a change in epigenetic aging, bivariate mixed models were used [32]. Bivariate mixed models optimally accommodate the current data as it allows the use of all data points (i.e., 1, 2, or 3 assessments) in a single analysis in a statistically optimal way. Furthermore, it can decompose the covariance between epigenetic aging and the correlate into a **subject-level contribution** and a **wave-level contribution.** Intuitively speaking, the **subject-level contribution** captures the correlation between the mean subject-specific epigenetic aging across all waves and the mean correlate values across all waves. The **wave-level contribution** captures the correlation between epigenetic aging and correlate values at each wave after taking into account the subject-specific epigenetic aging and correlate means across all waves. In other words, if the wave-level contribution is not significantly different from zero, a change in the correlate is not associated with a change in epigenetic aging.

Proportions of subject- and wave-level variance

Table 2 shows the proportion of subject-level (**columns 1-2**) and wave-level variance (**columns 3-4**). The proportion of subject-level variance (also known as the intra-class correlation) of epigenetic aging and each correlate indicates the stability over time. Correlates of physical development show high levels of stability (i.e., subject-level variance >50%), presumably partly due to underlying genetic control [33], while psychiatric correlates show more change than stability over time (i.e., subject-level variance of ~23%). The latter also holds true for the lifestyle variables (i.e., subject-level variances ranging from 2% to 32%), presumably due to different ages of initiation of substance use with general absence in childhood and peaks in adolescence and young adulthood. With respect to epigenetic aging and cumulative childhood trauma, approximately half of the total variance can be attributed to differences between subjects, while the other half can be explained by changes between waves. In other words, epigenetic aging and exposure to childhood trauma are relatively stable but also show dynamic potential. Taken together, these results show that all study variables show both stability and change over time.

Decomposition of covariances into subject- and wave-level contributions

The observed covariances were decomposed into subject- and wave-level contributions, and estimates were standardized by the total variance to obtain contributions to the correlations. The subject- (**columns 5-6**) and wave-level contributions (**columns 7-8**) between epigenetic aging and correlates are presented in **Table 2**. When looking into specific correlates, we found significant subject-level contributions for weight (r=0.14, P<0.01), BMI (r=0.15, P<0.001), and cumulative childhood trauma exposure (r=0.06, P=0.04). Higher weight, BMI, and childhood trauma exposure were correlated to higher epigenetic aging. Cannabis use (r=0.04, P=0.07) and smoking tobacco (r=0.04, P=0.08) were trending towards significance at the subject-level.

Several significant wave-level contributions were observed. That is, wave-level contributions of epigenetic aging and height (r=0.02, P=0.01), anxiety symptoms (r=0.02, P=0.04), and cannabis use (r=0.03, P=0.02) were significant, and cumulative childhood trauma exposure (r=0.02, P=0.051) and alcohol use (r=0.02, P=0.06) were trending towards significance. Taller height, more anxiety symptoms, and cannabis use were thus correlated to higher epigenetic aging on the wave-level.

The overall test showed a higher mean subject-level contribution of r=0.05 (P=0.07, range=0.02-0.15, SD=0.05) compared to the mean wave-level contribution of r=0.01 (P=0.18, range=0.00-0.03, SD=0.01). When only considering correlates significant at either the subject- or wave-level, the overall test showed a mean subject-level contribution of r=0.07 (P=0.01) compared to the mean wave-level contribution r=0.02 (P=0.09).This indicates that, on average, there is only weak evidence for significant wave-level contributions.

Higher correlations at the subject-level remained non-significant (e.g. cannabis use, r=0.04, P=0.07), while lower correlations at the wave-level were significant (e.g. cannabis use, r=0.03, P=0.02). Thus, our study design has better power to detect wave-level contributions. The longitudinal within-person design increased the reliability and precision of measuring changes over time and generally added statistical power to detect small effects

Table 2. Decomposition of covariance between epigenetic aging and correlate into subject- and wave-level contributions.

	Proportion subject-level variance		Proportion wave-level variance		Subject-level contribution		Wave-level contribution	
	Epigenetic aging	Correlate	Epigenetic aging	Correlate	r	P	r	P
Weight	0.46	0.78	0.54	0.22	0.14	**<0.001**	0.01	0.27
Height	0.46	0.50	0.54	0.50	0.03	0.25	0.02	**0.01**
BMI	0.45	0.80	0.55	0.20	0.15	**<0.001**	0.00	0.43
Depression	0.44	0.24	0.56	0.76	-0.01	0.70	0.00	0.63
Anxiety	0.44	0.23	0.56	0.77	0.04	0.12	0.02	**0.04**
Childhood trauma	0.44	0.50	0.56	0.50	0.06	**0.04**	0.02	0.05
Impairments	0.44	0.16	0.56	0.84	0.02	0.27	-0.01	0.86
Poverty	0.44	0.23	0.56	0.77	0.04	0.13	0.00	0.49
Smoking	0.44	0.32	0.56	0.68	0.04	0.08	0.01	0.13
Cannabis	0.44	0.09	0.56	0.91	0.04	0.07	0.03	**0.02**
Alcohol	0.44	0.02	0.56	0.98	0.02	0.27	0.02	0.06

Abbreviations: BMI, Body Mass Index. All models were corrected for linear and quadratic age terms, sex, Tanner pubertal stage, race/ethnicity, estimated cell counts, and lab technical covariates. Significant P-values <0.05 are indicated in bold.

DISCUSSION

In this study we used longitudinal data to examine whether there is a potential causal link between a wide range of correlates of epigenetic aging and epigenetic aging. We attempted to elucidate whether correlates make the epigenetic clock “tick faster” (i.e., accelerate), by decomposing the correlations between each correlate and epigenetic aging into a subject- and wave-level contribution. If the wave-level contribution is not significantly different from zero, changes in correlates do not parallel changes in epigenetic aging, and a causal relationship will be rather unlikely. In general, we found higher subject-level (mean r=0.05) compared to wave-level contributions (mean r=0.01), but also found that some epigenetic correlates trended towards significant contributions on both subject- and wave-levels (i.e., cumulative childhood trauma exposure, cannabis use). However, as many of the wave-level changes in correlates were unaccompanied by a change in epigenetic aging, caution is warranted in interpreting cross-sectional correlations with epigenetic aging as causal. It is important to bear in mind that the commonly used term “accelerated” epigenetic aging in cross-sectional studies may often be incorrect.

Alternative explanations exist for the limited support of potential causal effects. The model used in the current study correlated temporally coinciding measurements of epigenetic aging and correlates. However, it might be possible that changes in epigenetic correlates during childhood or adolescence may only be followed by changes in epigenetic aging at later time points. For example, the initial impact of trauma may magnify over time due to subsequent increases in health-risk behaviors such as smoking, substance use, and high-risk activities [34, 35] that then over time accelerate aging processes. If so, we may not have detected the wave-level correlation between epigenetic aging and corresponding correlate due to the time needed for acceleration to take place. Furthermore, the wave-level correlations are more affected by measurement error than the subject-level correlations, attenuating the correlations and resulting in more severe underestimates of the wave-level contributions.

The current study can be expanded and its results may be followed up in several ways. For example, although we included individuals with multiple assessments, only ~55% and ~18% of the current sample had 2 or 3 linked measurements, respectively. Ideally, to distinguish correlates from causes of epigenetic aging [36], individuals need to be tracked over longer periods of time with more frequent sampling of methylation and health measurements to estimate their covariance with more precision [16]. The sequencing-based

methylation data considered in the current study did not allow us to apply other established epigenetic clocks. More work is thus needed to determine the generalizability of findings using other epigenetic clocks. In addition, it will be useful to study the generalizability of findings to other age ranges (e.g., late adulthood) and populations (e.g., other ethnicities). The temporal complexities in the current study also highlight the need for further modelling work in larger datasets that allow more hypothesis-free data mining to examine alternative time lags (e.g., correlating epigenetic aging at a certain wave with correlates from preceding or later waves). Future studies including individuals with a clinical diagnosis, rather than symptom counts, are also needed to determine whether psychiatric disorders accelerate epigenetic aging in patient populations. Finally, a natural progression of this work is to investigate which factors might be driving the subject-level contributions to epigenetic aging correlations, for example, by examining time invariant variables (e.g., genetics).

In summary, cross-sectional studies have shown correlations between epigenetic aging and a wide variety of variables. Using a longitudinal design, we found weak evidence for potential causal links between psychiatric problems, lifestyle variables, and adversities and biological aging, as changes in epigenetic correlates at a particular wave were often unaccompanied by parallel changes in epigenetic aging. Such covariance is a necessary, but not sufficient, condition for causality. Thus, caution is needed against causal interpretations of cross-sectional correlates of epigenetic aging.

REFERENCES

1. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.
2. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. Mol Cell. 2013;49:359–367.
3. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
4. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging . 2018;10:573–591.
5. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16:25.
6. Chen BH, Marioni RE, Colicino E, Peters MJ, Ward-Caviness CK, Tsai P-C, et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging . 2016;8:1844–1865.
7. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A, et al. Traumatic stress and accelerated DNA methylation age: A meta-analysis. Psychoneuroendocrinology. 2018;92:123–134.
8. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic Aging in Major Depressive Disorder. Am J Psychiatry. 2018;175:774–782.
9. Whalley HC, Gibson J, Marioni R, Walker RM, Clarke T-K, Howard DM, et al. Accelerated epigenetic ageing in depression. 2017. 2017.
10. Fries GR, Bauer IE, Scaini G, Wu MJ, Kazimi IF, Valvassori SS, et al. Accelerated epigenetic aging and mitochondrial DNA copy number in bipolar disorder. Transl Psychiatry. 2017;7.
11. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging . 2017;9:419–446.
12. Luo A, Jung J, Longley M, Rosoff DB, Charlet K, Muench C, et al. Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2. Neuropsychopharmacology. 2020;45:327–336.
13. Simons RL, Lei MK, Beach SRH, Philibert RA, Cutrona CE, Gibbons FX, et al. Economic hardship and biological weathering: The epigenetics of aging in a U.S. sample of black women. Social Science and Medicine. 2016;150:192–200.
14. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. Elife. 2020;9.
15. Marioni RE, Suderman M, Chen BH, Horvath S, Bandinelli S, Morris T, et al. Tracking the Epigenetic Clock Across the Human Life Course: A Meta-analysis of Longitudinal Cohort Data. J Gerontol A Biol Sci Med Sci. 2019;74:57–61.
16. Moffitt TE, Belsky DW, Danese A, Poulton R, Caspi A. The Longitudinal Study of Aging in Human Young Adults: Knowledge Gaps and Research Agenda. J Gerontol A Biol Sci Med Sci. 2017;72:210–215.
17. Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME, Ryan J. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Clin Epigenetics. 2019;11:62.
18. Ryan J, Wrigglesworth J, Loong J, Fransquet PD, Woods RL. A Systematic Review and Meta-analysis of Environmental, Lifestyle, and Health Factors Associated With DNA Methylation Age. J Gerontol A Biol Sci Med Sci. 2020;75:481–494.

19. Costello EJ, Angold A, Burns BJ, Stangl DK, Tweed DL, Erkanli A, et al. The Great Smoky Mountains Study of Youth. Goals, design, methods, and the prevalence of DSM-III-R disorders. Arch Gen Psychiatry. 1996;53:1129–1136.
20. Angold A, Jane Costello E. The Child and Adolescent Psychiatric Assessment (CAPA). Journal of the American Academy of Child & Adolescent Psychiatry. 2000;39:39–48.
21. Angold A, Cox A, Prendergast M, Rutter M, Simonoff E, Costello EJ, et al. The Young Adult Psychiatric Assessment (YAPA). Durham, NC: Duke University Medical Center. 1999. 1999.
22. Canino G, Costello EJ, Angold A. Assessing Functional Impairment and Social Adaptation for Child Mental Health Services Research: A Review of Measures. Ment Health Serv Res. 1999;1:93–108.
23. Dorn LD, Susman EJ, Nottelmann ED, Inoff-Germain G, Chrousos GP. Perceptions of puberty: Adolescent, parent, and health care personnel. Dev Psychol. 1990;26:322.
24. Aberg KA, Dean B, Shabalin AA, Chan RF, Han LKM, Zhao M, et al. Methylome-wide association findings for major depressive disorder overlap in blood and brain and replicate in independent brain samples. Mol Psychiatry. 2018. September 21, 2018. https://doi.org/10.1038/s41380-018-0247-6.
25. Aberg KA, Chan RF, van den Oord EJCG. MBD-seq - realities of a misunderstood method for high-quality methylome-wide association studies. Epigenetics. 2020;15:431–438.
26. Shabalin A, Clark S, Hattab M, Aberg K, van den Oord E. ramwas: Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms. R package version 1.0.0, https://bioconductor.org/packages/ramwas/. 2017.
27. Houseman E, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics. 2012;13:86.
28. Hattab MW, Shabalin AA, Clark SL, Zhao M, Kumar G, Chan RF, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. Genome Biol. 2017;18.
29. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw. 2010;33.
30. Kuhn M. The caret package. R Foundation for Statistical Computing, Vienna, Austria URL Https://Cranr-Project Org/Package= Caret. 2012. 2012.
31. Clark SL, Hattab MW, Chan RF, Shabalin AA, Han LKM, Zhao M, et al. A methylation study of long-term depression risk. Mol Psychiatry. 2019;25:1334–1343.
32. Rasbash J, Browne W, Steele F. A User's guide to MLwiN, Version 2.0. Institute of Education, University of London; 2003.
33. Dubois L, Ohm Kyvik K, Girard M, Tatone-Tokuda F, Pérusse D, Hjelmborg J, et al. Genetic and environmental contributions to weight, height, and BMI from birth to 19 years of age: an international study of over 12,000 twin pairs. PLoS One. 2012;7:e30153.
34. Hughes K, Bellis MA, Hardcastle KA, Sethi D, Butchart A, Mikton C, et al. The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. Lancet Public Health. 2017;2:e356–e366.

35. Bellis MA, Hughes K, Ford K, Ramos Rodriguez G, Sethi D, Passmore J. Life course health consequences and associated annual costs of adverse childhood experiences across Europe and North America: a systematic review and meta-analysis. The Lancet Public Health. 2019;4:e517–e528.
36. Nelson PG, Promislow DEL, Masel J. Biomarkers for Aging Identified in Cross-sectional Studies Tend to Be Non-causative. J Gerontol A Biol Sci Med Sci. 2020;75:466–472.

SUPPLEMENTARY MATERIALS

Do psychiatric problems, lifestyle variables and adversities accelerate epigenetic aging?

SUPPLEMENTARY METHODS

Quality control of MBD-seq data in GSMS

DNA extraction and QC

DNA extraction was extracted from dry blood spots using the QIAamp DNA Mini Kit (Qiagen) and the concentration of double stranded DNA was assessed with the Qubit 2.0 Fluorometer (Invitrogen). Depending on the size of (the part of) the blood spot available the amount of extracted DNA spanned a broad range from ~10 ng to nearly 1 ug that was used for downstream methylomic analysis.

MBD-seq

We used components of the MethylMiner™ Kit (Invitrogen) to enrich for DNA fragments with methylated CpGs (mCpG) via affinity purification with the methyl-CG binding domain protein (MBD2). DNA was sonicated to 100-150 bp using a Covaris S2 ultra-sonicator. For each capture reaction, 15 µL (10 µL per µg DNA) of prepared MBD-beads (10 µg/uL beads; 350 ng/uL MBD-biotin protein) was incubated with 1.5 µg of fragmented DNA (200 µL final volume in 1x Bind-Wash Buffer) for 1 hours, at room temperature, on an orbital shaker at 650 rpm. Each capture reaction was washed three times with 1x Bind-Wash Buffer. The bound methylated fragments were recovered in three pooled elutions of 500 mM NaCl buffer (25% High Salt Elution Buffer, 75% Low Salt Elution Buffer v/v), and purified by ethanol precipitation. This optimal protocol was empirically determined, where we found that increased wash stringency, and elution with a low salt concentration improves the sensitivity/specificity of the assay for loci with modest numbers of CpG sites, giving better methylome-wide representation [1–3]. The MBD enriched fractions were used to generate indexed libraries with the TruSeq Nano DNA HT Library Prep Kit (Illumina). Libraries were size-selected using SPRI beads to obtain a mean insert size of 150 bp. The 75-bp single-end libraries were then pooled and sequenced on the NextSeq 500 using High-Output v2 chemistry (Illumina).

Quality control of methylation data and CpG score calculation

Reads were aligned (build hg19/GRCh37) with Bowtie2[4] using a seed-and-extend approach combined with local alignment while allowing for gaps. Specifically, we used a 20 bp seed with zero mismatches. Rather than considering the entire read, local alignment was used to improve sensitivity by finding the maximum similarity score between the reference sequence and a substring of the extension that may be "trimmed" at both ends. Gaps were allowed to account for small indels.

We performed thorough quality control of samples, reads, and CpGs [5] using the RaMWAS Bioconductor package [6], which is specifically designed for large-scale methylation studies. The complete set of methylation data included methylation profiles from 1,202 dry blood spots from 571 unique individuals. Of the generated methylation profiles, 10 were excluded due to failed libraries or sequencing (mainly poor library quality or low number of reads) and 41 profiles were excluded because of poor or failed enrichment (peak skewness, unexpected peak size and/or high background levels). After calling SNPs from the methylation sequencing reads using GATK [7], we tested for agreement of genotypes between different blood samples from the same subject [8]. We found 6 samples for which the genotype information did not match with the GWAS genotype information. This indicated that a sample swap, or sample contamination may have occurred. As it was impossible to determine whether the problem was caused by the GWAS or MWAS data, we conservatively excluded all six samples from further analysis. We used the R function 'pcout' in the 'mvoutliers' package (with the upper boundary for outlier detection set to 15, the scaling constant set to 0.5, and the boundary for final outliers set to 0.2) to identify multidimensional outliers using principal components of the methylation data as input. Fourteen samples were multidimensional outliers and omitted. Next, we compared methylation age, as estimated from the profiles [9], with reported chronological age. One sample swap was corrected. That is, for an individual the methylation age estimate indicated that the oldest and the youngest time points had been exchanged. The middle time point remained correct. Lab records further supported the correction of this swap. Furthermore, the reported sex in the phenotype files were checked for agreement with the overall amount of methylation detected on the sex chromosomes. No further sample swaps, or errors in the phenotype file were detected. However, for two samples, from a single individual, we observed a possible karyotypic abnormality of the sex chromosomes. Both samples from this individual were excluded from further analysis. Cell type proportion were estimated using a MBD specific reference panel [10] after cell sorting to obtain

DNA from the four common cell-types in blood[10]: T-cells (CD3+), monocytes (CD14+), granulocytes (CD15+), and B-cells (CD19+). Houseman method [11] was used to generate priors (the estimated means and twice the estimated standard deviations) to obtain final estimates by empirical Bayes using the R rstanarm package. We used the estimated cell-type proportions and cell-type means in the reference panel to predict methylation levels in whole blood. These predicted methylation levels were correlated. A total of 84 samples were excluded because of low correlations (r<0.32). This left 1,045 samples, of which 11 samples did not have any phenotypic data, resulting in 1,034 samples. For this project we further limited the selection to individuals who had a maximum of 3 DNA methylation assessments, as we could not run random permutations while preserving the subject-level dependence due to lack of other participants with >3 observations. This left a total of 1,029 samples from 539 participants.

The mean number of reads for samples used in this study was 59.7 million (SD=7.4 million) of which, on average, 99% aligned. Aligned reads were checked for excessive duplicate reads (>3 reads starting at the same location were reset to 1) and reads located in loci where alignment is challenging, determined by an in-silico experiment described elsewhere[5], were excluded. This left an average of 49.4 million (SD=7.4 million) reads per sample (=82.7% of all reads).

To identify CpGs, we combined reference genome sequence (hg19/GRCh37) with common SNPs calculated on the European super population from 1000 Genomes (Phase 3). To avoid including sites that are CpGs in only a very small proportion of subjects, we excluded CpGs created by SNPs with minor allele frequency <1%. This resulted in 27,916,990 CpGs. CpGs in loci prone to alignment errors, e.g. in repetitive regions, were eliminated prior to the analysis. To identify these CpGs, we used RaMWAS to perform the in-silico alignment experiment outlined elsewhere [5]. In this experiment, the vast majority of CpGs (89.3%) were located in regions that showed perfect alignment coverage and only 1.3% (365,223 CpGs) showed evidence of alignment problems defined as 15% or more reads from this locus not aligning properly. Finally, akin to filtering SNPs with low minor allele frequency, we excluded rarely methylated sites (average read coverage <0.3). This left 22,670,747 autosomal CpGs for MWAS, which corresponds to 81% of all common CpGs in the human genome.

Quantifying methylation

A natural way to quantify methylation for MBD-seq is to count the number of fragments covering a CpG site. However, with single-end libraries the fragment sizes are not observed. Counting the number of reads instead, seriously underestimates the amount of methylation as the sequenced fragment is

usually longer than the read. RaMWAS therefore first uses a non-parametric approach to estimate the fragment size distribution from the sequencing data using isolated CpGs [12]. The fragment size distribution is used to calculate the probability that a sequenced fragment will cover the CpG under consideration. For example, this probability is 1.0 for fragments with reads starting within one read-length of the CpG, but is ≤1.0 for fragments with reads starting more than one read-length away. The CpG score is then calculated by taking the sum of probabilities for all fragments aligning within proximity of the CpG.

Cell type measurements

To estimate cell type proportions, we used reference methylomes [11, 13]. Whole blood samples of six subjects were used to isolated cells with 5 clusters of differentiation (CD3, CD19, CD20, CD14, and CD15) that capture the most common cell types in blood (T-cells, B-cells, monocytes, and granulocytes). Cell populations were isolated by positive selection using EasySep™ kits (Stemcell technologies) that apply magnetic nanoparticles coated with antibodies against a particular surface antigen (CD molecules). All reference methylomes were generated using MBD-seq. In a previous paper we showed that the estimated cell type proportions effectively controlled for cell type heterogeneity in methylome-wide association studies[10].

Assessed measures

Tanner Pubertal Status

Self-ratings of pubertal status were made using Tanner stage pictorial assessments of breast and pubic hair development [14]. Such ratings show moderate correlations with physical examination based on Tanner stages [15]. With parental agreement, each child was provided with sex-appropriate schematic drawings and asked to rate her current status on each dimension. There was no evidence of differential associations of breast development and pubic hair with other pubertal measures (e.g., timing, sex steroid levels). The mean of the two ratings (breast development and pubic hair) was used as an overall index of morphological development (ranging from I-V for pre-pubertal to full maturity).

Cumulative childhood trauma exposure

Cumulative childhood trauma exposure was assessed by taking the sum of the following list of events in the following categories: 1) Violence (violent death of loved one/sibling or peer, war, terrorism, cause of death or severe harm, victim of physical violence, physical abuse by relative, captivity), 2) Sexual trauma (sexual abuse, rape, coercion), and 3) other injury or trauma (diagnosis of physical illness, serious accident).

Number of functional impairments

Psychosocial impairment secondary to psychiatric symptomatology was also rated according to a series of criteria specified in the CAPA glossary and the interview schedule [16]. Broadly, some decrement in actual function had to be described for a positive rating to be given [17] for a full description of definitions and concept of impairment implemented in the CAPA). A cumulative score of the total number of functional impairments was measured by summarizing dichotomous indicators across 17 areas of disrupted functioning in areas such as relationship with parents, teachers, peers, ability to complete chores at home, and disrupted schoolwork [18]. Impairments have previously been related to emotional and behavioral reported symptoms.

DNA methylation age estimation

To avoid analyzing all CpGs, of which the majority will not be associated with outcome and only add "noise" to the model, we increased the number of sites included in the elastic net in steps (100, 1000, 2500, 5000, 10,000, 25,000, 50,000, 75,000) until the explained variance of age did not improve anymore (**Supplementary Figure S1)**. We previously performed tests where the number of CpGs/genes was included in the loop over the k-folds. However, as it produced very similar results but is much more computer intensive[19], this latter approach was not used. Of note, we also tested whether the previously built model in the Netherlands Study of Depression and Anxiety (NESDA) would generalize to the current dataset, even though the sample properties (e.g. adult age range of 18-64 years) were different, but this resulted in poorer prediction accuracy (r=0.83, R^2=-0.80, MAE=6.61 years vs. r=0.93, R^2=0.85, MAE=1.85 years; Supplementary **Figure S2**).

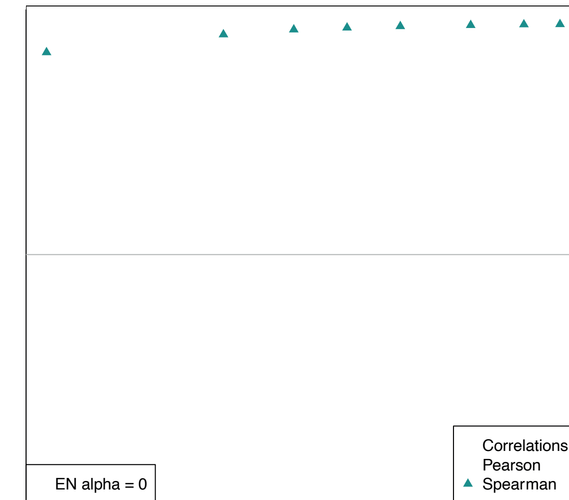


Figure S1. Stepwise selection of methylation sites to be included in DNA methylation age estimator. We increased the number of sites included in the elastic net in steps until the explained variance of chronological age did not improve anymore, resulting in 25,000 methylation sites included in the final prediction model (r=0.93 between chronological age vs. predicted DNA methylation age, R^2=0.85).

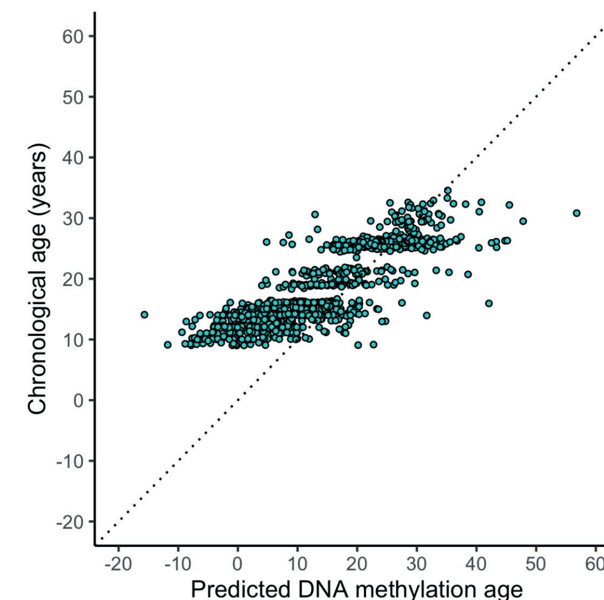


Figure S2. Generalization of the NESDA (18-64 years) DNAm age estimator to the GSMS (9-35 years) sample. Application of the NESDA model[9] to the current dataset led to underestimated predictions resulting in poorer prediction accuracy (r=0.83, R^2=-0.80, MAE=6.61).

Bivariate mixed model

We have longitudinal data for DNAm age and the correlates of interest. This translates to a 3-level mixed model as responses Y are nested in wave (i.e., we have DNAm age and correlate information for each wave), which in turn is nested in subject (multiple longitudinal measurements per individual). Let be the response for variable *i*=1..2 (i.e., DNAm age and correlates), at wave *j* (*j*=1.. for number of subjects) and subject *k* (*k*=1..number of subjects). Assume dummy variable D_{1jk} is coded 1 if for DNAm age and 0 for the correlate variable and dummy variable D_{2jk} = 1- D_{1jk} which is coded 1 if for the correlate variable and 0 for DNAm age. The model can now be written as:

$$Y_{\mathrm{ijk}} = b_{\mathrm{ijk}} D_{\mathrm{ijk}} + c_{\mathrm{i1}} Age + \sum_{p=2}^{n_p} c_{\mathrm{ip}} Cov_p, \text{ with } b_{\mathrm{ijk}} = b_{\mathrm{i}} + u_{\mathrm{ijk}} + v_{\mathrm{ik}}$$

Chronological age (Age) needs to be regressed out so that we study the residuals of that represent the biogical age of subject *k* at wave *j*. In addition, the model includes a set of $p = 2..n_p$ other covariates (Cov_p) with fixed effects c_{ip} such as Age^2, sex or lab technical variables.

To decompose the correlation between epigenetic aging and health correlates in a subject and wave contribution, we first write the model as:

$$Y_{1\mathrm{jk}} = b_{1\mathrm{jk}} D_{1\mathrm{jk}} + c_{11} Age + \sum_{p=2}^{n_p} c_{1\mathrm{p}} Cov_p, \text{ with } b_{1\mathrm{jk}} = b_1 + u_{1\mathrm{jk}} + v_{1\mathrm{k}}$$

$$Y_{2\mathrm{jk}} = b_{2\mathrm{jk}} D_{2\mathrm{jk}} + c_{21} Age + \sum_{p=2}^{n_p} c_{2\mathrm{p}} Cov_p, \text{ with } b_{2\mathrm{jk}} = b_2 + u_{2\mathrm{jk}} + v_{2\mathrm{k}}$$

Assume,

$$\begin{bmatrix} v_{1\mathrm{k}} \\ v_{2\mathrm{k}} \end{bmatrix} \sim (\mathrm{N}, \Omega_v),\ \Omega_v = \begin{bmatrix} \sigma_{\mathrm{v1}} & \\ \sigma_{\mathrm{v12}} & \sigma_{\mathrm{v2}} \end{bmatrix}$$

Thus, σ_{v1} represents the subject level contribution to the variance of DNAm age that remains after regressing out the covariates, σ_{v2} represents the contribution to the variance of the correlate variable that remains after regressing out the covariates, and σ_{v12} represents the subject level contribution to the covariance between DNAm age and the health correlate variable after regressing out the covariates. Denote the total variance-covariance matrix after regressing out the n_p covariates as:

$$\Omega_t = \begin{bmatrix} \sigma_{\mathrm{t1}} & \\ \sigma_{\mathrm{t12}} & \sigma_{\mathrm{t2}} \end{bmatrix}$$

We can now estimate the wave contribution to this overall variance-covariance matrix by subtraction:

$$\Omega_t - \Omega_v = \Omega_u = \begin{bmatrix} \sigma_{\mathrm{u1}} & \\ \sigma_{\mathrm{u12}} & \sigma_{\mathrm{u2}} \end{bmatrix}$$

where and $\sigma_{\mathrm{u12}} z$ represents the wave level contribution to the covariance between DNAm age and the health correlate variable after regressing out the covariates. The estimates σ_{v12} and σ_{u12} can be further standardized (e.g., $r_{\mathrm{v12}} = \sigma_{\mathrm{v12}} / \sqrt{\sigma_{\mathrm{v1}} \sigma_{\mathrm{v1}}}$) to obtain subject- and wave-level contributions to the correlation between epigenetic aging and the correlate variable.

The model was estimated using the R nlme package by maximum likelihood, leaving the covariance structure unrestricted through a Cholesky decomposition. The outcome variables were randomly permuted to perform significance tests of σ_{v12} and σ_{u12}.

SUPPLEMENTARY RESULTS

Participants either had between 1 and 3 methylation and linked correlate assessments collected from 18 different waves. Demographics and assessed correlates of the current study sample breakdown per wave assessment can be found in **Supplementary Table S1.**

Supplementary Table S1. Participant Characteristics and Number of Measurements Breakdown per Wave Assessment

Characteristic	N	Wave 1, N = 80	Wave 2, N = 163	Wave 3, N = 70
Chronological age (years)	1,029	11.48 ± 1.66 (9.02-13.86)	13.01 ± 1.64 (9.98-14.98)	12.95 ± 1.59 (10.97-15.58)
Sex (Female)	1,029	38 (48%)	78 (48%)	36 (51%)
Weight (kg)	971	47.29 ± 15.14 (22.72-81.00)	57.73 ± 20.02 (24.40-125.40)	53.29 ± 18.11 (20.00-114.10)
Height (cm)	979	148.16 ± 12.10 (125.21-172.35)	157.18 ± 12.52 (127.20-181.00)	157.08 ± 13.01 (125.35-187.85)
Body Mass Index (kg/m^2)	968	21.19 ± 4.79 (13.03-33.17)	22.84 ± 5.87 (14.43-41.47)	21.12 ± 4.97 (12.73-36.98)
Depression	1,029	0.44 ± 0.69 (0.00-3.00)	0.75 ± 0.93 (0.00-4.00)	0.83 ± 1.26 (0.00-5.00)
Anxiety	1,029	0.92 ± 1.45 (0.00-6.00)	1.07 ± 1.74 (0.00-7.00)	1.10 ± 1.73 (0.00-7.00)
Childhood trauma	1,029	0.21 ± 0.41 (0.00-1.00)	0.47 ± 0.50 (0.00-1.00)	0.69 ± 0.47 (0.00-1.00)
Impairments	1,029	0.70 ± 1.87 (0.00-10.00)	1.06 ± 2.07 (0.00-10.00)	1.91 ± 3.01 (0.00-10.00)
Poverty (yes)	959	22 (29%)	43 (28%)	18 (29%)
Smoking (yes)	1,029	3 (3.8%)	21 (13%)	14 (20%)
Cannabis (yes)	1,029	1 (1.2%)	3 (1.8%)	2 (2.9%)
Alcohol (yes)	1,029	0 (0%)	3 (1.8%)	3 (4.3%)

Statistics presented: mean ± SD (minimum-maximum); n (%); N reflects number of measurements.

(*continued on next page*)

Characteristic	Wave 4, N = 157	Wave 5, N = 29	Wave 6, N = 32
Chronological age (years)	13.91 ± 1.21 (11.81-16.69)	15.23 ± 0.32 (14.91-16.02)	14.99 ± 1.03 (13.92-16.43)
Sex (Female)	80 (51%)	14 (48%)	17 (53%)
Weight (kg)	63.29 ± 19.71 (30.90-140.00)	62.38 ± 16.12 (42.00-115.00)	74.31 ± 19.93 (47.00-140.00)
Height (cm)	161.73 ± 8.26 (143.60-179.45)	165.33 ± 8.41 (143.10-182.00)	167.38 ± 9.04 (151.30-191.20)
Body Mass Index (kg/m^2)	23.94 ± 6.39 (14.20-47.25)	22.84 ± 5.95 (16.16-45.60)	26.42 ± 6.35 (17.02-42.29)
Depression	0.92 ± 1.02 (0.00-5.00)	0.76 ± 1.06 (0.00-4.00)	0.72 ± 0.89 (0.00-3.00)
Anxiety	0.90 ± 1.60 (0.00-7.00)	0.62 ± 1.01 (0.00-4.00)	0.75 ± 1.61 (0.00-7.00)
Childhood trauma	0.68 ± 0.47 (0.00-1.00)	0.83 ± 0.80 (0.00-2.00)	1.19 ± 0.86 (0.00-3.00)
Impairments	1.69 ± 2.77 (0.00-10.00)	1.83 ± 2.90 (0.00-10.00)	1.69 ± 2.76 (0.00-10.00)
Poverty (yes)	45 (31%)	7 (25%)	3 (11%)
Smoking (yes)	25 (16%)	7 (24%)	7 (22%)
Cannabis (yes)	2 (1.3%)	3 (10%)	3 (9.4%)
Alcohol (yes)	0 (0%)	1 (3.4%)	1 (3.1%)

Statistics presented: mean ± SD (minimum-maximum); n (%); N reflects number of measurements.

(*continued on next page*)

Characteristic	Wave 7, N = 65	Wave 8, N = 16	Wave 9, N = 59
Chronological age (years)	16.18 ± 1.73 (14.92-19.95)	16.17 ± 0.17 (15.94-16.44)	20.12 ± 1.14 (18.78-21.77)
Sex (Female)	32 (49%)	9 (56%)	24 (41%)
Weight (kg)	70.34 ± 17.57 (35.80-128.20)	80.42 ± 26.62 (53.00-144.89)	78.16 ± 20.79 (42.30-144.89)
Height (cm)	167.65 ± 9.10 (147.50-186.20)	166.39 ± 8.34 (150.00-178.80)	169.92 ± 9.18 (150.00-190.00)
Body Mass Index (kg/m^2)	24.97 ± 5.75 (16.46-43.33)	28.42 ± 7.97 (19.83-47.25)	27.09 ± 6.96 (16.26-47.25)
Depression	1.14 ± 1.37 (0.00-5.00)	1.56 ± 1.75 (0.00-5.00)	0.90 ± 1.08 (0.00-5.00)
Anxiety	1.17 ± 1.92 (0.00-7.00)	1.81 ± 2.69 (0.00-7.00)	0.88 ± 1.70 (0.00-7.00)
Childhood trauma	0.83 ± 0.74 (0.00-2.00)	1.69 ± 1.01 (0.00-4.00)	0.97 ± 0.91 (0.00-4.00)
Impairments	1.82 ± 2.77 (0.00-10.00)	2.88 ± 3.54 (0.00-10.00)	0.63 ± 1.41 (0.00-7.00)
Poverty (yes)	24 (44%)	2 (22%)	33 (57%)
Smoking (yes)	21 (32%)	8 (50%)	38 (64%)
Cannabis (yes)	13 (20%)	4 (25%)	17 (29%)
Alcohol (yes)	11 (17%)	4 (25%)	24 (41%)

Statistics presented: mean ± SD (minimum-maximum); n (%); N reflects number of measurements.

(*continued on next page*)

Characteristic	Wave 11, N = 44	Wave 13, N = 26	Wave 14, N = 70
Chronological age (years)	20.09 ± 1.19 (18.31-21.95)	21.08 ± 0.23 (20.73-21.49)	26.44 ± 0.46 (25.98-28.39)
Sex (Female)	23 (52%)	11 (42%)	42 (60%)
Weight (kg)	82.42 ± 23.18 (44.50-136.40)	85.18 ± 23.46 (56.20-144.89)	84.13 ± 23.23 (44.00-144.89)
Height (cm)	170.30 ± 10.80 (150.00-202.40)	168.47 ± 11.04 (150.00-185.00)	170.21 ± 11.60 (152.00-193.00)
Body Mass Index (kg/m^2)	28.33 ± 7.68 (17.89-47.25)	30.05 ± 7.70 (19.94-47.25)	28.86 ± 6.57 (17.99-47.25)
Depression	0.98 ± 1.45 (0.00-5.00)	0.81 ± 1.10 (0.00-5.00)	0.93 ± 1.24 (0.00-5.00)
Anxiety	1.00 ± 2.03 (0.00-7.00)	0.73 ± 1.71 (0.00-7.00)	0.89 ± 1.77 (0.00-7.00)
Childhood trauma	1.41 ± 1.35 (0.00-4.00)	1.92 ± 1.57 (0.00-4.00)	1.41 ± 1.16 (0.00-4.00)
Impairments	0.45 ± 1.69 (0.00-10.00)	1.15 ± 2.85 (0.00-10.00)	0.10 ± 0.64 (0.00-5.00)
Poverty (yes)	17 (44%)	12 (46%)	9 (13%)
Smoking (yes)	22 (50%)	12 (46%)	51 (73%)
Cannabis (yes)	14 (32%)	6 (23%)	26 (37%)
Alcohol (yes)	12 (27%)	5 (19%)	20 (29%)

Statistics presented: mean ± SD (minimum-maximum); n (%); N reflects number of measurements.

(*continued on next page*)

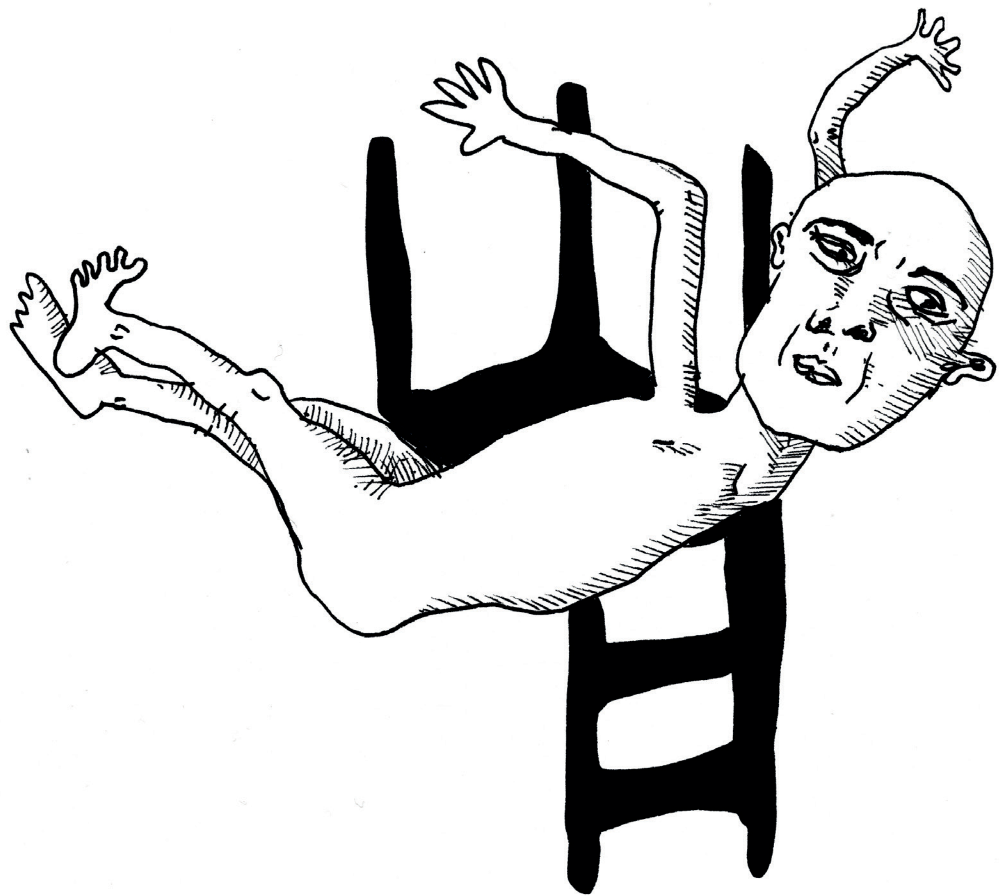
Characteristic	Wave 15, N = 82	Wave 16, N = 75	Wave 17, N = 22	Wave 18, N = 39
Chronological age (years)	25.57 ± 0.47 (23.51-26.73)	25.41 ± 0.57 (24.45-26.47)	28.48 ± 1.48 (26.03-31.77)	30.96 ± 1.35 (29.37-34.55)
Sex (Female)	44 (54%)	45 (60%)	10 (45%)	18 (46%)
Weight (kg)	86.23 ± 22.84 (42.00-144.89)	86.94 ± 25.28 (44.00-144.89)	77.95 ± 16.19 (50.00-102.00)	NA
Height (cm)	171.89 ± 9.62 (154.00-195.00)	171.29 ± 11.49 (152.00-195.00)	174.02 ± 10.99 (157.00-202.40)	NA
Body Mass Index (kg/m^2)	29.02 ± 7.32 (17.04-47.25)	29.60 ± 7.02 (18.55-47.25)	25.60 ± 4.26 (16.33-34.48)	NA
Depression	0.96 ± 1.20 (0.00-5.00)	0.89 ± 0.92 (0.00-3.00)	0.55 ± 1.10 (0.00-5.00)	0.90 ± 1.35 (0.00-5.00)
Anxiety	0.84 ± 1.58 (0.00-7.00)	0.80 ± 1.19 (0.00-5.00)	0.32 ± 1.29 (0.00-6.00)	1.23 ± 2.30 (0.00-7.00)
Childhood trauma	2.05 ± 1.36 (0.00-4.00)	1.59 ± 1.42 (0.00-4.00)	1.59 ± 1.33 (0.00-4.00)	1.97 ± 1.06 (0.00-4.00)
Impairments	0.30 ± 1.12 (0.00-7.00)	0.08 ± 0.51 (0.00-4.00)	0.00 ± 0.00 (0.00-0.00)	0.18 ± 0.85 (0.00-5.00)
Poverty (yes)	18 (22%)	15 (20%)	6 (27%)	5 (15%)
Smoking (yes)	52 (63%)	47 (63%)	12 (55%)	21 (54%)
Cannabis (yes)	37 (45%)	27 (36%)	9 (41%)	13 (33%)
Alcohol (yes)	36 (44%)	30 (40%)	12 (55%)	26 (67%)

Statistics presented: mean ± SD (minimum-maximum); n (%); N reflects number of measurements.

REFERENCES

1. Aberg KA, Chan RF, Shabalin AA, Zhao M, Turecki G, Staunstrup NH, et al. A MBD-seq protocol for large-scale methylome-wide studies with (very) low amounts of DNA. Epigenetics. 2017;12:743–750.
2. Aberg KA, Chan RF, van den Oord EJCG. MBD-seq - realities of a misunderstood method for high-quality methylome-wide association studies. Epigenetics. 2020;15:431–438.
3. Chan RF, Shabalin AA, Xie LY, Adkins DE, Zhao M, Turecki G, et al. Enrichment methods provide a feasible approach to comprehensive and adequately powered investigations of the brain methylome. Nucleic Acids Res. 2017;45:e97.
4. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat. 940. Methods. 2012;9:541.
5. Aberg KA, Mcclay JL, Nerella S, Xie LY, Clark SL, Hudson AD, et al. MBD-seq as a cost-effective approach for methylome-wide assocciation studies: demonstration in 1500 case-control samples. Epigenomics. 2012;4:605–621.
6. Shabalin A, Clark S, Hattab M, Aberg K, van den Oord E. ramwas: Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms. R package version 1.0.0, https://bioconductor.org/packages/ramwas/. 2017.
7. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–1303.
8. Costello EJ, Eaves L, Sullivan P, Kennedy M, Conway K, Adkins DE, et al. Genes, environments, and developmental research: methods for a multi-site study of early substance abuse. Twin Res Hum Genet. 2013;16:505–515.
9. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic Aging in Major Depressive Disorder. Am J Psychiatry. 2018;175:774–782.
10. Hattab MW, Shabalin AA, Clark SL, Zhao M, Kumar G, Chan RF, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. Genome Biol. 2017;18.
11. Houseman E, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics. 2012;13:86.
12. van den Oord EJCG, Bukszar J, Rudolf G, Nerella S, McClay JL, Xie LY, et al. Estimation of CpG coverage in whole methylome next-generation sequencing studies. BMC Bioinformatics. 2013;14:50.
13. Koestler DC, Christensen B, Karagas MR, Marsit CJ, Langevin SM, Kelsey KT, et al. Blood-based profiles of DNA methylation predict the underlying distribution of cell types: a validation analysis. Epigenetics. 2013;8:816–826.
14. Tanner JM. The evaluation of growth and maturity in children. Protein Metabolism. 1962:361–382.
15. Dorn LD, Biro FM. Puberty and Its Measurement: A Decade in Review. J Res Adolesc. 2011;21:180–195.
16. Angold A, Jane Costello E. The Child and Adolescent Psychiatric Assessment (CAPA). Journal of the American Academy of Child & Adolescent Psychiatry. 2000;39:39–48.
17. Angold A, Prendergast M, Cox A, Harrington R, Simonoff E, Rutter M. The Child and Adolescent Psychiatric Assessment (CAPA). Psychol Med. 1995;25:739–753.

18. Canino G, Costello EJ, Angold A. Assessing Functional Impairment and Social Adaptation for Child Mental Health Services Research: A Review of Measures. Ment Health Serv Res. 1999;1:93–108.
19. Clark SL, Hattab MW, Chan RF, Shabalin AA, Han LKM, Zhao M, et al. A methylation study of long-term depression risk. Mol Psychiatry. 2019;25:1334–1343.



CHAPTER 5

Brain Aging in Major Depressive Disorder: Results from the ENIGMA Major Depressive Disorder Working Group

Published in *Molecular Psychiatry* 2020, 1-16.

Laura K.M. Han
Richard Dinga
Tim Hahn
Christopher R.K. Ching
Lisa T. Eyler
Lyubomir Aftanas,
Moji Aghajani
André Aleman
Bernhard T. Baune
Klaus Berger
Ivan Brak
Geraldo Busatto Filho
Angela Carballedo
Colm G. Connolly
Baptiste Couvy-Duchesne
Kathryn Cullen
Udo Dannlowski
Christopher G. Davey
Danai Dima
Fabio L.S. Duran
Verena Enneking
Elena Filimonova
Stefan Frenzel
Thomas Frodl
Cynthia H.Y. Fu
Beata R. Godlewska
Ian H. Gotlib
Hans J. Grabe
Nynke A. Groenewold
Dominik Grotegerd
Oliver Gruber
Geoffrey B. Hall
Ben J. Harrison
Sean N. Hatton
Marco Hermesdorf
Ian B. Hickie
Tiffany C. Ho
Norbert Hosten
Andreas Jansen
Claas Kähler
Tilo Kircher
Bonnie Klimes-Dougan
Bernd Krämer
Axel Krug
Jim Lagopoulos
Ramona Leenings
Frank P. MacMaster
Glenda MacQueen
Andrew McIntosh
Quinn McLellan
Katie L. McMahon
Sarah E. Medland
Bryon A. Mueller
Benson Mwangi
Evgeny Osipov
Maria J. Portella
Elena Pozzi
Liesbeth Reneman
Jonathan Repple
Pedro G.P. Rosa
Matthew D. Sacchet
Philipp G. Sämann
Knut Schnell
Anouk Schrantee
Egle Simulionyte
Jair C. Soares
Jens Sommer
Dan J. Stein
Olaf Steinsträter
Lachlan T. Strike
Sophia I. Thomopoulos
Marie-José van Tol
Ilya M. Veer
Robert R.J.M. Vermeiren
Henrik Walter
Nic J.A. van der Wee
Steven J.A. van der Werff
Heather C. Whalley
Nils R. Winter
Katharina Wittfeld
Margaret J. Wright
Mon-Ju Wu
Henry Völzke
Tony T. Yang
Vasileios Zannias
Greig I. de Zubicaray
Giovana B. Zunta-Soares
Christoph Abé
Martin Alda
Ole A. Andreassen
Erlend Bøen
Caterina M. Bonnin
Erick J. Canales-Rodriguez
Dara Cannon
Xavier Caseras
Tiffany M. Chaim-Avancini
Torbjørn Elvsåshagen
Pauline Favre
Sonya F. Foley
Janice M. Fullerton
Jose M. Goikolea
Bartholomeus C.M. Haarman
Tomas Hajek
Chantal Henry
Josselin Houenou
Fleur M. Howells
Martin Ingvar
Rayus Kuplicki
Beny Lafer
Mikael Landén
Rodrigo Macha-Vieira
Ulrik F. Malt
Colm McDonald
Philip B. Mitchell
Leila Nabulsi
Maria Concepcion Garcia Otaduy
Bronwyn J. Overs
Mircea Polosan

Edith Pomarol-Clotet
Joaquim Radua
Maria M. Rive
Gloria Roberts
Henricus G. Ruhe
Raymond Salvador
Salvador Sarró
Theodore D. Satterthwaite
Jonathan Savitz
Aart H. Schene
Peter R. Schofield
Mauricio H. Serpa
Kang Sim
Marcio Gerhardt Soeiro-de-Souza
Ashley N. Sutherland
Henk S. Temmingh
Garrett M. Timmons
Anne Uhlmann
Eduard Vieta
Daniel H. Wolf
Marcus V. Zanetti
Neda Jahanshad
Paul M. Thompson
Dick J. Veltman
Brenda W.J.H. Penninx
Andre F. Marquand
James H. Cole
Lianne Schmaal

ABSTRACT

Major depressive disorder (MDD) is associated with an increased risk of brain atrophy, aging-related diseases, and mortality. We examined potential advanced brain aging in adult MDD patients, and whether this process is associated with clinical characteristics in a large multi-center international dataset. We performed a mega-analysis by pooling brain measures derived from T1-weighted MRI scans from 19 samples worldwide. Healthy brain aging was estimated by predicting chronological age (18-75 years) from 7 subcortical volumes, 34 cortical thickness and 34 surface area, lateral ventricles and total intracranial volume measures separately in 952 male and 1,236 female controls from the ENIGMA MDD working group. The learned model coefficients were applied to 927 male controls and 986 depressed males, and 1,199 female controls and 1,689 depressed females to obtain independent unbiased brain-based age predictions. The difference between predicted "brain age" and chronological age was calculated to indicate brain predicted age difference (brain-PAD). On average, MDD patients showed a higher brain-PAD of +1.08 (SE 0.22) years (Cohen's d=0.14, 95% CI 0.08-0.20) compared to controls. However, this difference did not seem to be driven by specific clinical characteristics (recurrent status, remission status, antidepressant medication use, age of onset or symptom severity). This highly-powered collaborative effort showed subtle patterns of age-related structural brain abnormalities in MDD. Substantial within-group variance and overlap between groups were observed. Longitudinal studies of MDD and somatic health outcomes are needed to further assess the clinical value of these brain-PAD estimates.

INTRODUCTION

Major Depressive Disorder (MDD) is associated with an increased risk of cognitive decline [1], metabolic dysregulation [2], and cellular aging [3, 4] indicating that the burden of MDD goes beyond mental ill-health and functional impairment, and extends to poor somatic health [5], and age-related diseases [6]. Moreover, MDD increases the risk of mortality [7], and not only through death by suicide [8]. Simultaneously, depression and aging have been linked to poor quality of life and increased costs for society and healthcare [9]. This underscores the importance of identifying brain aging patterns in MDD patients to determine whether and how they deviate from healthy patterns of aging.

Current multivariate pattern methods can predict chronological age from biological data (see Jylhava, Pedersen, and Hagg for a review) [10] with high accuracy. Similarly, chronological age can be predicted from brain images, resulting in an estimate known as "brain age" [11]. Importantly, by calculating the difference between a person's estimated brain age and their chronological age, one can translate a complex aging pattern across the brain into a single outcome: brain-predicted age difference (brain-PAD). A positive brain-PAD represents having an 'older' brain than expected for a person of their chronological age, whereas a negative brain-PAD signals a 'younger' brain than expected at the given chronological age. Higher brain-PAD scores have been associated with greater cognitive impairment, increased morbidity, and exposure to cumulative negative fateful life events [11, 12]. For a review summarizing brain age studies from the past decade, see Franke & Gaser (2019) [13]).

Prior studies from the Enhancing NeuroImaging Genetics through Meta-analysis (ENIGMA)-MDD consortium with sample sizes over 9,000 participants have shown subtle reductions in subcortical structure volumes in major depression that were robustly detected across many samples worldwide. Specifically, smaller hippocampal volumes were found in individuals with earlier age of onset and recurrent episode status [14]. In addition, different patterns of cortical alterations were found in adolescents vs. adults with MDD, suggesting that MDD may affect brain morphology (or vice versa) in a way that depends on the developmental stage of the individual [15]. Thus, subtle structural brain abnormalities have been identified in MDD. However, whether a diagnosis of MDD is associated with the multivariate metric of brain aging in a large dataset, and which clinical characteristics further impact this metric, remains elusive.

Accumulating evidence from studies suggests that, at the group level, MDD patients follow advanced aging trajectories, as their functional (e.g.

walking speed, handgrip strength) [16] and biological state (e.g. telomeres, epigenetics, mitochondria) [17–20] reflects what is normally expected at an older age (i.e. biological age "outpaces" chronological age).[21] It is important to examine whether biological aging findings in depression can be confirmed in a large heterogeneous dataset consisting of many independent samples worldwide, based on commonly derived gray matter measures. Only a handful of studies have investigated brain-PAD in people with psychiatric disorders, showing older brain-PAD in schizophrenia, borderline personality disorder, and first-episode and at-risk mental state for psychosis, yet findings were less consistent in bipolar disorder (for an overview, see Cole et al., 2018) [22].

Only three studies to date specifically investigated machine-learning-based brain aging in MDD - using relatively small samples of <211 patients, with inconsistent findings of a brain-PAD of +4.0 years vs. no significant differences [23–25]. The current study is the first to examine brain aging in over 6,900 individuals from the ENIGMA MDD consortium (19 cohorts, 8 countries worldwide), covering almost the entire adult lifespan (18-75 years). Our additional aim was to build a new multi-site brain age model based on FreeSurfer regions of interest (ROIs) that generalizes well to independent data to promote brain age model deployability and shareability. We hypothesized higher brain-PAD in MDD patients compared to controls. We also conducted exploratory analyses to investigate whether higher brain-PAD in MDD patients was associated with demographics (age, sex) and clinical characteristics such as disease recurrence, antidepressant use, remission status, depression severity, and age of onset of depression.

METHODS

Samples

Nineteen cohorts from the ENIGMA-MDD working group with data from MDD patients and controls (18-75 years of age) participated in this study (**Supplementary Table S1**). MDD was ascertained using the clinician-rated HDRS-17 in one cohort and diagnostic interviews in all other cohorts. Details regarding demographics, clinical characteristics, and exclusion criteria for each cohort may be found in **Supplementary Tables S1-4**. Because the literature suggests differential brain developmental trajectories by sex [26], we estimated brain age models separately for males and females. Sites with less than ten healthy controls were excluded from the training dataset and subsequent analyses (for exclusions see **supplementary material**). In total,

we included data from N=6,989 participants, including N=4,314 controls (N=1,879 males; N=2,435 females) and N=2,675 individuals with MDD (N=986 males; N=1,689 females). All sites obtained approval from the appropriate local institutional review boards and ethics committees, and all study participants or their parents/guardians provided written informed consent.

Training and test samples

To maximize the variation of chronological age distribution and scanning sites in the training samples, and to maximize the statistical power and sample size of patients for subsequent statistical analyses, we created balanced data splits within scanning sites preserving the chronological age distribution, **Figure 1A**. The full motivation to our data partition approach can be found in the **supplementary material**. Structural brain measures from 952 males obtained from 16 scanners and 1,236 female controls obtained from 22 scanners were included in the training samples. The top panel in **Figure 1B** shows the age distribution in the training sample. A hold-out dataset comprising controls served as a test sample to validate the accuracy of the brain age prediction model; 927 male and 1,199 female controls from the same scanning sites were included. Likewise, 986 male and 1,689 female MDD patients from the corresponding scanning sites were included in the MDD test sample. The two bottom panels in **Figure 1B** show the age distributions across the test samples.

Image processing and analysis

Structural T1-weighted scans of each subject were acquired at each site. To promote data sharing and to maximize the efficiency of pooling existing datasets, we used standardized protocols to facilitate harmonized image analysis and feature extraction (N=153) across multiple sites (http://enigma.ini.usc.edu/protocols/imaging-protocols/). Cortical parcellations were based on the Desikan/Killiany atlas [27]. Briefly, the fully-automated and validated segmentation software FreeSurfer was used to segment 14 subcortical gray matter regions (nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus), 2 lateral ventricles, 68 cortical thickness, and 68 surface area measures, and total intracranial volume (ICV). Segmentations were visually inspected and statistically examined for outliers. Further details on cohort type, image acquisition parameters, software descriptions, and quality control may be found in **Supplementary Table S3**. Individual-level structural brain measures and clinical and demographic measures from each cohort were pooled at a central site to perform the mega-analysis.

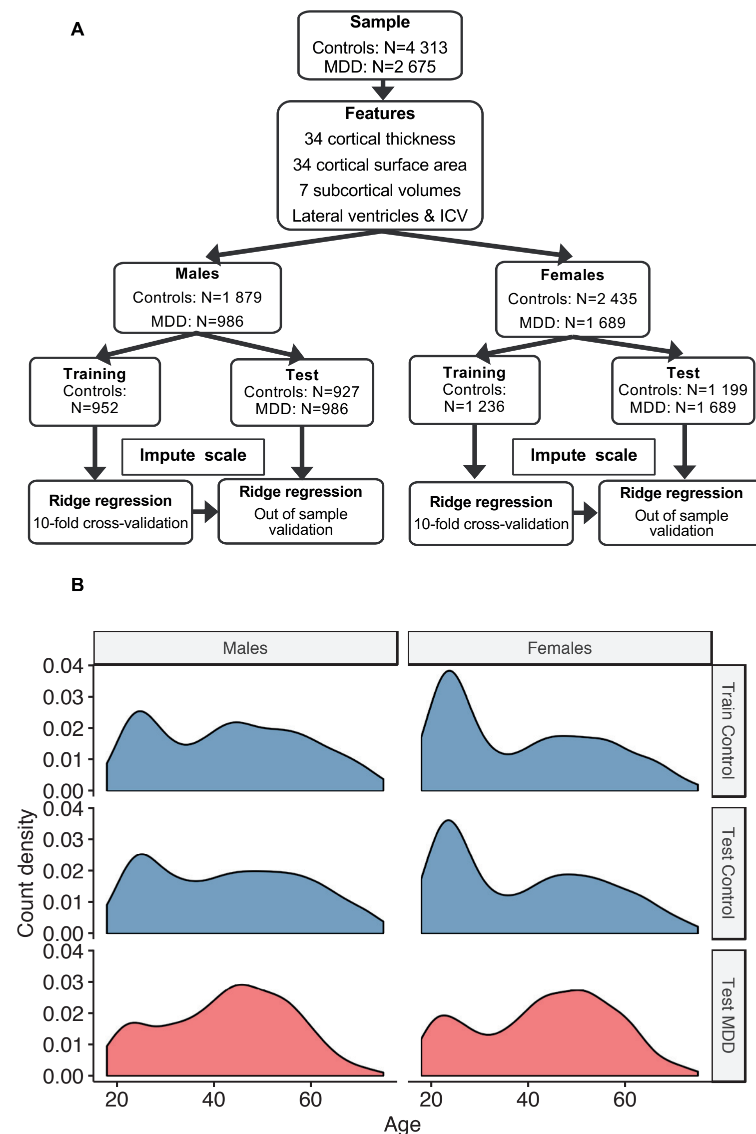


Figure 1. Data partition approach. (**A**) Schematic illustration of features used and data partition into training and test samples, separately for males and females. A full list of features can be found in the supplement (**B**) Data from control groups (blue) were partitioned into balanced 50:50 splits within each scanning site following random sampling but preserving the overall chronological age distribution. Major depressive disorder (MDD) groups are shown in red. The top panel illustrates the male (*left)* and female (*right*) training samples. The middle and bottom panels show the male (controls: mean [SD] in years, 43.1 [15.3]; MDD: 42.8 [13.1]) and female test samples (controls: 39.4 [15.7]; MDD: 43.2[14.0]). ICV, intracranial volume.

FreeSurfer brain age prediction model

To estimate the normative brain age models, we combined the FreeSurfer measures from the left and right hemispheres by calculating the mean ((left+right)/2) of volumes for subcortical regions and lateral ventricles, and thickness and surface area for cortical regions, resulting in 77 features (**Supplementary Table S5**). Using a mega-analytic approach, we first estimated normative models of the association between the 77 average structural brain measures and age in the training sample of controls (separately for males and females) using Ridge Regression, from the Python-based *sklearn* package [28]. All brain measures were combined as predictors in a single multivariate model. To assess model performance, we performed 10-fold cross-validation. To quantify model performance, we calculated the mean absolute error (MAE) between predicted brain age and chronological age. The literature suggests nonuniform age-related changes for cortical thickness, surface area, and subcortical volumes [29], which is further supported by empirical evidence showing that brain morphology is under control of distinct genetic and developmental pathways [30–33]. We therefore included all three feature modalities in our brain age prediction framework. Of note, we also tested whether reducing feature space by including only single modalities (only cortical thickness vs. cortical surface area vs. subcortical volume features) would improve model fit, but this resulted in poorer performance accuracy than combining all 77 features. Moreover, we also: (1) estimated a model including left and right hemisphere features separately, (2) compared the Ridge Regression to other machine learning methods, (3) regressed features on ICV instead of including ICV as a separate feature, none of which resulted in significantly superior prediction accuracy (results are provided in **Supplementary Table S6).**

Model validation

Model performance was further validated in the test sample of controls. The parameters learned from the trained model in controls were applied to the test sample of controls and to the MDD test samples to obtain brain-based age estimates. To assess model performance in these test samples, we calculated: a) MAE; b) Pearson correlation coefficients between predicted brain age and chronological age; and c) the proportion of the variance explained by the model (R^2). To evaluate generalizability to completely independent test samples (acquired on completely independent scanning sites), we applied the training model parameters to control subjects (males, N=610; females, N=720) from the ENIGMA Bipolar Disorder (BD) working group.

Statistical analyses

All statistical analyses were conducted in the test samples only. Brain-PAD (predicted brain-based age - chronological age) was calculated for each individual and used as the outcome variable. While different prediction models were built for males and females, the generated brain-PAD estimates were pooled for statistical analyses.

Each dependent measure of the ith individual at j^{th} scanning site were modelled as follows:

1. $\text{Brain-PAD}_{ij} = \text{Intercept} + \beta_1(Dx) + \beta_2(Sex) + \beta_3(Age) + \beta_4(Age^2) + \beta_5(Dx \times Age) + \beta_6(Dx \times Sex) + \beta_7(Age \times Sex) + \beta_8(Dx \times Age \times Sex) + U_j + \varepsilon_{ij}$
2. $\text{Brain-PAD}_{ij} = \text{Intercept} + \beta_1(Dx) + \beta_2(Sex) + \beta_3(Age) + \beta_4(Age^2) + \beta_5(Dx \times Age) + \beta_6(Dx \times Sex) + U_j + \varepsilon_{ij}$
3. $\text{Brain-PAD}_{ij} = \text{Intercept} + \beta_1(Dx) + \beta_2(Sex) + \beta_3(Age) + \beta_4(Age^2) + U_j + \varepsilon_{ij}$

Intercept, Dx (MDD diagnosis), sex, and all age effects were fixed. The term U_j and ε_{ij} are normally distributed and represent the random intercept attributed to the scanning site and the residual error, respectively.

Following Le and colleagues [34], we post hoc corrected for the residual age effects on the brain-PAD outcome in the test samples by adding age as a covariate to our statistical models. However, we found remaining nonlinear age effects on our brain-PAD outcome [35], and included both linear and quadratic age covariates as it provided significantly better model fit to our data compared to models with a linear age covariate only ($\chi^2(2)=9.73$, $p<0.002$). For more details see **supplementary material**.

Within MDD patients, we also used linear mixed models to examine associations of brain-PAD with clinical characteristics, including recurrence status (first vs. recurrent episode), antidepressant use at time of scanning (yes/no), remission status (currently depressed vs. remitted), depression severity at study inclusion (the 17-item Hamilton Depression Rating Scale (HDRS-17) and the Beck Depression Inventory (BDI-II)), and age of onset of depression (categorized as: early, <26 years; middle adulthood, >25 & <56 years; and late adulthood onset, >55 years). Analyses were tested two-sided and findings were False Discovery Rate (FDR) corrected and considered statistically significant at $p<0.05$.

Finally, to gain more insight into the importance of features for making brain age predictions we: a) calculated structure coefficients (i.e. Pearson correlations between predicted brain age and each feature) in the test samples only for illustrative purposes, b) explored single modality (either subcortical volumes or cortical thickness or cortical surface area features) trained models, and c) perturbed features (either subcortical volumes or cortical thickness or cortical surface area) by setting their values to zero in the test samples and examining the changes in performance.[36]

RESULTS

Brain age prediction performance

Supplementary Figure S1 and **Supplementary Table S7** illustrate the systematic bias in brain age estimation and the correction we applied. Within the training set of controls, under cross-validation, the structural brain measures predicted chronological age with a MAE of 6.32 (SD 5.06) years in males and 6.59 (5.14) years in females. When applying the model parameters to the test samples of controls, the MAE was 6.50 (4.91) and 6.84 (5.32) years for males and females, respectively. Similarly, within the MDD group, the MAE was 6.72 (5.36) and 7.18 (5.40) years for males and females, respectively. **Figure 2** shows the correlation between chronological age (y-axis) and predicted brain age (x-axis) [37] in the cross-validation training sample (males $r=0.85$, $p<0.001$ and females and $r=0.854$, $p<0.001$, both $R^2=0.72$), out-of-sample controls (males $r=0.85$, $p<0.001$; $R^2=0.72$ and females $r=0.83$, $p<0.001$; $R^2=0.69$), and MDD test samples (males $r=0.77$, $p<0.001$; $R^2=0.57$ and females $r=0.78$, $p<0.001$; $R^2=0.59$), and the generalization to completely independent healthy control samples of the ENIGMA Bipolar Disorder working group (MAE=7.49 [SD 5.89]; $r=0.71$, $p<0.001$; $R^2=0.45$ for males and MAE=7.26 [5.63]; $r=0.72$, $p<0.001$; $R^2=0.48$, for females).

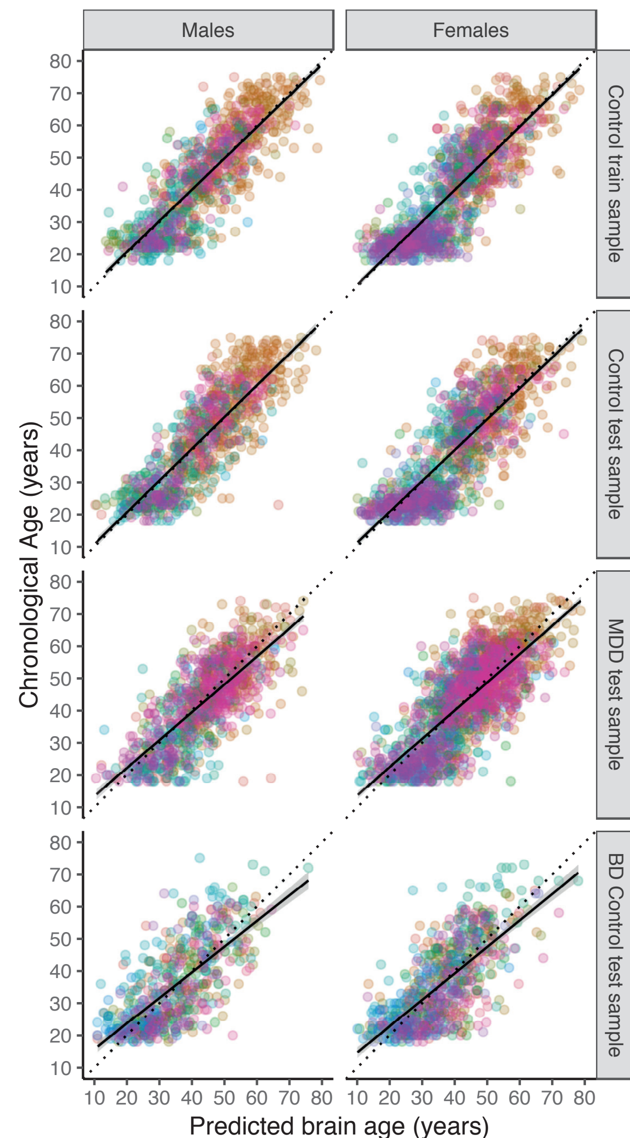


Figure 2. Brain age prediction based on 7 FreeSurfer subcortical volumes, lateral ventricles, 34 cortical thickness and 34 surface area measures, and total intracranial volume. The plots show the correlation between chronological age and predicted brain age in the 10-fold cross-validation of the ridge regression in the control train sample, the out-of-sample validation of the test samples (controls and MDD patients) from the ENIGMA MDD working group, and generalizability to completely independent test samples (controls only) from the ENIGMA BD working group (top to bottom). The colors indicate scanning sites and each circle represents an individual subject. Diagonal dashed line reflects the line of identity (x=y).

Added brain aging in MDD

Uncorrected mean brain-PAD scores were -0.20 (SD 8.44) years in the control and +0.68 years (SD 8.82) in the MDD group. Individuals with MDD showed +1.08 (SE 0.22) years higher brain-PAD than controls ($p<0.0001$, Cohen's d=0.14, 95% CI 0.08-0.20) adjusted for age, age^2, sex, and scanning site (**Figure 3**). Additionally, we found significant main effects for age (b=-0.28, $p<0.0001$) and age^2 (b=-0.001 $p<0.01$). Our analyses revealed no significant three-way interaction between diagnosis-by-age-by-sex, nor significant two-way interactions (diagnosis-by-age or diagnosis-by-sex). Of note, since there were no significant interactions with age or age^2 and MDD status, and the residual age effects in the brain-PAD estimates did not influence our primary finding. Given that our model showed higher errors in individuals >60y, we performed a sensitivity analysis by including only participants within the 18-60y age range. Here, we found a slightly increased effect of diagnostic group (MDD +1.16y [SE 0.24] higher brain-PAD than controls [$p<0.0001$, Cohen's d=0.15, 95% CI 0.09-0.21]).

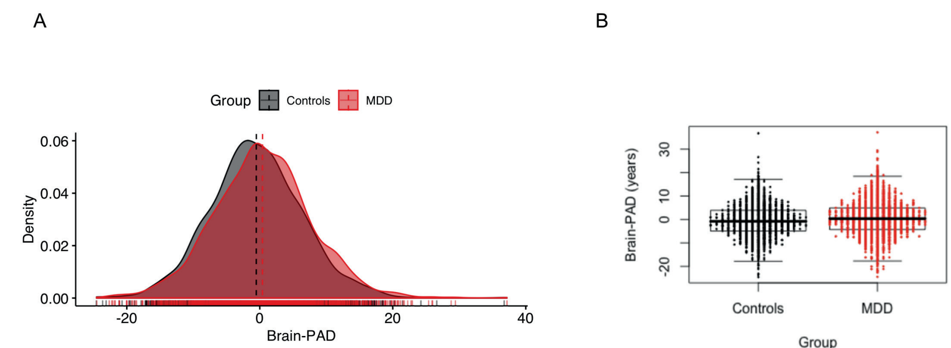


Figure 3. Case-control differences in brain aging. Brain-PAD (predicted brain age - chronological age) in patients with major depressive disorder (MDD) and controls. Group level analyses showed that MDD patients exhibited significantly higher brain-PAD than controls (b=1.08, $p<0.0001$), although large within-group variation and between-group overlap is observed as visualized in (**A**) the density plot and (**B**) the Engelmann-Hecker plot. The brain-PAD estimates are adjusted for chronological age, age^2, sex and scanning site.

The relative importance of thickness features

All features, except the mean lateral ventricle volume, and entorhinal and temporal pole thickness showed a negative correlation with predicted brain age, and are visualized in **Figure 4**. Widespread negative correlations

with average cortical thickness and surface area were observed, although thickness features resulted in stronger negative correlations (mean Pearson r [SD]: -0.44 [0.21]) than surface area features (-0.17 [0.08]). On average, subcortical volumes were slightly less negatively correlated to predicted brain age as thickness features (-0.34 [0.34]). Single modality models and ICV performed worse than a combined model including all modalities. Test performance was most negatively affected by the perturbation of thickness features (**Supplementary Tables 8-9**).

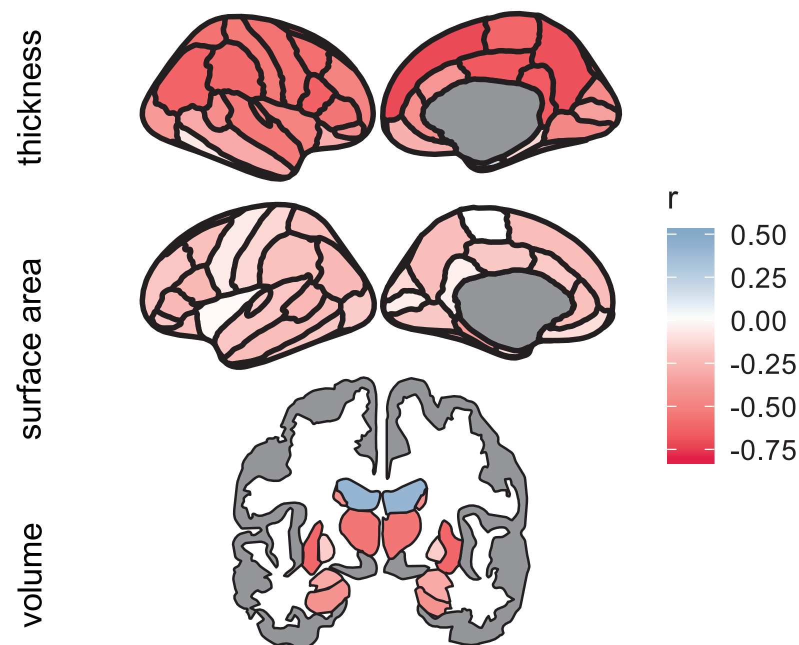


Figure 4. Structure coefficients of predicted brain age and FreeSurfer features across control and major depressive disorder (MDD) groups. Bivariate correlations are shown for illustrative purposes and to provide a sense of importance of features in the brain age prediction. The figure shows Pearson correlations between predicted brain age and cortical thickness features (top row), cortical surface areas (middle row), and subcortical volumes (bottom row). The negative correlation with ICV was excluded from this figure for display purposes.

Brain aging and clinical characteristics

Compared to controls, significant brain-PAD differences in years were observed in patients with a remitted disease status (+2.19 years, p<0.0001, d=0.18), with a current depression (+1.5y, p<0.0001, d=0.18), in those that were using antidepressant medication at the time of scanning (+1.4y, p<0.0001, d=0.15), medication-free depressed patients (+0.7y, p=0.0225, d=0.07), patients with a late adult-onset of depression (+1.2y, p=0.01, d=0.12), patients with an age of onset of MDD in mid-adulthood (+0.9y, p=0.0005, d=0.11), patients with an early age of onset of depression (<26 years; +1.0y, p=0.0004, d=0.11), first-episode patients (+1.2y, p=0.0002, d=0.13) and recurrent depressed patients (+1.0y, p=0.0002, d=0.11)(**Table 1**). Importantly, post hoc comparisons between the MDD subgroups did not show any significant differences (i.e., first vs. recurrent episode, antidepressant medication-free vs. antidepressant users, remitted vs. currently depressed patients, or early vs. adult vs. late age of onset of depression). Mean brain-PAD was above zero in all MDD subgroups, indicating that all MDD subgroups were estimated to be older than expected based on the brain age model compared to controls. Finally, there were no significant associations with depression severity or current depressive symptoms (self-reported BDI-II [b=0.04, p=0.06] or clinical-based HDRS-17 [b=-0.02, p=0.48] questionnaires) at the time of scanning within the MDD sample.

DISCUSSION

Using a new parsimonious multi-site brain age algorithm based on FreeSurfer ROIs from over 2,800 males and 4,100 females, we found subtle age-associated gray matter differences in adults with major depressive disorder (MDD). At the group level, patients had, on average, a +1.08 years greater discrepancy between their predicted and actual age compared to controls. Significantly larger brain-PAD scores were observed in all patient subgroups compared to controls (with Cohen's d effect sizes ranging from 0.07-0.18), indicating that the higher brain-PAD in patients was not driven by specific clinical characteristics (recurrent status, remission status, antidepressant medication use, age of onset or symptom severity). This study confirms previously observed advanced cellular aging in MDD at the brain level of analysis, however, it is important to mention the large within-group and small between-group variance, demonstrating that many patients did not show advanced brain aging. We were not able to investigate all potential clinical,

biological and other sources that could explain the large within-group variance of brain-PAD in MDD patients. Future studies, ideally with in-depth clinical phenotyping and longitudinal information on mental and somatic health outcomes (e.g., genomic variation, omics profiles, comorbidities, duration of illness, lifestyle, inflammation, oxidative stress, chronic diseases), are required to further evaluate the predictive value of the brain-PAD estimates, potentially by using our publicly available brain age model (https://www.photon-ai.com/enigma_brainage).

Perhaps surprisingly, we found higher brain-PAD in antidepressant users (+1.4y) compared to controls and antidepressant-free patients (+0.7y) and controls, although the difference between patient groups was not significant. Antidepressants are suggested to exert a neuroprotective effect, for example by promoting brain-derived neurotrophic factor (BDNF) [38]. However, patients taking antidepressant medication at the time of scanning likely had a more severe or chronic course of the disorder [14, 15]. Therefore, the larger brain-PAD in antidepressant users may be confounded by severity or course of the disorder. Unfortunately, the cross-sectional nature of the current study and the lack of detailed information on lifetime use, dosage and duration of use of antidepressants, do not allow us to draw any conclusions regarding the direct effects of antidepressants on brain aging. In addition, it remains to be elucidated how adaptable brain-PAD is in response to pharmacotherapy. Randomized controlled intervention studies are needed to develop an understanding of how reversible or modifiable brain aging is in response to pharmacological and non-pharmacological strategies (e.g., psychological, exercise and/or nutritional interventions), as seen in other biological age indicators [21, 39].

Our brain-PAD difference (+1.1y) is attenuated in contrast to earlier work showing +4.0 years of brain aging in a smaller sample of MDD patients in a study by Koutsouleris et al. (N=104) [23]. However, a recent study by Kaufmann and colleagues (2019) found a similar effect size to ours in 211 MDD patients (18-71 years), albeit non-significant [25]. Although the MAE of our models (6.6y in age range of 18-75y) is higher than in e.g. the study by Koutsouleris et al, 2014 (4.6y in age range of 18-65y), a simple calculation shows that, when scaled to covered age range, the studies show comparable MAE (0.11 vs. 0.10, respectively) [40]. As the range of possible predictions (age range) carry a strong bearing on prediction accuracy, increasingly wider ranges of outcomes become more challenging to predict [11]. Several methodological differences may underlie the inconsistencies or differences in magnitude of brain age effects in MDD, including, but not limited to: (1) the use of high-dimensional features such as whole-brain gray matter maps in the Koutsouleris et al. study vs. a much lower number of input features (Freesurfer ROIs) in our study, although the Kaufmann et al. study included multimodal parcellations and found similar brain age effects in MDD as we observed; (2) the composition of training and test data, including number of scanners in both sets, with 5 scanners included in the Koutsouleris et al. study vs. 22 in our study vs. 68 scanners in Kaufmann et al.; (3) sample sizes of training and test data (N=800 in training set and N=104 in MDD test set in Koutsouleris et al. vs. N>950 in training set and N>980 in MDD test set in our current study vs. N>16K training set and N=211 in MDD test set in Kaufmann et al.); and (4) heterogeneity of MDD and differences in patient characteristics between the studies. The inconsistencies between brain-PAD findings in MDD might be due to any (combination) of the sources of variation outlined above and precludes a direct comparison of these studies. Unfortunately, a methodological comparison is beyond the scope of our study and beyond our capability given data access limitations within ENIGMA MDD. Nevertheless, the current results are based on the largest MDD sample to date and likely provide more precise estimates regardless of the size of the effect [41, 42].

The current findings in MDD also show lower brain aging than previously observed in schizophrenia (SCZ) (brain-PAD ranges from +2.6 - +5.5y) [23, 40], even in the early stages of first episode SCZ. Inconsistent findings have been reported in bipolar disorder (BD), with “younger” brain age or no differences compared to controls [11]. While the same sources of variation described above in comparing our findings to previous brain aging findings in MDD also apply here, brain abnormalities might be subtler in MDD compared to BD or SCZ. This is in line with previous ENIGMA studies in SCZ, BD and MDD, showing the largest effect sizes of structural brain alterations in SCZ [43, 44] (highest Cohen’s d effect size -0.53), followed by BD [45, 46] (highest Cohen’s d -0.32) and MDD (highest Cohen’s d -0.14) [14, 15]. Conceivably more in line with MDD pathology [47], Liang and colleagues (2019) showed significantly higher brain-PAD in post-traumatic stress disorder (PTSD) using similar ridge regression and bias correction methods to the current paper [48]. This is consistent with similar effect sizes of structural alterations of individual brain regions observed across MDD and PTSD in large scale studies (highest Cohen’s d -0.17) [49].

Inflammation may be a common biological mechanism between MDD and brain aging [50]. Neuroimmune mechanisms (e.g. pro-inflammatory cytokines) influence biological processes (e.g. synaptic plasticity), and inflammatory biomarkers are commonly dysregulated in depression [51]. One study showed that brain-PAD was temporarily reduced by 1.1 years due to the probable acute anti-inflammatory effects of ibuprofen, albeit in healthy controls [52]. In MDD, both cerebrospinal fluid and peripheral blood interleukin (IL)-6 levels are

elevated [53]. Moreover, work by Kakeda and colleagues (2018) demonstrated a significant inverse relationship between IL-6 levels and surface-based cortical thickness and hippocampal subfields in medication-free, first-episode MDD patients [54]. This accords with the current study that increased brain-PAD was also observed in first-episode patients compared to controls, perhaps suggesting that neuroimmune mechanisms may be chief candidates involved in the brain morphology alterations, even in the early stage of illness. Further, the age-related structural alterations in MDD may also be explained by shared underlying (epi)genetic mechanisms involved in brain development and plasticity (thereby influencing brain structure) and psychiatric illness. For instance, Aberg and colleagues (2018) showed that a significant portion of the genes represented in overlapping blood-brain methylome-wide association findings for MDD was important for brain development, such as induction of synaptic plasticity by BDNF [55].

In terms of individual FreeSurfer measures that contributed most to the brain age prediction, we particularly found widespread negative correlations between predicted brain age and average cortical thickness and subcortical volume, and comparably weaker correlations with surface area features (**Figure 4**). We visualized these associations separately for controls and MDD patients, but findings were similar and suggest comparable structure coefficients in both groups (**Supplementary Figure S2**). Notably, we did not include a spatial weight map of our brain age model, as the weights (although linear) are obtained from a multivariable model, and do not allow for a straightforward interpretation of the importance of the brain regions contributing to the aging pattern. Instead, exploratory analyses pointed out that our model relied most on the cortical thickness features in order to make good predictions. This is consistent with existing literature that supports the importance and sensitivity of cortical thickness towards aging, different from surface areas [56]. However, models including the largest feature set demonstrated the best performance (**Supplementary Tables S8-10**).

Limitations and future directions

While our results are generally consistent with existing literature on advanced or premature biological aging and major depression using other biological indicators, we also have to acknowledge some limitations. First, limited information was available on clinical characterization due to the lack of harmonization of data collection across participating cohorts. However, we provided all participating sites with their brain-PAD estimates, and encouraged them to characterize brain-PAD determinants in more detail (for example,

using more in-depth phenotyping or examining associations with longitudinal outcomes). Second, we did not have access to raw individual-level data and future studies could include higher-dimensional gray matter features or additional modalities such as white matter volumes, hyperintensities and/or microstructure, or functional imaging data to examine whether model fit can be improved. However, we must also appreciate a pragmatic approach for collating data from such a large number of scanning sites. Here, we developed a parsimonious model based on FreeSurfer features collected with standardized ENIGMA extraction scripts to promote model sharing. While pooling harmonized data from many sites increases (clinical) heterogeneity, it also makes predictive models less susceptible to overfitting and more generalizable to other populations,[57] even though this might have come at the cost of lower accuracy [58]. Finally, the large within-group variance regarding the brain-PAD outcome in both controls and MDD (**Figure 3**), compared to the small between-group variance, renders the use of this brain aging indicator for discriminating patients and controls at the individual level difficult. As many of the MDD patients do not show advanced brain aging compared to controls, the clinical significance of the observed higher brain-PAD in MDD patients may be limited. Aberrant brain aging is not specific to MDD [11, 13, 22, 25], and it remains to be elucidated whether age-related brain atrophy is a consequence or cause of MDD. While currently brain age certainly would not constitute a viable biomarker for the diagnosis of depression based on our findings, it could potentially be used to identify those MDD patients at greater risk of poorer brain- or general health outcomes, given previous associations of older-appearing brains relating to cognitive decline, dementia, and death [59–62]. Future longitudinal studies examining the association between brain-PAD and mental, neurological or general health outcomes specifically in individuals with MDD are required to determine whether brain-PAD could provide a clinically useful biomarker.

Conclusions

In conclusion, compared to controls, both male and female MDD patients show advanced brain aging of around 1 year. This significant but subtle sign of advanced aging is consistent with other studies of biological aging indicators in MDD at cellular and molecular levels of analysis (i.e., telomere length and epigenetic age). The deviation of brain metrics from normative aging trajectories in MDD may contribute to increased risk for mortality and aging-related diseases commonly seen in MDD. However, the substantial within-group variance and overlap between groups signify that more (longitudinal)

work including in-depth clinical characterization and more precise biological age predictor systems are needed to elucidate whether brain age indicators can be clinically useful in MDD. Nevertheless, our work contributes to the maturation of brain age models in terms of generalizability, deployability, and shareability, in pursuance of a canonical brain age algorithm. Other research groups with other available information on longitudinal mental and somatic health outcomes, other aging indicators, and incidence and/or prevalence of chronic diseases may use our model to promote the continued growth of knowledge in pursuit of useful clinical applications.

REFERENCES

1. John A, Patel U, Rusted J, Richards M, Gaysina D. Affective problems and decline in cognitive state in older adults: a systematic review and meta-analysis. Psychol Med. 2018:1–13.
2. Vancampfort D, Correll CU, Wampers M, Sienaert P, Mitchell AJ, De Herdt A, et al. Metabolic syndrome and metabolic abnormalities in patients with major depressive disorder: a meta-analysis of prevalences and moderating variables. Psychol Med. 2014;44:2017–2028.
3. Verhoeven JE, Révész D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. Mol Psychiatry. 2013:1–7.
4. Verhoeven JE, Révész D, Picard M, Epel EE, Wolkowitz OM, Matthews KA, et al. Depression, telomeres and mitochondrial DNA: Between- and within-person associations from a 10-year longitudinal study. Mol Psychiatry. 2018;23:850–857.
5. Evans DL, Charney DS, Lewis L, Golden RN, Gorman JM, Krishnan KRR, et al. Mood disorders in the medically ill: scientific review and recommendations. Biol Psychiatry. 2005;58:175–189.
6. Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 2013;11:129.
7. Penninx BWJH. Depression and cardiovascular disease: Epidemiological evidence on their linking mechanisms. Neurosci Biobehav Rev. 2016. 2016. https://doi.org/10.1016/j.neubiorev.2016.07.003.
8. Nock MK, Hwang I, Sampson NA, Kessler RC. Mental disorders, comorbidity and suicidal behavior: results from the National Comorbidity Survey Replication. Mol Psychiatry. 2010;15:868–876.
9. Kessler RC, Bromet EJ, de Jonge P, Shahly V, Wilcox M. The Burden of Depressive Illness. Public Health Perspectives on Depressive Disorders (2017). 2017;40.
10. Jylhava J, Pedersen NL, Hagg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.
11. Cole J, Franke K, Cherbuin N. Quantification of the biological age of the brain using neuroimaging. Healthy ageing and longevity, Biomarkers of Human Aging; 2019.
12. Hatton SN, Franz CE, Elman JA, Panizzon MS, Hagler DJ Jr, Fennema-Notestine C, et al. Negative fateful life events in midlife and advanced predicted brain aging. Neurobiol Aging. 2018;67:1–9.
13. Gaser C, Franke K. 10 years of BrainAGE as an neuroimaging biomarker of brain aging: What insights did we gain? Front Neurol. 2019;10:789.
14. Schmaal L, Veltman DJ, van Erp TGM, Sämann PG, Frodl T, Jahanshad N, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry. 2015:1–7.
15. Schmaal L, Hibar DP, Sämann PG, Hall GB, Baune BT, Jahanshad N, et al. Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group. Mol Psychiatry. 2016;22:900.
16. Lever-van Milligen BA, Lamers F, Smit JH, Penninx BW. Six-year trajectory of objective physical function in persons with depressive and anxiety disorders. Depress Anxiety. 2017;34:188–197.
17. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic Aging in Major Depressive Disorder. Am J Psychiatry. 2018:appi.ajp.2018.1.

18. Darrow SM, Verhoeven JE, Révész D, Lindqvist D, Penninx BWJH, Delucchi KL, et al. The Association Between Psychiatric Disorders and Telomere Length: A Meta-Analysis Involving 14,827 Persons. Psychosom Med. 2016;78:776–787.
19. Lindqvist D, Wolkowitz OM, Picard M, Ohlsson L, Bersani FS, Fernström J, et al. Circulating cell-free mitochondrial DNA, but not leukocyte mitochondrial DNA copy number, is elevated in major depressive disorder. Neuropsychopharmacology. 2018;43:1557–1564.
20. Whalley HC, Gibson J, Marioni R, Walker RM, Clarke T-K, Howard DM, et al. Accelerated epigenetic ageing in depression. 2017. 2017.
21. Han LKM, E Verhoeven J, Tyrka AR, Penninx BW, Wolkowitz OM, Månsson KNT, et al. Accelerating Research on Biological Aging and Mental Health: Current Challenges and Future Directions. Psychoneuroendocrinology. 2019. 5 April 2019. https://doi.org/10.1016/j.psyneuen.2019.04.004.
22. Cole JH, Marioni RE, Harris SE, Deary IJ, Cole JH. Brain age and other bodily ‘ ages ’ : implications for neuropsychiatry. Mol Psychiatry. 2018. 2018. https://doi.org/10.1038/s41380-018-0098-1.
23. Koutsouleris N, Davatzikos C, Borgwardt S, Gaser C, Bottlender R, Frodl T, et al. Accelerated brain aging in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. Schizophr Bull. 2014;40:1140–1153.
24. Besteher B, Gaser C, Nenadić I. Machine-learning based brain age estimation in major depression showing no evidence of accelerated aging. Psychiatry Research: Neuroimaging. 2019;290:1–4.
25. Kaufmann T, van der Meer D, Doan NT, Schwarz E, Lund MJ, Agartz I, et al. Common brain disorders are associated with heritable patterns of apparent aging of the brain. Nat Neurosci. 2019;22:1617–1623.
26. Jahanshad N, Thompson PM. Mini-Review Multimodal Neuroimaging of Male and Female Brain Structure in Health and Disease Across the Life Span. 2017;379:371–379.
27. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage. 2006;31:968–980.
28. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine Learning in Python. J Mach Learn Res. 2011;12:2825–2830.
29. Lemaitre H, Goldman AL, Sambataro F, Verchinski B a., Meyer-Lindenberg A, Weinberger DR, et al. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiol Aging. 2012;33:617.e1–e9.
30. Fjell AM, Chen CH, Sederevicius D, Sneve MH. Continuity and Discontinuity in Human Cortical Development and Change From Embryonic Stages to Old Age. 2018. 2018.
31. Winkler AM, Kochunov P, Blangero J, Almasy L, Zilles K, Fox PT, et al. Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. Neuroimage. 2010;53:1135–1146.
32. Grasby KL, Jahanshad N, Painter JN, ColodroConde L. The genetic architecture of the human cerebral cortex. bioRxiv. 2018. 2018.
33. Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, et al. Distinct genetic influences on cortical surface area and cortical thickness. Cereb Cortex. 2009;19:2728–2735.
34. Le TT, Kuplicki RT, McKinney BA, Yeh H-W, Thompson WK, Paulus MP, et al. A Nonlinear Simulation Framework Supports Adjusting for Age When Analyzing BrainAGE. Front Aging Neurosci. 2018;10:317.
35. Smith SM, Vidaurre D, Alfaro-Almagro F, Nichols TE, Miller KL. Estimation of Brain Age Delta from Brain Imaging. 2019.
36. Breiman L. Random Forests. Mach Learn. 2001;45:5–32.
37. Piñeiro G, Perelman S, Guerschman JP, Paruelo JM. How to evaluate models: Observed vs. predicted or predicted vs. observed? Ecol Modell. 2008;216:316–322.
38. Castrén E, Kojima M. Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. Neurobiol Dis. 2017;97:119–126.
39. Puterman E, Weiss J, Lin J, Schilf S, Slusher AL, Johansen KL, et al. Aerobic exercise lengthens telomeres and reduces stress in family caregivers: A randomized controlled trial - Curt Richter Award Paper 2018. Psychoneuroendocrinology. 2018;98:245–252.
40. Schnack HG, Van Haren NEM, Nieuwenhuis M, Pol HEH, Cahn W, Kahn RS. Accelerated brain aging in schizophrenia: A longitudinal pattern recognition study. Am J Psychiatry. 2016;173:607–616.
41. Funder DC, Ozer DJ. Evaluating Effect Size in Psychological Research: Sense and Nonsense. Advances in Methods and Practices in Psychological Science. 2019;2:156–168.
42. Miller KL, Alfaro-Almagro F, Bangerter NK, Thomas DL, Yacoub E, Xu J, et al. Multimodal population brain imaging in the UK Biobank prospective epidemiological study. Nat Neurosci. 2016;19:1523–1536.
43. van Erp TGM, Walton E, Hibar DP, Schmaal L, Jiang W, Glahn DC, et al. Cortical Brain Abnormalities in 4474 Individuals With Schizophrenia and 5098 Control Subjects via the Enhancing Neuro Imaging Genetics Through Meta Analysis (ENIGMA) Consortium. Biol Psychiatry. 2018. 14 May 2018. https://doi.org/10.1016/j.biopsych.2018.04.023.
44. Van Erp TGM, Hibar DP, Rasmussen JM, Glahn DC, Pearlson GD, Andreassen OA, et al. Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. Mol Psychiatry. 2016;21:547–553.
45. Hibar DP, Westlye LT, van Erp TGM, Rasmussen J, Leonardo CD, Faskowitz J, et al. Subcortical volumetric abnormalities in bipolar disorder. Mol Psychiatry. 2016;21:1710–1716.
46. Hibar DP, Westlye LT, Doan NT, Jahanshad N, Cheung JW, Ching CRK, et al. Cortical abnormalities in bipolar disorder: an MRI analysis of 6503 individuals from the ENIGMA Bipolar Disorder Working Group. Mol Psychiatry. 2018;23:932–942.
47. Sartor CE, Grant JD, Lynskey MT, McCutcheon VV, Waldron M, Statham DJ, et al. Common heritable contributions to low-risk trauma, high-risk trauma, posttraumatic stress disorder, and major depression. Arch Gen Psychiatry. 2012;69:293–299.
48. Liang H, Zhang F, Niu X. Investigating systematic bias in brain age estimation with application to post-traumatic stress disorders. Hum Brain Mapp. 2019;10:1.
49. Logue MW, van Rooij SJH, Dennis EL, Davis SL, Hayes JP, Stevens JS, et al. Smaller Hippocampal Volume in Posttraumatic Stress Disorder: A Multisite ENIGMA-PGC Study: Subcortical Volumetry Results From Posttraumatic Stress Disorder Consortia. Biol Psychiatry. 2018;83:244–253.
50. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244–254.
51. Wohleb ES, Franklin T, Iwata M, Duman RS. Integrating neuroimmune systems in the neurobiology of depression. Nat Rev Neurosci. 2016;17:497–511.

52. Le TT, Kuplicki R, Yeh HW, Aupperle RL, Khalsa SS, Simmons WK, et al. Effect of Ibuprofen on BrainAGE: A Randomized, Placebo-Controlled, Dose-Response Exploratory Study. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2018:1–8.
53. Wang AK, Miller BJ. Meta-analysis of Cerebrospinal Fluid Cytokine and Tryptophan Catabolite Alterations in Psychiatric Patients: Comparisons Between Schizophrenia, Bipolar Disorder, and Depression. Schizophr Bull. 2018;44:75–83.
54. Kakeda S, Watanabe K, Katsuki A, Sugimoto K, Igata N, Ueda I, et al. Relationship between interleukin (IL)-6 and brain morphology in drug-naïve, first-episode major depressive disorder using surface-based morphometry. Sci Rep. 2018;8:10054.
55. Aberg KA, Dean B, Shabalin AA, Chan RF, Han LKM, Zhao M, et al. Methylome-wide association findings for major depressive disorder overlap in blood and brain and replicate in independent brain samples. Mol Psychiatry. 2018. 21 September 2018. https://doi.org/10.1038/s41380-018-0247-6.
56. Wang J, Li W, Miao W, Dai D, Hua J, He H. Age estimation using cortical surface pattern combining thickness with curvatures. Med Biol Eng Comput. 2014;52:331–341.
57. Woo C-W, Chang LJ, Lindquist MA, Wager TD. Building better biomarkers: brain models in translational neuroimaging. Nat Neurosci. 2017;20:365–377.
58. Schnack HG, Kahn RS. Detecting neuroimaging biomarkers for psychiatric disorders: Sample size matters. Front Psychiatry. 2016;7.
59. Cole JH, Ritchie SJ, Bastin ME, Valdés Hernández MC, Muñoz Maniega S, Royle N, et al. Brain age predicts mortality. Mol Psychiatry. 2017:1–8.
60. Franke K, Gaser C. Longitudinal Changes in Individual *BrainAGE* in Healthy Aging, Mild Cognitive Impairment, and Alzheimer's Disease 1Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni. GeroPsych . 2012;25:235–245.
61. Gaser C, Franke K, Klöppel S, Koutsouleris N, Sauer H. BrainAGE in Mild Cognitive Impaired Patients: Predicting the Conversion to Alzheimer's Disease. PLoS One. 2013;8.
62. Liem F, Varoquaux G, Kynast J, Beyer F, Kharabian Masouleh S, Huntenburg JM, et al. Predicting brain-age from multimodal imaging data captures cognitive impairment. Neuroimage. 2017;148:179–188.

SUPPLEMENTARY MATERIALS

Brain aging in Major Depressive Disorder

Image exclusion criteria

A neuroimaging expert at each scanning site inspected each image segmentation by overlaying the segmentation label of each structure on the T1-weighted brain scan. Additionally, study-wide statistics were collected (means and standard deviations) as well as histogram plots to identify non-normally distributed data and major outliers. Samples were excluded if its FreeSurfer feature was >2.698 standard deviations away from the global mean. If a sample was marked as a statistical outlier, the individual site was asked to re-inspect the subject's segmentation in order to verify that it was properly segmented. If a sample was a statistical outlier, yet properly segmented, it was kept in the dataset. Otherwise, the sample was removed.

Quality checking and sample exclusion criteria

The initial dataset included 35 scanning sites with an age range of 7-89 years old based on N=8,369 samples. However, due to scarcity of samples around the upper age boundary and to develop an adult model in the context of aging, we excluded those below 18 years and above 75 years old. Subsequently, the chronological age variable was floored, as some sites included one or two decimals in their age variable, while others did not. We checked individual FreeSurfer features for missings and excluded participant samples with >10% missing data, suggestive of poor reliability. The above criteria led to an exclusion of N=713 participants, resulting in the total sample of N=7,656. The total sample of (N=7,656) was partitioned into datasets of controls and major depressive disorder (MDD) patients, separately for males and females, including N=2,158 male controls and N=1,139 male MDD patients, and N=2,532 female controls and N=1,827 female MDD patients.

Data partitioning

We divided healthy controls from each of the scanning sites into separate training (subset to train the model) and test samples (subset to test the trained model) using a balanced split-half approach. The 50:50 data partitioning was performed following random sampling within each of these scanning sites while preserving the chronological age distribution between training and

test data using the createDataPartition function from the "*caret*" package in R. Whereas multiple approaches to partitioning datasets intro training and test samples were possible, we would like to explain our motivation for the specific approach used. By dividing the control subjects from each scanning site into equal parts of training and test data, we optimally maximize: a) the chronological age distribution and variety of scanning sites in our training set to increase generalizability, and b) the statistical power and sample size of MDD patients for subsequent statistical analyses in the test sets to answer our main research question. To clarify, not all scanning sites cover the wide age range (18-75 years of age) and chronological age and scanning site were therefore correlated.

Following an alternative data partition approach, we could train our model on control subjects from scanning sites (e.g. A, B, C) to test on a held-out set of other scanning sites (e.g. X, Y, Z). However, with that approach, we would need to exclude the corresponding MDD patients from those sites (A, B, C) from subsequent statistical analyses performed on the test data (i.e. controls vs. MDD patients). This is because the model would be trained on controls from the same scanning sites as those patients (both A, B, C), whereas the remaining patients and controls would be from completely independent scanning sites (X, Y, Z). If MDD patients from those scanning sites would be included, it would introduce a bias of learned patterns from the scanning sites for a part of the MDD patients (A, B, C) but not for the other part of MDD patients or any of the healthy controls (X, Y, Z). As a wider variety of scanners also improves the subsequent generalization to independent unseen data, we have opted for the data partition approach reported in the main manuscript. This is further supported by the generalization of our multisite models to the ENIGMA Bipolar Disorder working group dataset collected from 23 independent scanning sites.

Scanning sites with less than 20 control samples were excluded from the data partitioning, ensuring that training and test datasets both included at least 10 samples. Corresponding MDD patients from those scanning sites were also excluded. Thus, 16 scanning sites remained in the male sample (N=279 participants were excluded), compared to 22 scanning sites in the female sample (N=97 participants were excluded). The final training sample consisted of N=952 male controls and N=1,236 female controls. The final test samples consisted of N=927 male controls and N=986 depressed males and N=1,199 female controls and N=1,689 female depressed patients.

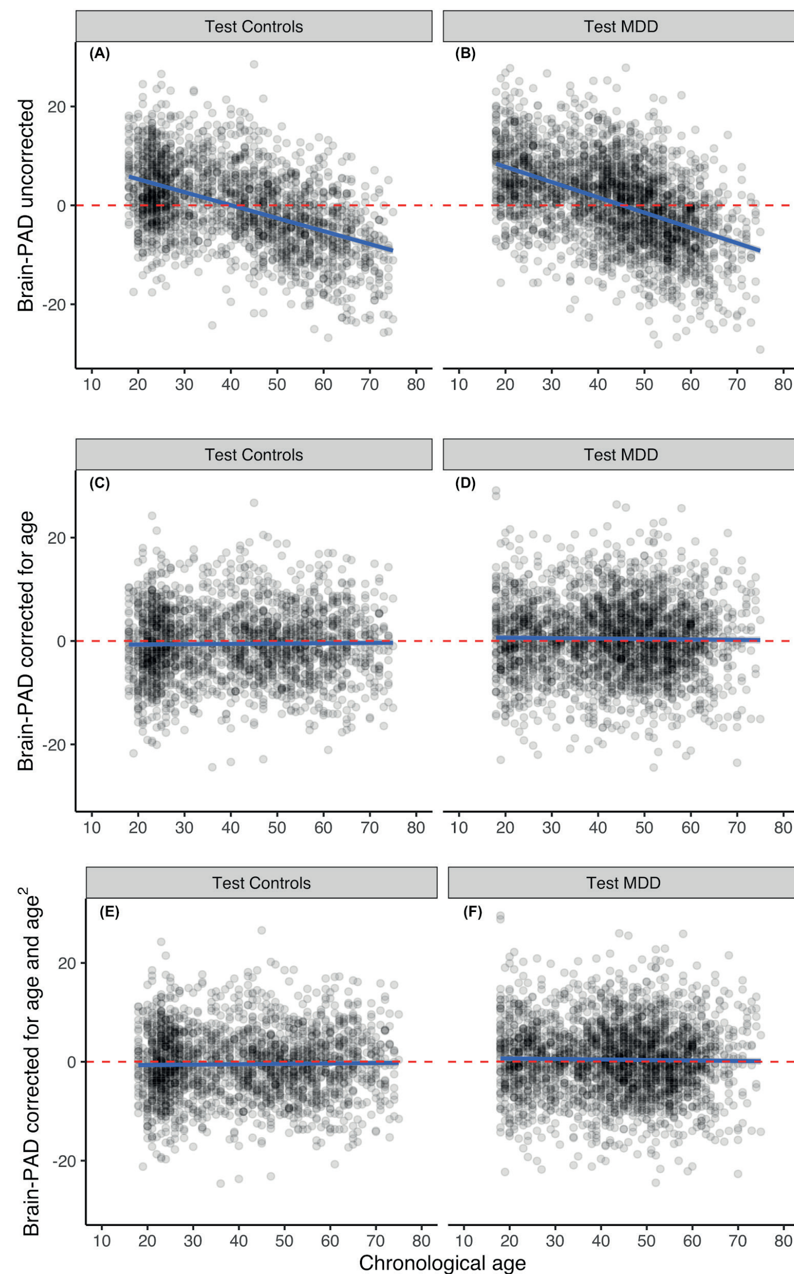
Brain age prediction framework

The correlation between brain-PAD and chronological age

We observed a significant correlation between brain-PAD and chronological age (**Supplementary Figure S1A and S1B; Supplementary Table S7**), which is a known phenomenon in any brain age prediction framework. Since age is the variable being predicted, the model is fitted to minimize the error around predicted age and not the error around observed age. This commonly leads to young people being systematically predicted to be older and old people to be systematically predicted younger. Currently, three excellent papers describe this "regression dilution" phenomenon in brain age prediction models and propose solutions to statistically deal with this bias [1–3]. We would like to emphasize that the aim and scope of our paper were to test whether MDD patients show higher brain-PAD than controls. To this aim we have applied a statistical method in which we account for this bias (further described below) to answer this research question, thus our conclusions are not affected by the systematic effect of the regression dilution.

Correcting the linear and nonlinear age dependence of brain-PAD

Regression dilution, described above, creates a dependence between chronological age and predicted age. To control for this effect, we have included chronological age as a covariate in subsequent analyses (**Supplementary Figure S1C and S1D)** as proposed in [1]. This removes all linear age dependence of our outcome variable. However, one can assume that not all aging effects are perfectly linear. More specifically, the morphology of the brain follows nonlinear trajectories in the young and adolescent [4], but we may potentially also expect an acceleration in older ages, specifically with respect to (mental) illness [2, 5]. As Smith and colleagues (2019) suggest the bias in brain age estimation and the nonlinear dependence can be adjusted for. To statistically correct for the nonlinear age effects on the brain-PAD metric, we included quadratic age terms in our models to test group differences in our test samples (**Supplementary Figure S1E and S1F**). Important to note, we formally tested the goodness of fit of models including age only, age and age^2, and age, age^2 and age^3 as covariates, and found that the model including age and quadratic age terms statistically showed the best model fit.



Supplementary Figure S1. Brain-PAD was significantly negatively correlated to chronological age (overall r=-0.48, p<0.0001) in both controls (A) and MDD patients (B). After linear correction brain-PAD was not significantly correlated to chronological age (r=-2.59E-15, p=1) in neither controls (C) nor MDD patients (D). Although difficult to visually detect, additional nonlinear correction for age^2 resulted in a better model fit in both controls (E) and MDD patients (F) compared to correction for linear age effects only ($\chi_{(2)}$=9.73, p<0.002).

Statistical analyses of case-control comparison of brain-PAD

By including age and nonlinear age effects as covariates in all statistical models, we statistically adjusted for the systematic age bias but also for any other potentially confounding effects of age in our analyses. Moreover, we demonstrate that there were no significant interactions with age or age^2 and MDD status. Thus, the residual age effects in the brain-PAD metric did not influence our main finding with regard to case-control differences.

Alternative machines/kernels

To explore the effect of different machines and kernels, we repeated the 10-fold cross-validation training using Support Vector Regression (SVR) and Random Forest Regression (RFR) in comparison to the Ridge Regression. To model non-linear multivariate patterns, we also explored radial basis function (RBF) kernels, as compared to linear kernel methods. Important to mention here is that all machine learning algorithms showed similar performances (**Supplementary Table S6**). Given the aim to make our model publicly available, we opted for the Ridge Regression emphasizing its deployability and shareability. In contrast to the RBF kernels, Ridge Regression allows for sharing model weights at the feature level for making predictions in new independent test samples, without sharing any actual data points or support vectors from the training data. This ensures that no individual level data is shared.

Alternative feature selection: single modality trained models

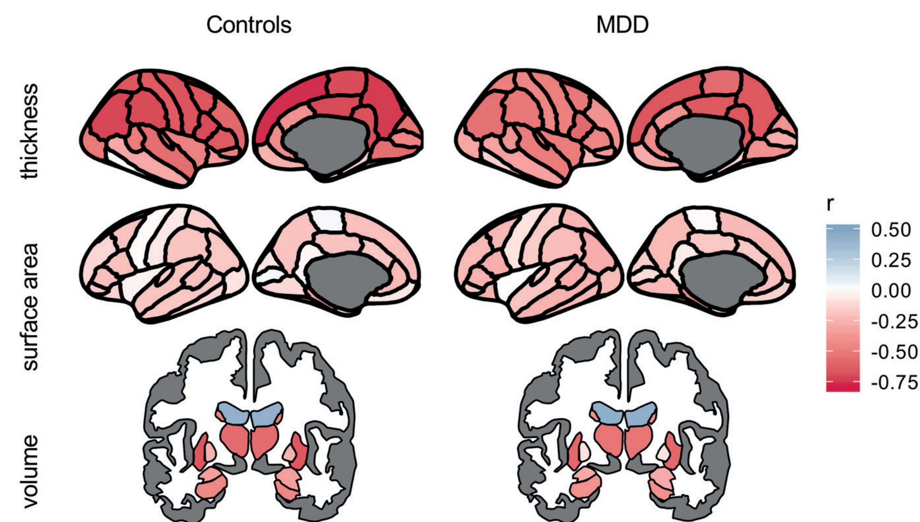
We explored if certain selections of features would optimally explain the age variance in the data. Therefore, we built three separate models with the aim to reduce feature space by including only single modalities (only cortical thickness vs. cortical surface area vs. subcortical volume features). The cortical thickness and intracranial volume (ICV) and subcortical volume and ICV showed reasonable performance accuracy (MAE=7.53-8.95), but a model only trained on surface area features and ICV performed the worst (MAE=10.9 years in both males and females). Combining all 77 features and maximizing the feature set resulted in the most superior performance accuracy (MAE=6.3-6.6), **Supplementary Table S8**. Please note that we used the following formula implemented in the *caret* package in *R* to calculate R^2:

$$R^2 = 1-\frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y}_i)^2}$$

This formula also allows negative expressions of R^2, indicating that despite the high correlation between chronological age and predicted brain age, the data are not well-explained by the model.

Feature importance: structure coefficients

All features, except the mean lateral ventricle volume, and entorhinal and temporal pole thickness showed a negative correlation with predicted brain age, and are visualized in **Figure 4** in the main manuscript. Widespread negative correlations with average cortical thickness and surface area were observed, although thickness features resulted in stronger negative correlations (mean Pearson r [SD]: -0.44 [0.21]) than surface area features (-0.17 [0.08]). On average, subcortical volumes were slightly less negatively correlated to predicted brain age as thickness features (-0.34 [0.34]). We also visualized these associations separately for controls and MDD patients, but findings were similar and suggest comparable structure coefficients in both groups (**Supplementary Figure S2**).



Supplementary Figure S2. Structure coefficients of predicted brain age and FreeSurfer features between control and major depressive disorder (MDD) groups. Bivariate correlations are shown for illustrative purposes and to show the similarity of patterns between controls and MDD patients. The figure shows Pearson correlations between predicted brain age and cortical thickness features (*top row*), cortical surface areas (*middle row*), and subcortical volumes (*bottom row*). The negative correlation with intracranial volume (ICV) was excluded from this figure for display purposes.

Feature importance of modalities

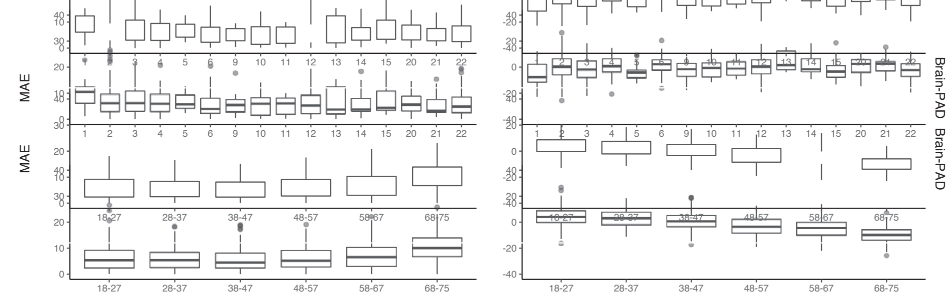
To get a sense of how important a feature is for prediction, we systematically set certain features to zero in all test samples. Overall, cortical thickness features seem to be important for obtaining good predictions, as the MAE increases to >98 years when perturbed (**Supplementary Table S9**). The MAEs were equally affected in control and MDD patients, suggesting that the features important for making accurate brain age predictions were similar across groups.

Qualitative comparison

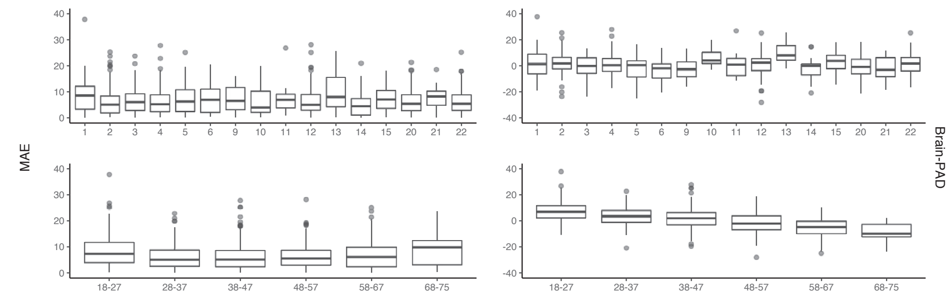
To qualitatively compare the MDD patients with the highest decile of brain-PAD to the bottom 90%, we have provided structure coefficients between predicted brain age and all features. In short, the parahippocampal surface area showed the largest differential structure coefficient, with a diminished reduction in the top 10% compared to the bottom 90%. The parahippocampal region is an essential input region to the hippocampus, a structure that is commonly implicated in MDD. The reported increased parahippocampal surface area in MDD by Qui and colleagues (2014) is in line with the current observation, however, this surface area difference could not be replicated by Peng et al. (2015).[6, 7] Taken together, we observed that particularly thickness features were more negatively associated with predicted brain age in the top 10% highest brain-PAD patients compared to the bottom 90%, while this was vice versa for surface area features (**Supplementary Table S10**).

Generalizability to independent test samples from the ENIGMA MDD working group

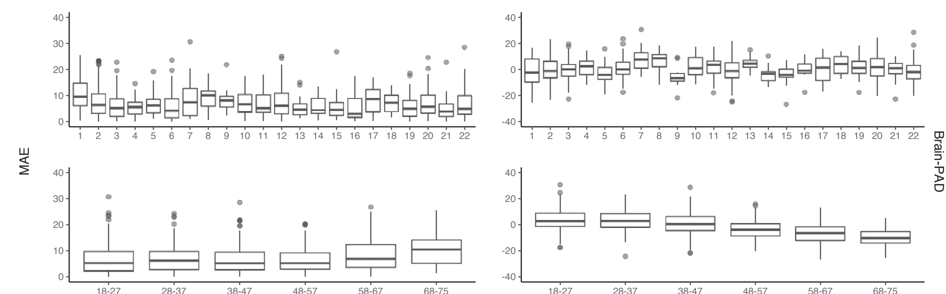
The brain age prediction model generalized well to unseen samples. The overall correlations between predicted brain age and chronological age in the out-of-sample test controls from the ENIGMA MDD working group were r=0.85, P<0.001; R^2=0.72 for males and r=0.83,p<0.001; R^2=0.69 for females. Similarly, the performances in the MDD test samples were r=0.77, p<0.001; R^2=0.57 for males, and r=0.78, p<0.001; R^2=0.59) for females. Of note here is that prediction errors were similar, but not equal between sites and age groups (**Supplementary Figures S3-6**). More specifically, the mean absolute error (MAE) was highest in the oldest age group (68-75 years old, mean 10.25 [6.59]), although this group was relatively small (N=166 out of N=4,801). Brain predicted age difference (brain-PAD) was significantly negatively associated with chronological age (overall r=-0.48, p<0.0001).



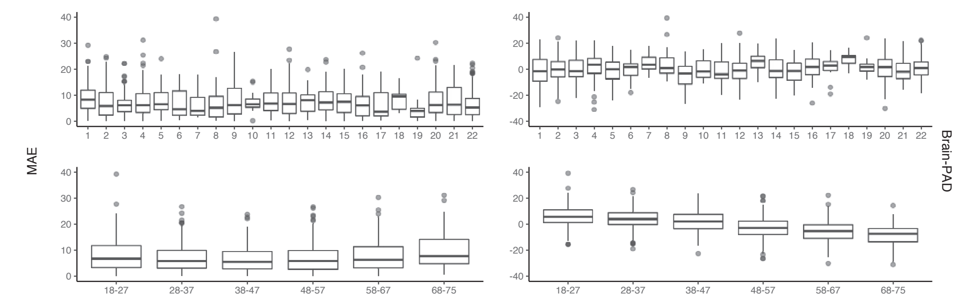
Supplementary Figure S3. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) across scanning site and age group for the male control test samples. Top row figures illustrate scanning sites on the x-axis. Prediction errors were examined across 16 different scanning sites and six different age groups of ten-year bins.



Supplementary Figure S4. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) across scanning site and age group for the male major depression disorder (MDD) test samples. Top row figures illustrate scanning sites on the x-axis. Prediction errors were examined across 16 different scanning sites and six different age groups of ten-year bins.



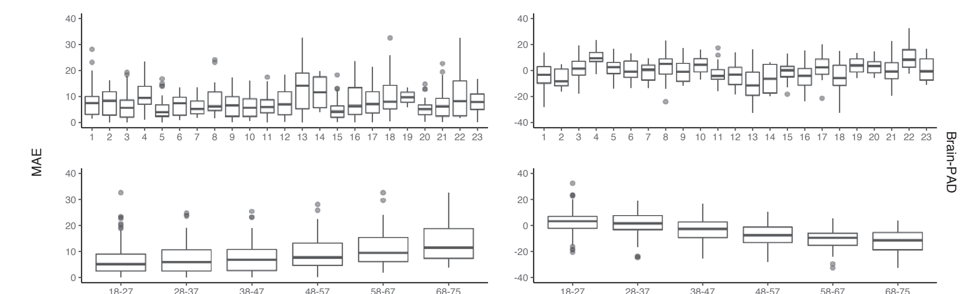
Supplementary Figure S5. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) across scanning site and age group for the female control test samples. Top row figures illustrate scanning sites on the x-axis. Prediction errors were examined across 22 different scanning sites and six different age groups of ten-year bins.



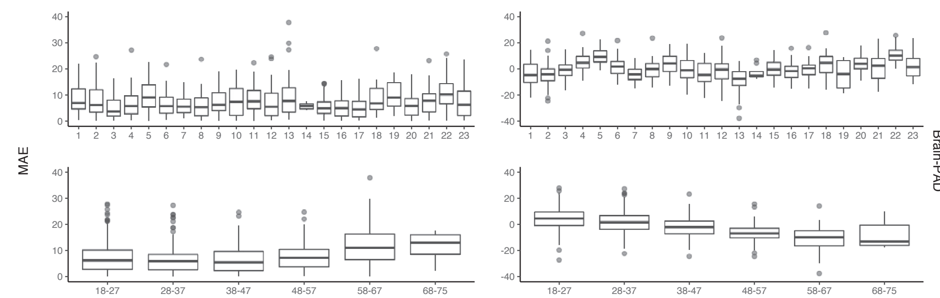
Supplementary Figure S6. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) across scanning site and age group for the female major depression disorder (MDD) test samples. Top row figures illustrate scanning sites on the x-axis. Prediction errors were examined across 22 different scanning sites and six different age groups of ten-year bins.

Generalizability to completely independent healthy controls from the ENIGMA BD working group

The brain age prediction models generalized well to healthy controls from completely independent samples (i.e. independent scanning sites) from the ENIGMA Bipolar Disorder (BD) working group (**Supplementary Figures 7-8**). The MAE was 7.49 (5.89) years in males and 7.26 (SD 5.63) in females, slightly higher than the MAE in the test samples of the ENIGMA MDD working group. The overall correlations between predicted brain age and chronological age in the out-of-sample controls were r=0.71, p<0.001; R^2=0.45 for males and r=0.72, p<0.001; R^2=0.48 for females.



Supplementary Figure S7. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) across scanning site and age group for the male control test sample from the ENIGMA Bipolar Disorder (BD) working group. Top row figures illustrate scanning sites on the x-axis. Prediction errors were examined across 23 different scanning sites and six different age groups of ten-year bins.



Supplementary Figure S8. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) across scanning site and age group for the female control test sample from the ENIGMA Bipolar Disorder (BD) working group. Top row figures illustrate scanning sites on the x-axis. Prediction errors were examined across 23 different scanning sites and six different age groups of ten-year bins.

153

ENIGMA MDD Brain Age Model publicly available

FreeSurfer is an automated and widely used software tool (http://surfer.nmr.mgh.harvard.edu/). Thus, our brain age algorithm can be easily applied to independent data, promoting validation and replication across different samples worldwide needed to mature modeling efforts, contributing to the development of canonical brain age models. To this aim, we will make our FreeSurfer-based brain age model publicly available at https://www.photon-ai.com/ upon publication. Detailed instructions and guidelines for its use will be made available on the webpage. It is, however, important to note that prediction errors were higher in older age groups (>60 years old) and brain-PAD was significantly negatively associated with chronological age (r=-0.53 males, r=-0.48 females, both p's<0.0001), with the latter being a known feature of the brain-PAD metric.[1] Thus, caution is warranted when applying our model to data from older participants (>60 years). We recommend to: a) only use our models to samples with an upper age limit of 60 years, and b) always include residual chronological age effects as covariates in the analyses.

REFERENCES

1. Le TT, Kuplicki RT, McKinney BA, Yeh H-W, Thompson WK, Paulus MP, et al. A Nonlinear Simulation Framework Supports Adjusting for Age When Analyzing BrainAGE. Front Aging Neurosci. 2018;10:317.
2. Smith SM, Vidaurre D, Alfaro-Almagro F, Nichols TE, Miller KL. Estimation of Brain Age Delta from Brain Imaging. 2019.
3. Liang H, Zhang F, Niu X. Investigating systematic bias in brain age estimation with application to post-traumatic stress disorders. Hum Brain Mapp. 2019;10:1.
4. Wierenga LM, Langen M, Oranje B, Durston S. Unique developmental trajectories of cortical thickness and surface area. Neuroimage. 2014;87:120–126.
5. Fjell AM, Westlye LT, Grydeland H, Amlien I, Espeseth T, Reinvang I, et al. Critical ages in the life course of the adult brain: nonlinear subcortical aging. Neurobiol Aging. 2013;34:2239–2247.
6. Qiu L, Lui S, Kuang W, Huang X, Li J, Li J, et al. Regional increases of cortical thickness in untreated, first-episode major depressive disorder. Transl Psychiatry. 2014;4:e378.
7. Peng D, Shi F, Li G, Fralick D, Shen T, Qiu M, et al. Surface vulnerability of cerebral cortex to major depressive disorder. PLoS One. 2015;10:e0120704.

BRAIN AGING IN MAJOR DEPRESSIVE DISORDER - SUPPLEMENTARY TABLES

Supplementary Table S1. ENIGMA - Major Depressive Disorder Working Group Demographics. Age (in years), and MDD patients-control breakdown per participating cohort, separately for males and females, and training and test samples.

	Cohort	Control training samples			
		Males		Females	
		N	Age (years)	N	Age (years)
1	Barcelona	NA	NA	12	45.67 ± 8.85
2	BiDirect	109	51.41 ± 8.17	110	53.32 ± 8.00
3	CLiNG	66	25.86 ± 6.20	96	24.66 ± 4.94
4	Dublin	39	34.09 ± 9.53	38	33.83 ± 10.42
5	Edinburgh (Bipolar Family Study)	NA	NA	22	22.55 ± 2.34
6	FOR2107 - Marburg	64	32.53 ± 11.02	100	33.20 ± 12.73
7	FOR2107 - Münster	20	27.65 ± 10.03	37	24.78 ± 6.19
8	Houston	18	39.28 ± 11.98	37	37.38 ± 13.28
9	BRDECC London	15	50.67 ± 8.49	17	50.88 ± 10.69
10	McMaster University Mood Disorders	NA	NA	15	32.07 ± 11.35
11	Melbourne	19	21.16 ± 2.65	20	20.95 ± 2.48
12	MPIP	47	47.42 ± 12.34	65	48.07 ± 13.41
13	Münster Neuroimaging Cohort	154	36.28 ± 11.92	205	35.07 ± 12.08
14	QTIM	NA	NA	100	21.68 ± 2.33
15	Sao Paolo (Wellcome)	NA	NA	20	32.40 ± 8.15
16	SHIP/TREND	254	50.05 ± 13.96	199	49.65 ± 13.78
17	SHIP	115	54.54 ± 11.47	95	53.92 ± 11.42
18	Stanford	12	36.67 ± 9.41	19	36.26 ± 10.50
19	Sydney	20	46.65 ± 22.36	29	43.28 ± 23.27
	Total	952		1236	

(*continued on next page*)

Supplementary Table S1. *(continued)*

	Cohort	Control test samples				MDD test samples			
		Males		Females		Males		Females	
		N	Age (years)	N	Age (years)	N	Age (years)	N	Age (years)
1	Barcelona	NA	NA	11	46.00 ± 7.90	NA	NA	49	46.67 ± 7.90
2	BiDirect	108	51.33 ± 8.17	108	52.61 ± 8.06	231	47.96 ± 7.41	346	49.51 ± 7.17
3	CLiNG	64	24.84 ± 3.53	95	25.26 ± 5.53	23	38.70 ± 9.74	26	34.19 ± 12.77
4	Dublin	36	32.95 ± 10.86	32	34.28 ± 12.19	40	36.65 ± 9.49	47	38.08 ± 10.61
5	Edinburgh (Bipolar Family Study)	NA	NA	20	22.65 ± 2.54	NA	NA	11	22.82 ± 2.99
6	FOR2107 - Marburg	63	33.29 ± 12.05	98	33.47 ± 13.51	105	37.12 ± 13.58	171	37.71 ± 13.53
7	FOR2107 - Münster	17	26.53 ± 8.27	35	26.49 ± 11.25	21	32.43 ± 11.38	27	33.22 ± 13.47
8	Houston	18	38.22 ± 11.02	35	37.26 ± 12.51	23	39.83 ± 13.82	55	38.67 ± 13.42
9	BRDECC London	14	52.00 ± 5.99	15	53.47 ± 5.37	22	45.64 ± 10.01	47	48.89 ± 8.26
10	McMaster University Mood Disorders	NA	NA	12	32.67 ± 13.27	NA	NA	26	35.85 ± 13.13
11	Melbourne	16	20.75 ± 2.32	19	20.68 ± 2.21	27	20.48 ± 2.31	35	20.14 ± 1.90
12	MPIP	45	47.70 ± 13.22	63	49.55 ± 12.20	157	47.81 ± 13.17	204	46.80 ± 13.31
13	Münster Neuroimaging Cohort	152	35.23 ± 11.23	202	35.65 ± 12.68	115	36.98 ± 11.24	156	38.63 ± 12.35
14	QTIM	NA	NA	97	21.58 ± 1.89	NA	NA	44	21.69 ± 2.06
15	Sao Paolo (Wellcome)	NA	NA	20	32.90 ± 9.53	NA	NA	17	28.59 ± 7.90
16	SHIP/TREND	252	49.89 ± 13.95	199	49.93 ± 13.14	106	47.33 ± 9.80	200	49.34 ± 12.40
17	SHIP	111	54.21 ± 12.23	93	53.53 ± 11.74	36	52.06 ± 9.55	95	52.89 ± 10.96
18	Stanford	11	35.27 ± 11.89	17	38.35 ± 11.11	23	37.22 ± 9.59	33	36.21 ± 10.75
19	Sydney	20	44.80 ± 21.83	28	42.93 ± 22.29	57	38.93 ± 21.13	100	40.20 ± 20.08
	Total	927		1199		986		1689	

Age reflects chronological age (mean ± SD in years). MDD, major depressive disorder. Total N=6,989.

Supplementary Table S2. **ENIGMA - Major Depressive Disorder Working Group Clinical characteristics of MDD patients.**

	Cohort	Males		Females		Males		Females		Males		Females	
		AD Free (%)	AD User (%)	AD Free (%)	AD User (%)	First (%)	Recurrent (%)	First (%)	Recurrent (%)	Current (%)	Remitted (%)	Current (%)	Remitted (%)
1	Barcelona	NA	NA	4	96	NA	NA	31	69	NA	NA	41	59
2	BiDirect	12	88	13	87	54	46	58	42	0	100	0	100
3	CLiNG	0	100	12	88	57	43	38	62	9	91	4	96
4	Dublin	59	41	17	83	31	69	28	72	0	100	0	100
5	Edinburgh (Bipolar Family Study)	NA	NA	73	27	NA	NA	NA	NA	NA	NA	NA	NA
6	FOR2107 - Marburg	41	59	35	65	26	74	28	72	17	83	25	75
7	FOR2107 - Münster	19	81	52	48	45	55	37	63	24	76	33	67
8	Houston	100	0	100	0	29	71	23	77	0	100	5	95
9	BRDECC London	23	77	30	70	0	100	0	100	NA	NA	NA	NA
10	McMaster University Mood Disorders	NA	NA	35	65	NA	NA	35	65	NA	NA	0	100
11	Melbourne	78	22	69	31	29	71	33	67	0	100	0	100
12	MPIP	15	85	17	83	31	69	25	75	13	87	14	86
13	Münster Neuroimaging Cohort	7	93	8	92	25	75	23	77	9	91	8	92
14	QTIM	NA	NA	80	20	NA	NA	NA	NA	NA	NA	NA	NA
15	Sao Paolo (Wellcome)	NA	NA	53	47	NA	NA	33	67	NA	NA	0	100
16	SHIP/TREND	87	13	81	20	44	56	33	68	NA	NA	NA	NA
17	SHIP	78	22	83	17	58	42	55	45	NA	NA	NA	NA
18	Stanford	60	40	56	44	9	91	13	87	0	100	0	100
19	Sydney	51	49	29	71	28	72	13	87	91	9	82	18

Percentage of MDD patients using antidepressant (AD) medication, percentage of first episode and recurrent episode MDD patients, percentage of acutely depressed and remitted MDD patients breakdown for participating sites, separately for male and female samples. *(continued on next page)*

Supplementary Table S2. *(continued)*

	Cohort	Males	Females	Males	Females	Males	Females
		Age of Onset MDD (years)		HDRS-17[a]		BDI-II[b]	
1	Barcelona	NA	32.43 ± 11.44	NA	12.61 ± 8.84	NA	NA
2	BiDirect	39.50 ± 9.97	37.86 ± 11.34	12.51 ± 6.76	14.33 ± 6.61	NA	NA
3	CLiNG	34.52 ± 9.99	26.69 ± 9.81	20.53 ± 4.47	19.33 ± 4.05	20.63 ± 14.47	22.64 ± 6.38
4	Dublin	29.29 ± 10.61	27.37 ± 11.29	23.58 ± 4.54	23.55 ± 5.37	21.42 ± 5.48	19.75 ± 4.43
5	Edinburgh (Bipolar Family Study)	NA	22.50 ± 2.78	NA	7.20 ± 6.86	NA	NA
6	FOR2107 - Marburg	26.68 ± 13.46	26.48 ± 12.58	8.43 ± 6.18	8.13 ± 6.52	19.76 ± 10.28	18.42 ± 11.54
7	FOR2107 - Münster	25.86 ± 10.12	24.93 ± 11.31	8.86 ± 7.23	9.15 ± 7.61	18.25 ± 12.28	15.26 ± 11.70
8	Houston	22.65 ± 9.80	21.12 ± 11.13	11.96 ± 8.50	9.67 ± 7.71	14.79 ± 13.85	17.49 ± 15.61
9	BRDECC London	17.56 ± 5.73	21.89 ± 10.42	NA	NA	17.11 ± 13.43	17.51 ± 11.46
10	McMaster University Mood Disorders	NA	24.54 ± 9.76	NA	12.00 ± 6.54	NA	NA
11	Melbourne	17.68 ± 2.63	17.03 ± 3.10	NA	NA	NA	NA
12	MPIP	35.28 ± 13.75	32.99 ± 12.89	25.99 ± 6.29	26.91 ± 7.10	13.44 ± 9.63	14.45 ± 11.31
13	Münster Neuroimaging Cohort	29.41 ± 11.81	30.14 ± 11.79	21.18 ± 6.79	21.81 ± 7.81	23.35 ± 9.31	26.29 ± 11.10
14	QTIM	NA	18.57 ± 2.86	NA	NA	NA	NA
15	Sao Paolo (Wellcome)	NA	NA	NA	15.64 ± 9.54	NA	NA
16	SHIP/TREND	35.84 ± 12.85	35.96 ± 14.44	NA	NA	12.05 ± 8.24	12.53 ± 8.02
17	SHIP	39.03 ± 12.90	37.67 ± 12.76	NA	NA	11.81 ± 9.37	12.01 ± 10.81
18	Stanford	20.43 ± 9.34	18.84 ± 9.25	NA	NA	29.13 ± 10.90	22.78 ± 8.29
19	Sydney	27.76 ± 19.43	24.76 ± 14.33	11.55 ± 6.47	12.53 ± 7.09	NA	NA

Age of onset of MDD and severity of symptoms breakdown for participating sites, separately for male and female samples.

[a] Measured with the Hamilton Depression Rating Scale (HDRS-17; range: 0-52)
[b] Measured with the Beck Depression Inventory (BDI-II; range: 0-63)

Supplementary Table S3. ENIGMA - Major Depressive Disorder Working Group Image acquisition and processing by cohort.

Cohort	Country	Scanner type	Sequence T1	FreeSurfer version	Slice orientation	Operating system
Barcelona	**Spain**	3T Philips Achieva	3D MPRAGE images (Whole-brain T1-weighted); TR=6.7ms, TE=3.2ms; 170 slices, voxel size 0.89X0.89X1.2 mm. Image dimensions 288X288X170; field of view: 256X256X204; slice thickness: 1.2 mm; with a sagittal slice orientation, T1 contrast enhancement, flip angle: 8°, grey matter as a reference tissue, ACQ matrix MXP = 256X240 and turbo-field echo shots (TFE) = 218.	6	Sagittal	Scientific Linux 5
BiDirect	**Germany**	3 T Philips Intera scanner	3D T1-weighted turbo field echo images were collected with a the following parameters: TR = 7.26, TE = 3.56, 9° flip angle, 160 sagittal slices, matrix dimension 256 x 256, FOV = 256 x 256mm, 2mm slice thickness (reconstructed to 1mm) and a resulting voxel size of 1x1x1mm	5.3	Sagittal	
Edinburgh (Bipolar Family Study)	**Scotland**	1.5T GE Signa	T1-weighted sequence. TR=500 msec; TE=4 msec; flip angle 8°; matrix 192 x 192; 180 slices; voxel size 1.25 mm x 1.25 mm x 1.20 mm; FOV=24, phase FOV 1	5.3	Coronal	linux 6, x86_64, kernel 2.6.32
BRCDECC London	**England**	1.5T GE Signa HDx	ADNI-1 MPRAGE pulse sequence (details at http://adni.loni.ucla.edu/research/protocols/mri-protocols/)	5.3	Sagittal	Linux-centos4_x86_64
CliNG	**Germany**	3T Siemens Tim Trio	T1-weighted 3D MPRAGE; TR/TE/TI/FA=2250 ms/3.26 ms/900 ms/9°; image matrix = 256 x 256; 192 sagittal slices; voxel size= 1 mm3	5.3	Sagittal	Linux
Dublin	**Ireland**	3T Phillips Achieva; 1.5T Siemens Vision	3T: A sagittal T1 3D TFE was used to scan all participants. TR=8.5 msec; TE=3.9 msec; FOV = 256 mm, AP: 256 mm, RL: 160 mm; matrix: 256×256. 1.5T: 3D-MPRAGE T1-weighted sequence. TR=11.6 msec; TE=4.9 msec; FOV=230 mm; matrix 512 x 512, slice thickness: 1.5 mm.	5.3	Sagittal (3T), Coronal (1.5T)	Mac OS
FOR2107 - Marburg	**Germany**	3T Siemens Magnetom TiroTim syngo MR B17	Sequence: 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) - Sagittal Acquisition Direction, # of Slices 176, 0.5mm Slice Gap, 1.0x1.0x1.0 Voxel Size (mm3), TI 900 ms, TE 2.26 ms, TR 1900 ms, Flip Angle 9.	5.3	Sagittal	Red Hat Enterprise Linux Server release 5.11 (Tikanga)
FOR2017 - Münster	**Germany**	3T Siemens PRISMA	Sequence: 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE). - Sagittal Acquisition Direction, # of Slices 192, 0mm Slice Gap, 1.0x1.0x1.0 Voxel Size (mm3), TI 900 ms, TE 2.28 ms, TR 1900 ms, Flip Angle 8	5.3	Sagittal	Red Hat Enterprise Linux Server release 5.11 (Tikanga)
Houston	**USA**	subjects in 20000s: 1.5 T Philips Medical Systems Gyroscan Intera; subjects in 30000s: 3T Siemens Allegra	Subjects in the 20000s: Fast field echo sequence-repetition time (TR) = 24 ms, echo time (TE) = 4.99 ms, flip angle = 40°, slice thickness = 1 mm, matrix size = 256 × 256 and 150 slices. Subjects in 30000s: MPRAGE- repetition time (TR) = 1750 ms, echo time (TE) = 4.39 ms, flip angle = 8°, slice thickness = 1 mm, matrix size = 208 × 256 and 160 slices.	5.3	Subjects in 20000s: Sagittal; Subjects in 30000s: Transverse	Fedora 19
McMaster University Mood Disorders	**Canada**	1.5T (GE); 3T(GE)	1.5-T. Sigma GE Genesis-based Echo-Speed scanner running version 5.7 software and using a standard 30-cm circularly polarized head coil. Sagittal anatomic images were acquired by using a 3D/FSPGR/20 sequence (flip angle=20; echo delay time in-phase (TE), minimum repetition time (TR)=300 ms; inversion recovery=300 ms; matrix=512x256; field of view (FOV)=24 cm; scan thickness=1.2 mm). 3-T MRI Sigma GE Genesis (General Electric Medical Systems, Milwaukee, WI). Sagittal T-1 weighted images were acquired using a 3D FSPGR-IR sequence, (TR/TE=10.3/2.1 ms; flip angle=20; inversion time=300; matrix=512x256; FOV=24; and slice thickness=1.2 mm.	5		
Melbourne	**Australia**	3T GE Signa Excite	3D BRAVO sequence 140; TR=7900 ms; TE=3000 ms; flip angle=13°; FOV=256 mm; matrix=256 x 256	5.3	Axial	Linux Debian x86 64

MPIP	**Germany**	1.5T GE and Siemens (the latter: only few cases)	#1: T1-weighted SPGR sagittal 3D volume. TR=1030 msec; TE=3.4 msec; 124 slices; matrix=256x256; FOV=23.0x23.0 cm2; voxel size=0.8975 mm x0.8975 mm x 1.2- 1.4 mm; flip angle=90°; birdcage resonator. #2: same scanner as #1, platform update Signa Excite, sagittal T1-weighted (spin echo sequence, TR=9.7 msec, TE=2.1 msec; FOV=25.0x25.0 cm2, voxel size=0.875 mm x0.875 mm x1.2 mm, 124- 132 slices, flip angle=90°. #3: Siemens 1.5 Tesla, Vario, 3D MPRAGE, TR=11.6 msec; TE=4.9 msec; FOV 23x23 cm2; matrix 512x512; 126 axial slices; voxel site 0.45 mm x 0.45 mm x 1.5 mm. (only N=2 subjects)	5.3	1.5 GE: sagittal. 1.5 Siemens: axial	Linux 2.6.37.1-1.2-desktop x86_64
Münster Neuroimaging Cohort	**Germany**	3T Philips Gyroscan Intera	3D fast gradient echo sequence (turbo field echo), repetition time = 7.4 milliseconds, echo time = 3.4 milliseconds, flip angle = 9°, two signal averages, inversion prepulse every 814.5 milliseconds, acquired over a field of view of 256 (feet -head [FH]) × 204 (anterior -posterior [AP]) × 160 (right -left [RL]) mm, phase encoding in AP and RL direction, reconstructed to cubic voxels of .5 mm × .5 mm × .5 mm	5.3	Sagittal	Red Hat Enterprise Linux Server release 5.11 (Tikanga)
QTIM	**Australia**	Bruker 4T Wholebody MRI	3D T1 weighted sequence. TR=1500 msec; TE=3.35 msec; flip angle=8°, 256 or 240 (coronal or sagittal) slices, FOV=240 mm, matrix 256x256x256 (or 256x256x240)	5.1	Coronal, then sagittal following software upgrade.	Linux- centos4_ x86_64- stable-pub-v5.1.0
Sao Paulo (Wellcome)	**Brasil**	1.5T General Eletric (GE)	Imaging data were acquired using two MRI scanners (at the Clinics Hospital of the University of Sa˜o Paulo 1.5 T GE Signa scanner, General Electric, Milwaukee Wisconsin, USA). T1-SPGR sequence providing 124 contiguous slices, voxel size 0.8660.8661.5 mm, echo time 5.2 ms, resolution time 21.7 ms, flip angle 20, field of vision 22, matrix 256x192)	5.3		

SHIP	**Germany**	1.5T Siemens Avanto	3D T1-weighted (MP-RAGE/ axial plane); TR=1900 msec; TE=3.4 msec; Flip angle=15°; voxel size 1 mm x 1 mm x 1 mm	5.3 (cortical), 5.1 (subcortical)	Axial	Centos6_x86_64
SHIP/TREND	**Germany**	1.5T Siemens Avanto	3D T1-weighted (MP-RAGE/ axial plane); TR=1900 msec; TE=3.4 msec; Flip angle=15°; voxel size 1 mm x 1 mm x 1 mm	5.3 (cortical), 5.1 (subcortical)	Axial	Centos6_x86_64
Stanford	**USA**	1.5T GE Signa Excite	Whole-brain T1-weighted images were collected using a spoiled gradient echo (SPGR) pulse sequence (116 sagittal slices; through-plane resolution = 1.5 mm; in-plane resolution = 0.86 x 0.86 mm; flip angle = 15 degrees; repetition time [TR] = 8.3-10.1 ms; echo time [TE] = 1.7-3.0; inversion time [TI] = 300 ms; matrix = 256 x 192).	5.3	Sagittal	Linux-centos6_ x86_64
Sydney	**Australia**	3T GE MR750	3D T1-weighted sequence. TR=7.2 msec; TE=2.78 msec; matrix =256; FOV=240; No. slices=196; thick=0.9mm; inplane resolution=0.9375	5.1 but rerunning it for 5.3	Coronal	Linux_Ubuntu16.04 lts 64bit

Supplementary Table S4. ENIGMA - Major Depressive Disorder Working Group Instrument for diagnosing Major Depressive Disorder and exclusion criteria by site.

Cohort	Country	Diagnosis measurment	Sample characteristics/Inclusion criteria	Exclusion criteria
Barcelona	**Spain**	DSM-IV-TR acc. to CIDI-interview and HAMD	Outpatients with MDD diagnosis (DSM-IV-TR), with a first episode, recurrent MDD or chronic MDD (TRD) age 18-65	The exclusion criteria for healthy participants were. lifetime psychiatric diagnoses, first-degree relatives with psychiatric diagnoses and clinically significant physical or neurological illnesses. Axis I comorbidity according to DSM-IV-TR criteria was an exclusion criteria for all participants.
BiDirect	**Germany**	M.I.N.I. Neuropsychiatric Interview, IDS, HAMD, CESD, ICD-10	Patients hospitalized for a first or recurrent episode of depression, population controls randomly selected in city registry	dementia, addiction
Edinburgh (Bipolar Family Study)	**Scotland**	SCID interview	The MDD group were originally people with a FHx of bipolar disorder	MDD subjects: presence of other axis I diagnoses. Control subjects: no medical history, including neurological and psychiatric history, as well as no previous or actual use of psychotropic medication All subjects: any major neurological disorder, learning disability, or any history of head injury that included loss of consciousness and any contraindications to MRI.
BRCDECC London	**England**	SCAN interview	Community based or outpatients, none were inpatients. MDD subjects: Less than two depressive episodes of at least moderate severity. Did not meet DSM-IV diagnostic criteria for recurrent major depressive disorder. Control group participants were clinically interviewed to ensure they had never experienced depressive symptoms. Exclusion criteria for all participants were for contraindications to MRI; other exclusion criteria were a diagnosis of neurological disorder, head injury leading to loss of consciousness or conditions known to affect brain structure or function (including alcohol or substance misuse), ascertained during clinical interview. Potential participants were also excluded if they or a first-degree relative had ever fulfilled criteria for mania, hypomania, schizophrenia or mood-incongruent psychosis.	Contraindications to MRI, diagnosis of neurological disorder, head injury leading to loss of consciousness or conditions known to affect brain structure or function (including alcohol or substance misuse), if they or a first-degree relative had ever fulfilled criteria for mania, hypomania, schizophrenia or mood-incongruent psychosis.
CliNG	**Germany**	ICD-10 interview	Patients met the diagnostic criteria for major depressive disorder according to ICD-10 classification standards and were aged between 18 and 60 years.	Exclusion criteria for MDD subjects were neurological and severe other medical conditions (in particular those that could be related to affective symptoms), lifetime diagnosis of substance dependence, substance abuse during the last month, cannabis abuse during the last 2 weeks, mental retardation as well as past or actual presence of other axis I diagnoses with exception of anxiety disorders. Exclusion criteria for control subjects were neurological, psychiatric and severe other medical conditions, lifetime diagnosis of substance dependence, substance abuse during the last month, cannabis abuse during the last 2 weeks, previous or actual use of psychotropic medication, and mental retardation.
Dublin	**Ireland**	SCID-1 interview		MDD subjects: comorbid psychiatric disorders (Axis I or Axis II, other than MDD), Treatment with antipsychotics or mood stabilizers, age 65, Control subjects: no Axis-I diagnosis, no medication use. All subjects: history of neurological or other severe medical illness, head injury or severe substance abuse in their lifetime history and general MRI contraindications.
FOR2107 - Marburg	**Germany**	SCID-1	Participants recruited by means of public advertisement and from the inpatient services. Inclusion criteria: age 18-65 years; patients were diagnosed with major depressive disorder by SCID-Interview, currently depressed or remitted.	Exclusion criteria all: any MRI contraindications; any neurological abnormalities. Exclusion criteria controls: any current or former psychiatric disorder; Exclusion criteria patients: substance dependence or current benzodiazepine treatment (wash out of at least three half-lives before study participation)"
FOR2017 - Münster	**Germany**	SCID-1	Participants recruited by means of public advertisement and from the inpatient services. Inclusion criteria: age 18-65 years; patients were diagnosed with major depressive disorder by SCID-Interview, currently depressed or remitted.	Exclusion criteria all: any MRI contraindications; any neurological abnormalities. Exclusion criteria controls: any current or former psychiatric disorder; Exclusion criteria patients: substance dependence or current benzodiazepine treatment (wash out of at least three half-lives before study participation)"

Houston	**USA**	SCID interview	Outpatients	MDD subjects: age below 18; lifetime or current diagnosis of psychotic disorder, or bipolar I or II disorder; substance abuse/dependence in 6 months prior to study inclusion; current major medical problems. Control subjects: age below 18; current major medical problems; current psychiatric or neurologic disorder; history of psychiatric disorders in a first-degree relative; current major medical problems. Both groups: MRI contra-indications
McMaster University Mood Disorders	**Canada**	SCID	Outpatients	Comorbid Axis 1 disorders excluded, including for example, psychosis, bipolar, PTSD substance dependence or current active eating disorder. Exclusion criteria included: i) treatment with anti-cholinergic or typical (first generation) anti-psychotic medication; ii) electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS) within the past year; iii) a history of substance dependence or significant and recent (< 1 year) substance abuse; iv) a history (within the past 12 months) of an endocrine or other medical disorder known to adversely affect cognition (e.g., Cushing's, uncontrolled diabetes, seizure disorder); and v) English comprehension lower than a grade 6 reading level.
Melbourne	**Australia**	SCID interview	Youth depression sample: 15-25 years of age. Recruited as part of 2 large RCTs (incl. YoDA-C - Davey et al., 2014; Trials) and scanned prior to treatment randomisation. 60 patients unmedicated (YoDA-C).	MDD subjects: lifetime or current SCID-I diagnosis of psychotic disorder, or bipolar I or II disorder. Control subjects: any SCID-I diagnosis or medication use. Both groups: Acute or unstable medical disorder; general MRI contraindications

MPIP	**Germany**	M-CIDI/SCAN interview	M. A. R. S. sample: both first and recurrent episodes; RUD sample: only recurrent episodes with some patients scanned in remission	Munich Antidepressant Response Signature (MARS) study MDD subjects (clinical consensus diagnosis or M-CIDI (since 2008)): depressive syndromes secondary to any medical or neurological condition (e. g., intoxication, drug abuse, stroke), the presence of manic, hypomanic or mixed affective symptoms, lifetime diagnosis of alcohol dependence, illicit drug abuse or the presence of severe medical conditions (e.g., ischemic heart disease). Patients with bipolar depression were excluded for the current MR study. Control subjects: age > 65, MMSE<27, presence of severe somatic diseases or lifetime history of the following axis I disorders as assessed by the M-CIDI interview: alcohol dependence, drug abuse or dependence, possible psychotic disorder, mood disorder, anxiety disorder including OCD and PTSD, somatoform disorder, dissociative disorder NOS, and eating disorder 2. Recurrent unipolar depression (RUD) study: MDD subjects (SCAN interview): presence of manic episodes, mood incongruent psychotic symptoms, the presence of a lifetime diagnosis of intravenous drug abuse and depressive symptoms only secondary to alcohol or substance abuse or to medical illness or medication.Control subjects: presence of severe somatic diseases or life-time history of anxiety and affective disorders according to the Composite International Diagnostic-Screener (CIDI-S). All subjects: gross incidental MR findings such as territorial infarction, tumor, hydrocephalus, malformations and anatomical deviations (e.g. enlarged ventricles) that prevent appropriate image processing were additional exclusion criteria. 3. MR images of 9 additional controls acquired at the LMU, Munich, meeting equivalent criteria as the RUD control sample were included.
Münster Neuroimaging Cohort	**Germany**	SCID interview	Participants recruited by means of public advertisement and from the inpatient services. Inclusion criteria: age 16-65 years; patients were diagnosed with major depressive disorder by SCID-Interview	MDD subjects: presence of bipolar disorder, schizoaffective disorders and schizophrenia; substancerelated disorders or current benzodiazepine treatment (wash out of at least three half-lives before study participation), and former electroconvulsive therapy. Control subjects: any current or former psychiatric disorder. Both groups: any neurological abnormalities, MRI contra-indications

QTIM	**Australia**	CIDI interview	Retrospective questionnaire about depression episodes combined with an MRI study. The best described MDD episode is defined as the worst one (according to individuals). We have up to 5 supplementary episodes (briefly) described. Sample composed of twins and relatives. Population-based sample	MDD subjects: presence of axis-I disorders other than MDD and anxiety disorders Control subjects: antidepressant use, psychiatric disorders All subjects: relatedness between subjects, left handedness, history of neurological or other severe medical illness, head injury or current or past diagnosis of substance abuse, use of cognition affecting medication and general MRI contraindications
Sao Paulo (Wellcome)	**Brasil**	Hamilton Rating Scale for Depression (HRSD)	Population-based study of incident (first-episode) psychosis in outpatient services. All subjects we provided were diagnosed with psychotic depression (and not schizophrenia, bipolar disorder or other psychotic diagnoses).	People with psychotic disorders due to a general medical condition or substance-induced psychosis were excluded. Additional exclusion criteria were: (a) history of head injury; (b) presence of neurological disorders or any organic disorders that could affect the central nervous system; and (c) contraindications for MRI. Exclusion cri- teria specific for the control group were personal history of psychosis or other Axis I disorders, except substance misuse or mild anxiety disorders.
SHIP	**Germany**	M-CIDI interview	Population based longitudinal cohort study	MDD subjects: presence of axis-I disorders other than MDD, anxiety disorders, conversion, somatization and eating disorder. Control subjects: no lifetime diagnosis of depression, no antidepressiva, and severity index=0 All subjects: We removed subjects with medical conditions (e.g. a history of cerebral tumor, stroke, Parkinson's diseases, multiple sclerosis, epilepsy, hydrocephalus, enlarged ventricles, pathological lesions) or due to technical reasons (e.g. severe movement artifacts or inhomogeneity of the magnetic field).
SHIP/TREND	**Germany**	M-CIDI interview	Population based longitudinal cohort study	MDD subjects: no special exclusion criteria Control subjects: no lifetime diagnosis of depression, no antidepressiva, and severity index=0 All subjects: We removed subjects with due to medical conditions (e.g. a history of cerebral tumor, stroke, Parkinson's diseases, multiple sclerosis, epilepsy, hydrocephalus, enlarged ventricles, pathological lesions) or due to technical reasons (e.g. severe movement artifacts or inhomogeneity of the magnetic field).

Stanford	**USA**	SCID interview	Community-based DSM-diagnosed sample	MDD subjects: presence of axis-I disorders other than MDD, anxiety and eating disorders . Control subjects: control individuals did not meet diagnostic criteria for any current psychiatric. Both groups: alcohol / substance abuse or dependence within six months prior to MRI scanning, history of head trauma with loss of consciousness > 5 min, aneurysm, or any neurological or metabolic disorders that require ongoing medication or that may affect the central nervous system (including thyroid disease, diabetes, epilepsy or other seizures, or multiple sclerosis), MRI contraindications, or bad MRI data (e.g., extreme movement).
Sydney	**Australia**	SCID interview		MDD subjects: presence of axis-I disorders other than MDD, panic disorder, social anxiety disorder, or generalized anxiety disorder. Control subjects: no Axis-I diagnosis, no medication use. Exclusion criteria for all subjects included medical instability (as determined by a psychiatrist), history of neurological disease (e.g. tumour, head trauma, epilepsy), medical illness known to impact cognitive and brain function (e.g. cancer), intellectual and/or developmental disability and insufficient English for neuropsychological assessment. All subjects were asked to abstain from drug or alcohol use for 48 hours prior to testing and informed about a drug screen protocol.

Supplementary Table S5. A full list of the 77 gray matter FreeSurfer features included in our brain age model.

Modality	Feature	Anatomical ROI
	1	ICV
Subcortical volume	2	Mvent
	3	Mthal
	4	Mcaud
	5	Mput
	6	Mpal
	7	Mhippo
	8	Mamyg
	9	Maccumb
Mean cortical thickness	10	M_bankssts_thickavg
	11	M_caudalanteriorcingulate_thickavg
	12	M_caudalmiddlefrontal_thickavg
	13	M_cuneus_thickavg
	14	M_entorhinal_thickavg
	15	M_fusiform_thickavg
	16	M_inferiorparietal_thickavg
	17	M_inferiortemporal_thickavg
	18	M_isthmuscingulate_thickavg
	19	M_lateraloccipital_thickavg
	20	M_lateralorbitofrontal_thickavg
	21	M_lingual_thickavg
	22	M_medialorbitofrontal_thickavg
	23	M_middletemporal_thickavg
	24	M_parahippocampal_thickavg
	25	M_paracentral_thickavg
	26	M_parsopercularis_thickavg
	27	M_parsorbitalis_thickavg
	28	M_parstriangularis_thickavg
	29	M_pericalcarine_thickavg
	30	M_postcentral_thickavg
	31	M_posteriorcingulate_thickavg
	32	M_precentral_thickavg
	33	M_precuneus_thickavg
	34	M_rostralanteriorcingulate_thickavg
	35	M_rostralmiddlefrontal_thickavg
	36	M_superiorfrontal_thickavg
	37	M_superiorparietal_thickavg
	38	M_superiortemporal_thickavg
	39	M_supramarginal_thickavg
	40	M_frontalpole_thickavg
	41	M_temporalpole_thickavg
	42	M_transversetemporal_thickavg
	43	M_insula_thickavg
Mean cortical surface area	44	M_bankssts_surfavg
	45	M_caudalanteriorcingulate_surfavg
	46	M_caudalmiddlefrontal_surfavg
	47	M_cuneus_surfavg
	48	M_entorhinal_surfavg
	49	M_fusiform_surfavg
	50	M_inferiorparietal_surfavg
	51	M_inferiortemporal_surfavg
	52	M_isthmuscingulate_surfavg
	53	M_lateraloccipital_surfavg
	54	M_lateralorbitofrontal_surfavg
	55	M_lingual_surfavg
	56	M_medialorbitofrontal_surfavg
	57	M_middletemporal_surfavg
	58	M_parahippocampal_surfavg
	59	M_paracentral_surfavg
	60	M_parsopercularis_surfavg
	61	M_parsorbitalis_surfavg
	62	M_parstriangularis_surfavg
	63	M_pericalcarine_surfavg
	64	M_postcentral_surfavg
	65	M_posteriorcingulate_surfavg
	66	M_precentral_surfavg
	67	M_precuneus_surfavg
	68	M_rostralanteriorcingulate_surfavg
	69	M_rostralmiddlefrontal_surfavg
	70	M_superiorfrontal_surfavg
	71	M_superiorparietal_surfavg
	72	M_superiortemporal_surfavg
	73	M_supramarginal_surfavg
	74	M_frontalpole_surfavg
	75	M_temporalpole_surfavg
	76	M_transversetemporal_surfavg
	77	M_insula_surfavg

Left and right features were averaged across hemisphere.

Supplementary Table S6. Mean absolute error (MAE) and brain predicted age difference (brain-PAD) per age group.

Age group	Brain-PAD		MAE	
	Male test samples (N=2,256)	Female test samples (N=3,370)	Male test samples (N=2,256)	Female test samples (N=3,370)
18-27 years	5.56 (7.91)	4.65 (7.72)	7.40 (6.22)	7.11 (5.53)
28-37 years	3.10 (6.97)	3.65 (7.62)	6.11 (4.56)	6.77 (5.04)
38-47 years	1.34 (7.41)	1.75 (7.95)	5.90 (4.66)	6.49 (4.90)
48-57 years	-2.37 (7.13)	-3.18 (7.61)	6.15 (4.30)	6.61 (4.93)
58-67 years	-5.06 (7.30)	-6.14 (7.76)	7.07 (5.38)	7.93 (5.92)
68-75 years	-9.51 (6.77)	-9.55 (8.29)	10.00 (6.02)	10.47 (7.08)

Values were calculated in the overall test samples of controls and major depressive disorder (MDD) patients.

Supplementary Table S7. Alternative machines and kernels in the brain age prediction framework.

Machine learning algorithm	R		R^2		MAE	
	Male training sample	Female training sample	Male training sample	Female training sample	Male training sample	Female training sample
Ridge regression	0.85	0.84	0.73	0.71	6.75	6.86
SVR linear	0.85	0.84	0.72	0.71	6.86	6.91
SVR RBF	0.85	0.87	0.73	0.75	6.50	6.09
RFR	0.79	0.80	0.67	0.64	6.81	7.22

Performance metrics in the training samples of males and females across four different machine learning algorithms/kernels are displayed here. R, Pearson's correlation; R2, explained variance; MAE, mean absolute error.

Supplementary Table S8. Alternative feature selection in the brain age prediction framework.

Ridge Regression	R		R^2		MAE	
	Male training sample	Female training sample	Male training sample	Female training sample	Male training sample	Female training sample
Subcortical volumes and ICV	0.75	0.72	0.56	0.51	8.01	8.95
Cortical thickness and ICV	0.78	0.75	0.61	0.56	7.53	8.24
Cortical surface areas and ICV	0.48	0.53	0.23	0.28	10.93	10.85

Performance metrics under 10-fold cross-validation in the training samples of males and females using features from three different modalities are displayed here. R, Pearson's correlation; R^2, explained variance; MAE, mean absolute error.

Supplementary Table S9. Feature importance of modalities.

Feature importance	Sex	R		R^2		MAE	
		Control test sample	MDD test sample	Control test sample	MDD test sample	Control test sample	MDD test sample
Full model	M	0.85	0.77	0.72	0.57	6.50	6.72
	F	0.83	0.78	0.69	0.59	6.84	7.18
Subcortical volumes	M	0.79	0.71	-6.71	-10.03	41.36	42.52
	F	0.78	0.72	-3.41	-4.22	31.34	30.44
Cortical thickness	M	0.72	0.63	-40.61	-56.45	98.10	98.79
	F	0.69	0.62	-40.21	-48.91	99.98	98.32
Cortical surface area	M	0.82	0.73	-3.01	-4.74	29.35	30.09
	F	0.81	0.75	-1.65	-2.22	23.81	23.39

The full model includes all orginal testdata and 77 features. The rows indicate which features (subcortical volumes [8 features] or cortical thickness [34 features] or cortical surface area [34 features]) were perturbed (values set to zero) in the test samples. Test performance is most negatively affected by the perturbation of cortical thickness features. R, Pearson correlation coefficient; R2, explained variance; MAE, mean absolute error; M, males; F, females.

Supplementary Table S10. A qualitative comparison between structure coefficients.

FreeSurfer Feature	Top 10%	Bottom 90%	Difference
M_parahippocampal_surfavg	-0.18	-0.41	-0.23
M_superiorparietal_surfavg	-0.12	-0.25	-0.14
M_fusiform_surfavg	-0.25	-0.39	-0.14
M_cuneus_surfavg	-0.03	-0.15	-0.12
M_precentral_thickavg	-0.37	-0.49	-0.12
M_paracentral_thickavg	-0.46	-0.58	-0.12
M_parstriangularis_surfavg	-0.15	-0.26	-0.11
M_precuneus_surfavg	-0.12	-0.23	-0.11
M_superiorfrontal_thickavg	-0.58	-0.67	-0.09
M_lingual_surfavg	-0.13	-0.22	-0.09
M_lateraloccipital_surfavg	-0.13	-0.22	-0.09
Mamyg	-0.19	-0.28	-0.09
M_frontalpole_surfavg	-0.04	-0.12	-0.09
Mvent	0.50	0.41	-0.09
M_pericalcarine_surfavg	-0.02	-0.10	-0.08
Maccumb	-0.51	-0.59	-0.07
M_parsopercularis_thickavg	-0.51	-0.58	-0.07
M_posteriorcingulate_surfavg	-0.10	-0.17	-0.07
M_entorhinal_surfavg	-0.04	-0.11	-0.07
M_bankssts_thickavg	-0.32	-0.39	-0.06
M_precuneus_thickavg	-0.60	-0.66	-0.06
M_cuneus_thickavg	-0.33	-0.39	-0.06
M_superiortemporal_surfavg	-0.13	-0.19	-0.06
M_lateralorbitofrontal_surfavg	-0.20	-0.25	-0.05
M_parsorbitalis_surfavg	-0.12	-0.18	-0.05
M_rostralmiddlefrontal_surfavg	-0.17	-0.22	-0.05
M_inferiorparietal_surfavg	-0.22	-0.27	-0.05
M_postcentral_surfavg	-0.11	-0.16	-0.04
Mcaud	-0.35	-0.39	-0.04
M_postcentral_thickavg	-0.41	-0.45	-0.04
M_supramarginal_surfavg	-0.18	-0.21	-0.04
M_transversetemporal_surfavg	-0.14	-0.18	-0.04
M_rostralanteriorcingulate_thickavg	-0.40	-0.43	-0.03
M_middletemporal_surfavg	-0.22	-0.25	-0.03
M_supramarginal_thickavg	-0.49	-0.52	-0.03
ICV	-0.10	-0.13	-0.03
M_superiorfrontal_surfavg	-0.20	-0.22	-0.03
Mthal	-0.50	-0.53	-0.03
M_caudalmiddlefrontal_thickavg	-0.49	-0.51	-0.03
M_superiorparietal_thickavg	-0.46	-0.49	-0.02
M_caudalanteriorcingulate_surfavg	-0.14	-0.16	-0.02
M_insula_surfavg	0.02	0.00	-0.02
M_isthmuscingulate_surfavg	-0.04	-0.05	-0.02
M_isthmuscingulate_thickavg	-0.64	-0.66	-0.01
M_bankssts_surfavg	-0.26	-0.28	-0.01
M_superiortemporal_thickavg	-0.49	-0.50	-0.01
M_temporalpole_surfavg	-0.07	-0.08	-0.01
M_inferiorparietal_thickavg	-0.54	-0.55	0.00
M_caudalmiddlefrontal_surfavg	-0.22	-0.22	0.00
Mput	-0.59	-0.59	0.00
M_parstriangularis_thickavg	-0.46	-0.46	0.00
M_rostralanteriorcingulate_surfavg	-0.21	-0.20	0.00
M_caudalanteriorcingulate_thickavg	-0.39	-0.39	0.01
M_pericalcarine_thickavg	-0.27	-0.27	0.01
M_medialorbitofrontal_surfavg	-0.14	-0.13	0.01
M_parsopercularis_surfavg	-0.28	-0.26	0.01
M_posteriorcingulate_thickavg	-0.66	-0.65	0.01
Mpal	-0.09	-0.08	0.01
M_rostralmiddlefrontal_thickavg	-0.41	-0.39	0.02
M_frontalpole_thickavg	-0.35	-0.33	0.02

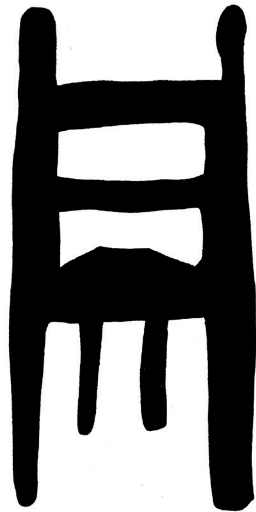
M_inferiortemporal_surfavg	-0.25	-0.22	0.02
M_transversetemporal_thickavg	-0.49	-0.46	0.03
M_lingual_thickavg	-0.43	-0.39	0.03
Mhippo	-0.42	-0.39	0.04
M_parsorbitalis_thickavg	-0.40	-0.36	0.04
M_paracentral_surfavg	-0.05	-0.01	0.04
M_insula_thickavg	-0.55	-0.50	0.05
M_medialorbitofrontal_thickavg	-0.34	-0.28	0.05
M_precentral_surfavg	-0.13	-0.07	0.06
M_temporalpole_thickavg	-0.01	0.05	0.06
M_lateraloccipital_thickavg	-0.39	-0.29	0.10
M_parahippocampal_thickavg	-0.21	-0.11	0.10
M_middletemporal_thickavg	-0.43	-0.31	0.12
M_lateralorbitofrontal_thickavg	-0.34	-0.22	0.12
M_fusiform_thickavg	-0.40	-0.26	0.15
M_inferiortemporal_thickavg	-0.30	-0.13	0.17
M_entorhinal_thickavg	-0.02	0.18	0.20

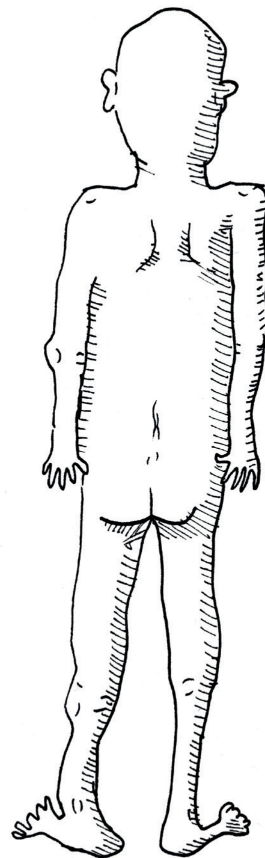
The top 10% column indicates major depressive disorder patients with the highest decile of brain-PAD values. The difference is sorted from smallest to largest and reflects the difference compared to the bottom 90% of brain-PAD values

Contributing Factors to Advanced Brain Aging in Depression and Anxiety Disorders

Submitted for publication

Laura K.M. Han
Hugo G. Schnack
Rachel M. Brouwer
Dick J. Veltman
Nic J.A. van der Wee
Marie-José van Tol
Moji Aghajani
Brenda W.J.H. Penninx





ABSTRACT

Depression and anxiety are common and often comorbid mental health disorders that represent risk factors for aging-related conditions. Brain aging has shown to be more advanced in patients with Major Depressive Disorder (MDD). Here, we extend prior work by investigating multivariate brain aging in patients with MDD and/or anxiety disorders and examine which factors contribute to older appearing brains. Adults aged 18-57 years from the Netherlands Study of Depression and Anxiety underwent structural MRI. A pre-trained brain age prediction model based on >2,000 samples from the ENIGMA consortium was applied to obtain brain-predicted age differences (brain-PAD, predicted brain age minus chronological age) in 65 controls and 220 patients with current MDD and/or anxiety. Brain-PAD estimates were associated with clinical, somatic, lifestyle, and biological factors. After correcting for antidepressant use, brain-PAD was significantly higher in MDD (+2.78 years, Cohen's d=0.25, 95% CI -0.10-0.60) and anxiety patients (+2.91 years, Cohen's d=0.27, 95% CI -0.08-0.61), compared to controls. There were no significant associations with lifestyle or biological stress systems. A multivariable model indicated unique contributions of higher severity of somatic depression symptoms (b=4.21 years per unit increase on average sum score) and antidepressant use (-2.53 years) to brain-PAD. Advanced brain aging in patients with MDD and anxiety was most strongly associated with somatic depressive symptomatology. We also present clinically relevant evidence for a potential neuroprotective antidepressant effect on the brain-PAD metric that requires follow-up in future research.

INTRODUCTION

Depression and anxiety are common and often comorbid mental health disorders, and their effects can broadly impact a person's life. There is a plethora of evidence showing poorer quality of life, functional disability, and increased mortality burden in these patients[1, 2]. Depression and anxiety disorders further represent a risk factor for aging-related conditions[3–5], as studies show consistent evidence for poorer somatic and chronic disease profiles in these patient groups[6], often with a premature onset. Importantly, the incidence and burden of these disorders are a strain on society, which has an important challenge to face in the coming years, as the number of people aged >65 is expected to reach 1.6 billion in 2050[7]. Advancing mental health and well-being across the lifespan and into old age should, therefore, be a major priority on the research agenda.

Multivariate pattern recognition techniques, and especially machine learning methods, have promoted a steep increase in the development of ways to measure and quantify aging[8]. Central to this field is that multivariate (biological) patterns are utilized and integrated into a single score: the biological age. Biological age can be derived from, for instance, omics-data (e.g. epigenetic clocks), but also clinical biomarkers obtained from, for example, blood chemistries[9]. In the current study, we focus on biological age based on a validated method of MRI-derived brain structure[10, 11] with brain-predicted age difference (brain-PAD, predicted brain age minus chronological age)[12] as the main outcome. This metric is relative to one's chronological age, such that positive values indicate an older appearing brain, and negative values resemble a younger appearing brain than normally expected at that age.

A handful of studies have investigated brain-PAD in depression, with studies showing +4.0 years[13], as well as no significantly increased brain age[14, 15]. Recent findings from the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium using a more than ten-fold larger pooled sample of MDD patients than the largest previous study suggest a 1.1 year higher brain-PAD in MDD patients as compared to controls[16]. However, this difference did not seem to be driven by specific clinical characteristics (recurrent status, remission status, antidepressant medication use, age of onset, or symptom severity). An important aspect that remains relatively unknown is thus which underlying mechanisms cause the brain age metric to advance in depression, and, despite the increase of brain age studies in the past decade, in general[11].

Large pooled datasets from global consortia offer the statistical power needed to detect small effect sizes usually observed in MDD, but a limitation of consortium data is that its collection is commonly not harmonized across all sites and cohorts. Here, we underline the complementary value of a more homogeneous and clinically well-characterized sample from the Netherlands Study of Depression and Anxiety, to gain more insight into the observed brain-PAD difference between MDD patients and controls. We extend prior work by exploring which specific symptom clusters (mood/cognition, immunometabolic, somatic) of MDD are associated with brain-PAD. To the best of our knowledge, there are currently no brain age studies in anxiety disorders, although higher brain-PAD has been observed in posttraumatic stress disorder[17]. Given the frequent co-occurrence and correlated symptoms[18] of depression and anxiety (i.e. family of internalizing disorders)[19], we also extend prior work by including patients with MDD and/or anxiety disorders in the current study.

Evidence is starting to emerge that brain-PAD is associated with reduced mental and somatic health, such as with stroke history, diabetes diagnosis, smoking, alcohol consumption, and some cognitive measures[20], but also intrinsic measures such as genetic variants[21, 22]. This study seeks to further address the research gaps, by examining whether three commonly dysregulated biological stress systems in depression and anxiety disorders (inflammation, hypothalamic pituitary adrenal [HPA]-axis, autonomic nervous system [ANS]) were predictive of brain aging. Disruptions and dysregulations in these stress systems were hypothesized to result in advanced brain aging across diagnostic groups. We further associated various clinical, lifestyle, and somatic health indicators with the brain-PAD metric for our primary hypothesis to identify unique contributing factors to brain aging.

METHODS AND MATERIALS

Study Sample

A subsample of subjects of the Netherlands Study of Depression and Anxiety (NESDA) were included for the MRI substudy (total N=301). Twelve participants were excluded due to poor image quality, two because of claustrophobia, one control subject due to high depression rating (Montgomery Asberg Depression Rating Scale score >8), and one due to the large time difference between the psychiatric and biological and MRI measurements (total excluded, N=16). For the current study, we therefore included N=65 controls (65% female, aged 21-55) and N=220 patients with a current depressive and/or anxiety disorder

(69% female, aged 18-57). The current study was approved by the ethical review boards of the three participating centers (Amsterdam, Groningen, Leiden) and informed consent of all participants was obtained.

Image Processing and Analysis

Magnetic resonance imaging (MRI) data were obtained using three independent 3T Philips MRI scanners (Philips Healthcare, Best, The Netherlands) located at different participating centers. Scanners were equipped with a SENSE 8-channel (Leiden University Medical Center and University Medical Center Groningen) and a SENSE 6-channel (Academic Medical Center) receiver head coil (Philips Healthcare). Standardized image segmentation and feature extraction protocols, using the FreeSurfer processing software, developed by the ENIGMA consortium were used (http://enigma.ini.usc.edu/protocols/imaging-protocols/) to extract 153 features from regions of interest, including the volumes of 14 subcortical gray matter regions (bilateral nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus) and the 2 lateral ventricles, cortical thickness and surface area from 68 cortical regions, and total intracranial volume (ICV). Segmentations were statistically examined for outliers and the FreeSurfer feature was excluded if it was >2.698 standard deviations away from the global mean. However, if a sample was a statistical outlier, but visual inspection showed that it was properly segmented, it was kept in the dataset.

FreeSurfer Brain Age Prediction Model

We used a publicly available brain age model (https://www.photon-ai.com/enigma_brainage/) that was trained to predict age from 77 ((left+right hemisphere features)/2 and ICV) FreeSurfer features (for more detail, see[16]. Briefly, the Ridge Regression coefficients learned from 952 male and 1,236 female control subjects (aged 18-75 years) from the ENIGMA MDD working group were applied to the features of the current samples (N=285). Of note, NESDA was not part of the development of this model. The model's generalization performance was assessed by calculating several metrics: a) the correlation between predicted brain age and chronological age, b) the amount of chronological age variance explained by the model (R^2), c) the mean absolute error (MAE) between predicted brain age and chronological age, and d) Root Mean Squared Error (RMSE).

Diagnostic Ascertainment

Participants in the current study included control subjects (no lifetime history of psychiatric disorders) and patients with a current depression and/or current anxiety disorder (i.e. generalized anxiety disorder, panic disorder, social anxiety disorder) within a 6-month recency. The Composite International Diagnostic Interview (CIDI version 2.1) was used as a diagnostic instrument to ascertainment[23].

Clinical Assessment

We examined several clinical variables as predictors, including a) depressive symptoms as measured by the summary score of the Inventory for Depressive Symptoms (IDS) at time of scanning[24], but also b) three separate validated clusters of depressive symptoms (mood/cognition, somatic, and immunometabolic symptoms)[25], c) anxiety symptoms as measured by the summary score of the Beck Anxiety Inventory (BAI) at time of scanning[26], d) cumulative childhood trauma index[27] (before the age of 16) as measured by a childhood trauma interview, and e) recent negative life events in the past year as measured with the Brugha questionnaire[28]. Within the patients only, we also investigated associations with: a) duration of symptoms, b) age of onset of illness, and c) antidepressant medication use (selective serotonin reuptake inhibitors (ATC code N06AB) and other antidepressants (ATC codes N06AF, N06AG, N06AX). See **Supplement** for full details.

Somatic Health Assessment

Body Mass Index (BMI) was assessed during an interview by dividing a person's weight (in kilogram [kg]) by the square of their height (in meter [m]). The number of self-reported current somatic diseases (heart disease, epilepsy, diabetes, osteoarthritis, cancer, stroke, intestinal disorders, ulcers, and lung-, liver-, and thyroid disease) for which participants received medical treatment was counted.

Lifestyle Assessment

Smoking status was expressed by calculating the number of cigarettes smoked per day. Alcohol consumption was expressed as the mean number of drinks consumed per week, measured by the AUDIT[29]. Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) and expressed in total metabolic equivalent (MET) minutes per week[30].

Biological Stress Assessment

We included predictors from three major biological stress systems: a) the immune-inflammatory system (C-reactive protein [CRP], Interleukin-6 (IL6), and tumor necrosis factor-α (TNF-α), b) the hypothalamic pituitary adrenal (HPA)-axis (cortisol awakening response [CAR] and evening cortisol), and c) the autonomic nervous system (ANS: heart rate, respiratory sinus arrhythmia [RSA] and pre-ejection period [PEP]). Details can be found in **Supplement.**

Statistical Analysis

All statistical analyses were performed using R version 3.5.3 (R Core Team, 2019). First, we used linear regressions to examine brain-PAD differences between the control and patient groups and tested brain-PAD associations with several clinical characteristics within the patients only (i.e. duration of symptoms, age of onset of illness, AD use). Second, we used separate linear regression models with brain-PAD as measured outcome and variables of interest as a predictor to explore and select significant contributors in all participants irrespective of diagnostic group. Finally, stepwise regression with forward selection was used to successively add significant contributors to an intercept-only model, starting with the variable that explained most variance and stopping if the model fit did not improve anymore. The best subset of variables leading to the best model fit (i.e. lowest Aikaike's Information Criterion [AIC]) were selected to examine unique contributions to brain-PAD. Inflammatory predictors were $\log_e$-transformed due to highly skewed distributions and subsequently corrected for fasting status and anti-inflammatory medication use. ANS predictors were corrected for fasting status, heart medication use, and mean arterial blood pressure. HPA predictors were corrected for fasting status, awakening time, variable indicating whether it was a working day or not, and season. All biological stress markers >3*sd away from the mean were winsorized. Age, sex, education level (years), and two dummy variables for scan location were included as predictor variables in all models. Analyses were tested two-sided and findings were considered statistically significant at $p<0.05$. All b regression coefficients from all models may be interpreted as added brain aging in years in response to each unit increase of the predictor.

RESULTS

Sample Characteristics

Demographics and assessed phenotypes of the current study sample can be found in **Table 1**. Briefly, the patient group consisted of patients with a current MDD diagnosis but no anxiety (28.2%), patients with a current anxiety disorder but no depression (30.5%), and patients with a current comorbid depression and anxiety disorder (41.4%). The patient group (mean 37.37 ± SD 10.20 years) was younger than the control group (mean 40.81 ± SD 9.78 years) and had fewer years of education (mean 14.28 ± SD 2.86 years in controls vs. mean 12.39 ± SD 3.19 in patients). Control and patient groups were similar in terms of male/female ratios, but not distributed equally between scan locations (Amsterdam, Leiden, Groningen) ($\chi_{(2)}$=6.26, p=0.044).

Brain Age Prediction Performance

Using the ENIGMA brain age model (www.photon-ai.com/enigma_brainage) we obtained a correlation of r=0.73 in the control subjects and r=0.72 in the patient group between predicted and chronological age, but in both groups brain age predictions were overestimated (mean brain-PAD [SD]; 8.18 [7.27] years in controls and 10.86 [7.73] years in patients). To correct for the offset, we calculated the mean brain-PAD in the control group and subtracted these from all individual brain-PAD estimates. This correction resulted in an R^2 of 0.45 and MAE of 5.97 (SD 4.09) years in controls, and R^2 of 0.36 and MAE of 6.73 (4.64) years in patients. Of note, this linear correction does not affect subsequent statistics. **Figure 1A** shows the unaffected correlation between predicted brain age (x-axis) and chronological age (y-axis) in control subjects (r=0.73, p<0.0001) and in patients (r=0.72, p<0.0001). There also was a well-known and commonly described age-bias (i.e. correlation between brain-PAD and age)[17, 31, 32] in controls (r=-0.32, p=0.01) and patients (r=-0.37, p<0.0001) in the current sample (**Figure 1B)**, which was statistically dealt with by including age as a predictor variable in further analyses (**Figure 1C)**[31].

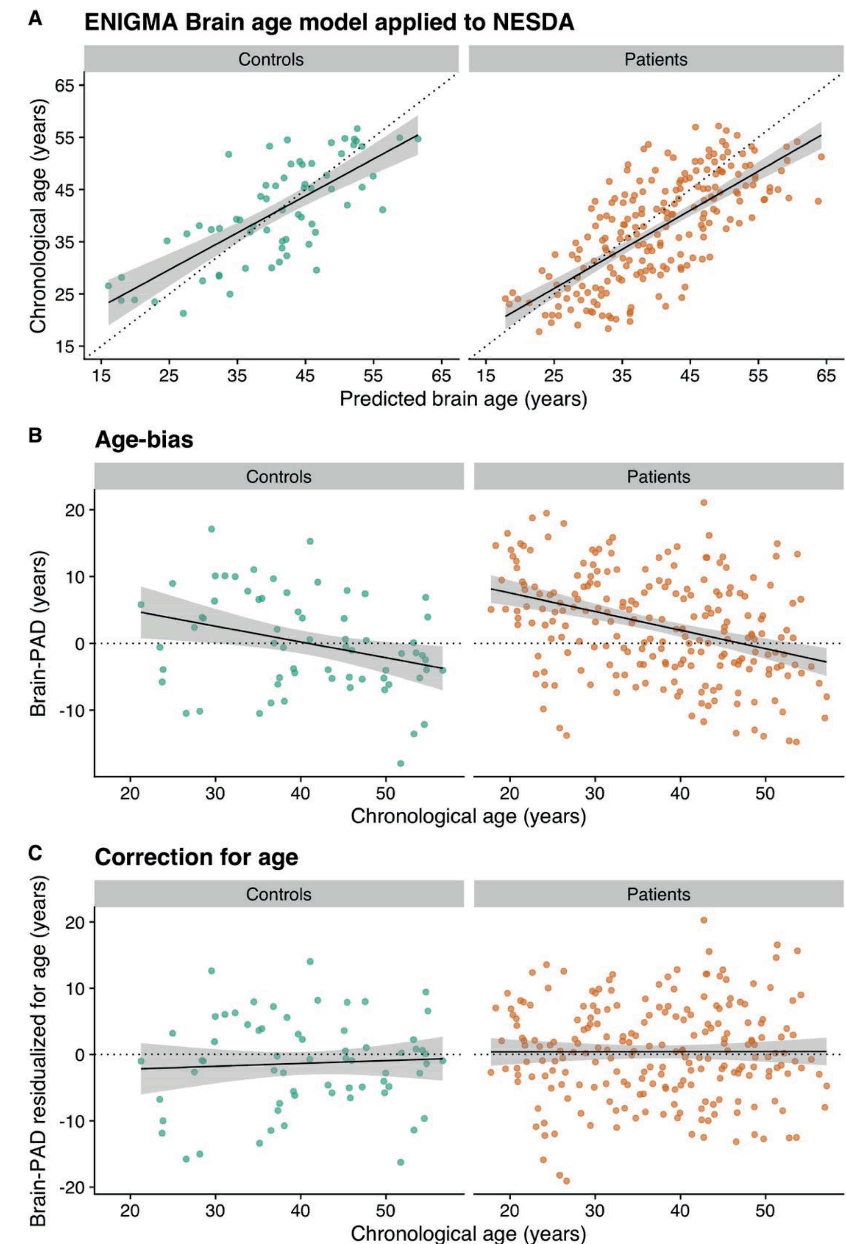


Figure 1. Brain age prediction. (**A**) Correlation between predicted brain age and chronological age in controls (r=0.73, R^2=0.45, p<0.0001) and patients (r=0.72, R^2=0.36, p<0.0001). Of note, predicted brain age reflects estimates corrected for the offset (brain $age_{corrected}$ = brain age - (brain-PAD - mean brain-$PAD_{controls}$). (**B**) There was a residual effect of age on the brain-PAD outcome in controls (r=-0.32, p=0.01) and patients (r=-0.37, p<0.0001), (**C**) which was statistically corrected for by adding age as a covariate in all models.

Table 1. Participant Characteristics of Controls and Patients.

Characteristic	N	Controls, N = 65[a]	Patients, N = 220[a]	p-value[b]
Demographics				
Age (years)	285	40.81 ± 9.78 (21.26-56.67)	37.37 ± 10.20 (17.76-57.17)	**0.02**
Female Sex	285	42 (65%)	152 (69%)	0.60
Education Level (years)	285	14.28 ± 2.86 (5.00-18.00)	12.39 ± 3.19 (5.00-18.00)	**<0.001**
Scanlocation	285			0.04
1		26 (40%)	66 (30%)	
2		27 (42%)	78 (35%)	
3		12 (18%)	76 (35%)	
Clinical characteristics				
Major depressive disorder			62 (28%)	
Anxiety disorder			67 (30%)	
Comorbid depression and anxiety			91 (41%)	
Total depression severity score	280	4 ± 4 (0-21)	23 ± 12 (1-57)	<0.001
Total anxiety severity score	278	2 ± 3 (0-11)	14 ± 10 (0-50)	**<0.001**
Mood/cognition symptom cluster	285	1.09 ± 0.13 (1.00-1.47)	1.86 ± 0.50 (1.00-3.27)	**<0.001**
Somatic depression symptom cluster	285	1.20 ± 0.21 (0.90-2.20)	1.64 ± 0.41 (0.80-2.80)	**<0.001**
Immunometabolic symptom cluster	285	1.11 ± 0.21 (0.80-1.80)	1.60 ± 0.48 (0.60-3.60)	<0.001
Childhood Trauma Index	285	1 ± 1 (0-8)	2 ± 2 (0-8)	**<0.001**
Recent negative life events	285	0.57 ± 0.83 (0.00-3.00)	0.89 ± 1.09 (0.00-3.00)	0.05
Within patients				
Antidepressant use	220		77 (35%)	
Duration of depressive symptoms (proportion of time in the past 4 years)	190		0.34 ± 0.28 (0.00-1.00)	
Duration of anxiety symptoms (proportion of time in the past 4 years)	192		0.42 ± 0.35 (0.00-1.00)	
Age of onset of depression (years)	191		23.75 ± 10.44 (4.00-54.00)	
Age of onset of anxiety (years)	170		18.15 ± 10.93 (4.00-52.00)	
Somatic health				
Body Mass Index (kg/m^2)	285	24.36 ± 3.73 (19.03-37.42)	25.14 ± 4.72 (18.04-42.21)	0.35
Number of somatic diseases	285	0 ± 1 (0-3)	0 ± 1 (0-3)	0.66
Lifestyle				
Alcohol intake (mean number of drinks per week)	285	6.2 ± 6.1 (0.0-25.0)	4.3 ± 6.5 (0.0-47.5)	**0.01**
Smoking behavior (cigarettes/day)	161	9.26 ± 7.61 (0.00-29.00)	12.56 ± 10.73 (0.00-70.00)	0.09
Physical activity (1,000 MET minutes per week)	271	3.8 ± 3.3 (0.3-16.5)	3.6 ± 3.5 (0.0-17.1)	0.19
Inflammation				
C-Reactive Protein (mg/l)	280	-0.03 ± 0.52 (-1.00-1.08)	0.11 ± 0.59 (-1.00-1.35)	0.17
Tumor Necrosis Factor-α (pg/ml)	279	-0.16 ± 0.26 (-1.00-0.63)	-0.12 ± 0.26 (-1.00-0.63)	0.39
Interleukin-6 (pg/ml)	280	-0.15 ± 0.31 (-1.12-0.57)	-0.14 ± 0.48 (-2.29-1.74)	0.89
Autonomic Nervous System				
Resting Heart Rate (bpm)	276	69 ± 8 (51-86)	68 ± 10 (44-96)	0.62
Respiratory Sinus Arrhythmia (ms)	276	51 ± 25 (14-130)	49 ± 26 (7-130)	0.59
Pre-injection Period (ms)	276	119 ± 17 (81-147)	119 ± 16 (75-168)	0.66
HPA-axis				
Cortisol Awakening Response Area under the curve with respect to the ground (nmol/l/hr)	197	0.57 ± 4.63 (-13.06-11.13)	2.44 ± 5.74 (-14.92-19.00)	0.10
Cortisol Awakening Response Area under the curve with respect to the increase (nmol/l/hr)	197	16.89 ± 4.88 (8.49-32.13)	18.51 ± 6.75 (5.36-37.97)	0.23
Evening cortisol (nmol/l)	207	5.05 ± 2.44 (2.12-13.33)	5.04 ± 2.43 (1.09-12.96)	0.90

[a] Statistics presented: mean ± SD (minimum-maximum); n (%)

[b] Statistical tests performed: Wilcoxon rank-sum test; chi-square test of independence

Advanced Brain Aging in Depression and Anxiety Disorders

Using diagnostic status as a dichotomous between-group predictor we found that patients exhibited +1.75 years higher brain-PAD than controls, but this difference did not reach statistical significance (Cohen's d=0.24). Within the patient group only, we found no significant associations with the age of onset of illness or duration of symptoms of either MDD or anxiety. However, brain-PAD was significantly lower in antidepressants (AD) using patients compared to AD-free patients (b=-2.58 years, p=0.01), but not control subjects (b=0.59 years, p=0.65) (**Figure 2A**). Given the significant difference in brain-PAD between AD-free and AD-using patients, we included AD status as an additional covariate when comparing controls to the patient group, resulting in significantly higher brain-PAD in patients (+2.63 year [SE 1.10 year], Cohen's d=0.34, 95% CI 0.06-0.62). We also added AD status as an additional covariate in a model to compare controls against specific MDD, anxiety, or comorbid patient groups (the proportion of subjects using AD in specific diagnostic groups was marginally different, $\chi_{(2)}$=5.91, p=0.052). This revealed significantly higher brain-PAD in MDD (+2.78 years, Cohen's d=0.25, 95 % CI -0.10-0.60, p=0.04) and anxiety patients (+2.91 years, Cohen's d=0.27, 95% CI -0.08-0.61, p=0.03), and a similar effect in the comorbid MDD and anxiety group (+2.23 years, Cohen's d=0.21, 95% CI 0.10-0.53) although only marginally significant (p=0.08) (**Table 2**). There were no post-hoc differences in brain-PAD corrected for AD use between specific patient groups (MDD vs. anxiety vs. comorbid patients; P's>0.46, Cohen's d's <0.07).

Table 2. Advanced Brain Aging in Depression and Anxiety with Correction for Antidepressant Use.

Ref	Predictor	b	SE	*t* value	*P*	Cohen's *d*	SE	95% CI
Controls	Any patient	2.63	1.10	2.39	0.02	0.34	0.14	0.06-0.62
	MDD	2.78	1.32	2.11	0.04	0.25	0.18	-0.09-0.6
	Anxiety	2.91	1.31	2.22	0.03	0.27	0.17	-0.08-0.61
	Comorbid MDD and anxiety	2.23	1.28	1.74	0.08	0.21	0.16	-0.11-0.53

Age, sex, education level (years) and two dummy variables for scanlocation were included in all models. Antidepressant status was additionally included as covariate.

To gain more insight into the differences in brain-PAD between AD-free and AD-using patients, we post-hoc calculated a derived daily dose by dividing the AD mean daily dose by the daily dose recommended by the World Health Organization (also see[33]). Brain-PAD was not significantly negatively associated with a derived daily dose of antidepressants in n=74 patients (b=-0.91 year, p=0.50) (**Supplementary Figure S1**). Of note, we excluded three subjects from this analysis as these AD-using patients were using Venlafaxine at doses higher than 150 mg/day, acting as a dual serotonin and norepinephrine reuptake inhibitor rather than acting as a Selective Serotonin Reuptake Inhibitor (SSRI) only[34]. Based on the above findings, both diagnostic and AD status were included in the multivariable model to test unique brain-PAD contributions.

Selection of Significant Associations with Clinical Variables in All Participants

Using a dimensional approach based on symptoms rather than diagnosis, we found that higher brain-PAD was associated with higher total depression (b=0.07 year per unit change on the Inventory of Depressive Symptoms, p=0.03) and anxiety severity scores (b=0.11 year per unit change on the Beck's Anxiety Inventory, p=0.01) across all participants (**Figure 2B-C**). No significant associations were found for the mood/cognition (b=0.89 year per unit increase on the average sum score, p=0.27, **Figure 2D**) or immunometabolic symptom clusters of depression (b=0.45 year per unit increase on the average sum score, p=0.62, **Figure 2E**), but higher brain-PAD was strongly associated with more somatic symptoms of depression (b=4.03 year per unit increase on the average sum score, p<0.0001, **Figure 2F**). There were no significant associations between brain-PAD and childhood trauma exposure (b=0.23 year per unit change on the childhood trauma index, p=0.26) or recent negative life events (b=0.35 year per negative life event, p=0.39).

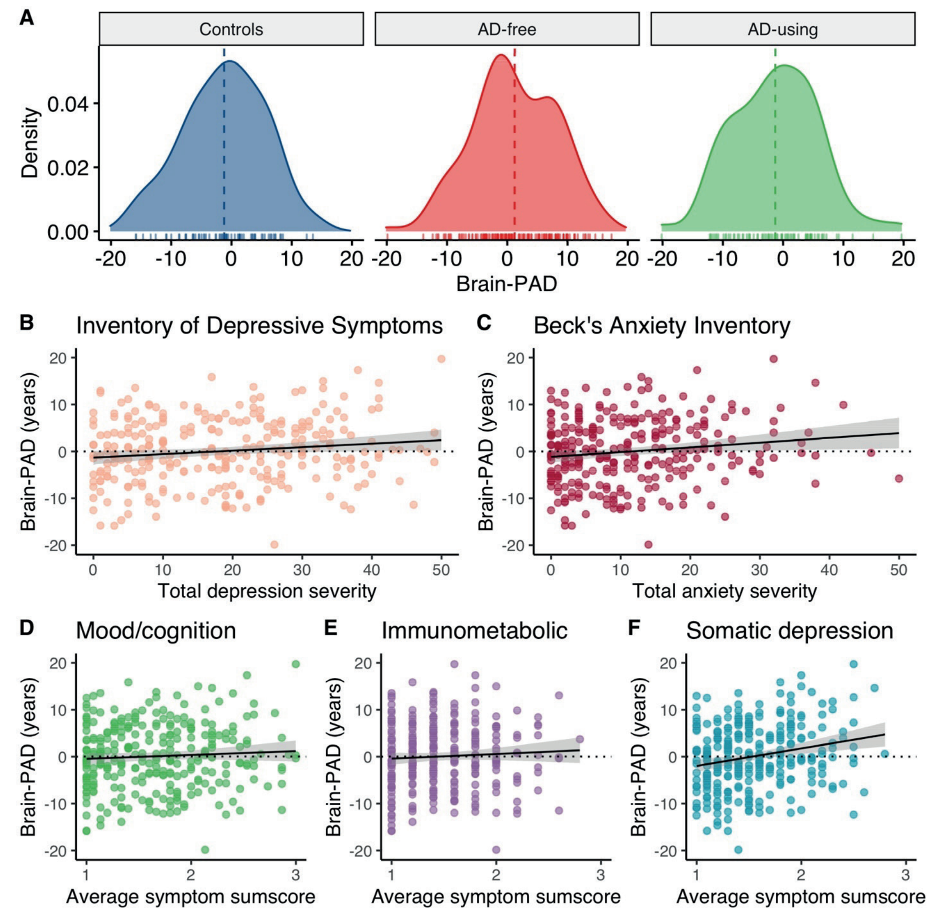


Figure 2. Brain-PAD differences and clinical characteristics. (**A**) AD-free patients showed significantly higher brain-PAD compared to AD-using patients (+2.58 year [SE 1.02 year], Cohen's d=0.36, 95% CI 0.09-0.64) and controls (+2.63 year [SE 1.10 year], Cohen's d=0.31, 95% CI 0.01-0.60). (**B**) Advanced brain aging was associated with overall higher total depressive symptoms (b=0.07 years per unit increase on the Inventory of Depressive Symptoms, p=0.03), (**C**) total anxiety symptoms (b=0.11 years per unit increase on the Beck's Anxiety Inventory, p=0.01)**,** but not specifically with (**D**) the mood/cognition (b=0.89 years per unit increase on average sum score, p=0.27) or (**E**) immunometabolic (b=0.45 years per unit increase on the average sum score, p=0.62) symptom cluster. The association in (B) seemed to be driven mostly by (**F**) a specific cluster of somatic symptoms in MDD (b=4.03 years per unit increase on the average sum score, p<0.0001)**.** Brain-PAD estimates (in years) were residualized for age, sex, education level (years) and two dummy variables for scanlocation.

191

Selection of Significant Associations with Somatic Health in All Participants

Higher brain-PAD was associated with both higher BMI (b=0.23 year per kg/m^2, p=0.02), as well as the number of somatic diseases under medical treatment (b=1.45 year per somatic disease, p=0.03). However, the latter association became non-significant if those with >2 chronic diseases (n=4) were truncated to two chronic diseases (b=1.29 year per somatic disease, p=0.08).

No Associations with Lifestyle or Biological Stress Variables

There were no significant associations with any of the lifestyle variables (smoking, alcohol, physical activity) or biological stress variables (inflammatory markers, ANS, HPA-axis). An overview of the separate linear regressions can be found in **Table 3**.

Multivariable Model

To characterize the unique contributions of the selected significant predictors on the brain-PAD outcome, we included diagnostic status (control vs. patient), MDD and anxiety symptom scores, BMI, AD use, and the number of somatic diseases under treatment as predictors in a stepwise regression model with forward selection. Thus, predictors were successively added to an intercept-only model (Akaike's Information Criterion [AIC] = 1115.81), only adding regression coefficients if they improved model fit (i.e. lower AIC). Using this method, we found that the best subset of variables to explain brain-PAD consisted of somatic depression symptoms and AD use (AIC=1098.79). In sum, unique contributions to brain-PAD were observed for the somatic depression symptom cluster (b=4.21 year per unit increase on average sum score, 95% CI 2.25 to 6.16, p<0.0001) and AD use (b=-2.53 year, 95% CI -4.36 to 0.70, p=0.007).

Table 3. Overview of the Brain-PAD Associations with Predictors of Interest.

Assessment	Predictor	b	SE	*t* value	*P*
Clinical	Depressive symptom severity	0.07	0.03	2.16	**0.03**
	Anxiety symptom severity	0.11	0.04	2.50	**0.01**
	Mood/cognition symptoms	0.89	0.81	1.10	0.27
	Somatic depression symptoms	4.03	1.04	3.87	**<0.00001**
	Immunometabolic symptoms	0.45	0.92	0.49	0.62
	Childhood trauma index	0.23	0.20	1.13	0.26
	Negative life events	0.35	0.41	0.86	0.39
Within patients	Antidepressant use	-2.58	1.02	-2.54	**0.01**
	Duration of depressive symptoms	-0.20	1.97	-0.10	0.92
	Duration of anxiety symptoms	-0.88	1.55	-0.56	0.57
	Age of onset of depression	0.04	0.06	0.61	0.55
	Age of onset of anxiety	0.01	0.05	0.09	0.93
Somatic health	BMI	0.23	0.10	2.31	**0.02**
	Number of somatic diseases	1.29	0.72	1.79	0.08
Lifestyle	Alcohol (mean drinks per week)	-0.09	0.07	-1.33	0.19
	Smoking (cigarettes per day)	-0.07	0.05	-1.26	0.21
	Physical exercise (MET-minutes)	-0.06	0.13	-0.48	0.63
Inflammation	CRP	0.57	0.76	0.75	0.46
	TNF-α	0.10	1.65	0.06	0.95
	IL6	0.60	0.98	0.61	0.54
ANS	Resting HR	0.08	0.05	1.51	0.13
	RSA	-0.01	0.02	-0.46	0.65
	PEP	-0.04	0.03	-1.45	0.15
HPA-axis	AUCi	-0.04	0.10	-0.34	0.74
	AUCg	-0.04	0.09	-0.42	0.67
	Evening	0.18	0.23	0.76	0.45

Age, sex, education level (years) and two dummy variables for scanlocation were included in all models. BMI, Body Mass Index; MET-minutes, ;CRP, C-reactive protein; TNF-α, Tumor Necrosis Factor-α; IL6, Interleukin-6; ANS, autonomic nervous system; HR, heart rate; RSA, respiratory sinus arrhythmia; PEP, pre-ejection period; AUCi, cortisol awakening response: area under the curve with respect to the increase; AUCg, cortisol awakening response: area under the curve with respect to the ground; Evening, Evening Cortisol.

DISCUSSION

The current study used a validated brain age prediction model to show that the previously observed findings of older appearing brains in MDD patients was associated with symptom severity and BMI. Moreover, antidepressant (AD) users exhibited similar average brain-PAD as control subjects, whereas those that were AD-free showed older appearing brains. Correcting for AD-use, we also showed that not only MDD patients, but also patients with anxiety disorder exhibited older appearing brains compared to controls. Surprisingly, there were no significant associations with lifestyle or biological stress systems. A multivariable model showed unique contributions of somatic depression symptom severity and AD-use on brain-PAD.

To the best of our knowledge, we are the first to report advanced brain aging in anxiety disorders (i.e. generalized anxiety disorder, panic disorder, social anxiety disorder) with an estimated +2.91 years on average, compared to controls, when correcting for AD use. This is consistent with the literature describing comparable effect sizes with respect to structural brain alterations in social anxiety disorder (Cohen's d=0.20)[35], and other anxiety-related disorders such as post-traumatic stress disorder (PTSD) (Cohen's d=-0.17)[36], with PTSD patients also showing advanced brain-PAD without correction for AD[17]. This observation may potentially offer an explanation as to why clinical anxiety is associated with an increased risk of dementia, even independent from depression[37], although further evidence is needed. The lack of any significant post-hoc differences between specific diagnostic groups can likely be explained due to, amongst others, the high genetic correlation between the disorders[38], shared environmental risks, and overlapping personality traits of patients with depression and anxiety disorders[39].

The most clinically relevant finding was that AD-using patients showed a similar brain age to controls, but not to AD-free patients, irrespective of specific depressive or anxiety disorder. This finding was previously overlooked in consortium data, presumably due to a lack of more detailed information on lifetime use, dosage and duration of use of AD[16], highlighting the complementary values of well-characterized local samples and large-scale consortia. The AD finding was particularly interesting as the AD-using patients constituted a more severely depressed and anxious group as indicated by higher symptom severities compared to AD-free patients, potentially suggesting compensatory or normalizing mechanisms of AD, at least on the brain-PAD metric. This accords with earlier work reporting brain-PAD associations with therapeutic drugs, suggesting neuroprotective effects of Lithium treatment in bipolar disorder patients (vs. no Lithium)[40] and ibuprofen (vs. placebo)

in healthy participants in a exploratory randomized controlled trial[41]. Yet, it remains unclear if and to what extent the brain age protective mechanisms overlap with, for example, increased neural progenitor cells[42], brain-derived neurotrophic factor (BDNF)[43], or other serotonergic neuroplasticity processes implicated in AD use[44], or, alternatively, whether neuropharmacology affects the MRI signal[45]. Brain-PAD was not positively associated with the duration of symptoms (either MDD or anxiety), suggesting that the AD effect was not driven by the duration of the disease and did not seem to be progressive. Taken together, these findings may suggest an age-related neuroprotective effect of AD, but interpretative caution is warranted as the current study was cross-sectional in nature and the dose-response association with AD not statistically significant. We also did not find associations with physical activity, while a previous study found an association between brain-PAD and the daily number of flights of stairs climbed[46]. Future clinical interventions are needed to examine the short and long-term effects of antidepressants and physical activity on biological aging, an objective currently pursued by the MOod treatment with antidepressants or running (MOTAR) study[47].

There were no associations with the cumulative childhood trauma index or the number of recent negative life events, different from the impact that adverse childhood experience commonly has on other biological age indicators such as telomere length[48], or epigenetic aging[49], albeit with small effects. Future studies with larger samples may potentially be more sensitive in picking up associations between brain-PAD and childhood trauma. However, taken together, the current study found that advanced brain aging was more associated with current disease states, likely related to current symptom severity, rather than the result of cumulative exposure (i.e. no association with childhood trauma history, age of onset of illness, duration of symptoms) or traits.

Furthermore, Cole and colleagues (2020) found significant associations between brain-PAD and several biomedical (e.g. blood pressure, diabetes, stroke) and lifestyle variables (e.g. smoking status, alcohol intake frequency), but not BMI, in the UK Biobank[20], albeit with a different, multi-modal brain age prediction model but in a much larger sample size (>14,000 subjects). Although the current findings with somatic health broadly support previously associated diabetes[50] and stroke findings in UK Biobank, as well as the null-finding with respect to physical activity, we did not identify associations with smoking or alcohol behavior[50]. More work is needed in terms of identifying unique or shared robust contributors to the brain-PAD metric, converging evidence across and between datasets, processing methods, and populations. Other previous studies, however, also identified associations with BMI[51]

and here we show that an increase of 1 kg/m^2 in BMI leads to +0.23 years of added brain aging, although not independent from depression or anxiety symptom severity. A previous study did show such an independent effect for obesity and first-episode schizophrenia[52], but here the obese group only constituted of 13% of the total current sample. Furthermore, each increase of 1 of the average sum score (range 0.80-4.00) of somatic depression symptoms, resulted in +4.20 years of added brain aging, independent from AD use. The somatic symptom cluster studied here consisted of items tapping into sleep, psychomotor, and other bodily symptom problems (see **Supplement** for all individual items within each cluster). This emphasizes the need to prevent and improve both mental and somatic conditions to promote healthy brain aging in psychiatric populations.

Surprisingly, none of the biological stress systems considered in the current study were predictive of brain aging, despite the strong association between brain-PAD and somatic symptoms. This suggests that the biological dysregulations that commonly link depression to somatic health[5], were not directly contributing to advanced brain aging. On the other hand, it might indicate that the brain-PAD metric is more responsive to psychological stressors, rather than biological stressors. With respect to the inflammatory markers, it might be possible that blood levels of inflammatory markers do not accurately mirror central neuroimmune levels, although there is some evidence that C-Reactive Protein (CRP) measured peripherally also reflects central inflammation, at least in MDD[53]. Alternatively, a different potential biological mechanism that may explain the observed advanced brain aging in depression and anxiety disorders is metabolic dysregulation. Future studies could characterize the brain-PAD metric in more detail with respect to metabolic factors (e.g. blood pressure, triglycerides, cholesterol), as these are well-established risk factors for unfavorable somatic conditions[54–57] and frequently co-occur with depression[58].

Given the richness of the current dataset, we additionally computed post-hoc intercorrelations between the brain-PAD metric and other available biological age indicators in NESDA. Briefly, we found low, non-significant (P's>0.13), correlations between brain-PAD, and three omics-based clocks (epigenetic, transcriptomic, metabolomic) and telomere length (with Pearson *r* in the range of -0.03 to 0.15, **Supplementary Figure S2**). Surprisingly, brain-PAD was negatively associated with the proteomic clock (r=-0.24, p=0.02) after correcting for age (albeit in a greatly reduced overlapping sample of N=98). Only a handful of studies have compared multiple biological age indicators side-by-side[59–62], but the current findings support most work showing the very little overlap between biological clocks from different types

of data[9]. However, the small but significant negative correlation between brain and proteomic aging suggests a further study with more focus on the interplay between this peripheral and central proxy of aging is needed. Aging remains a multifaceted and complex process that may manifest differently across multiple biological levels and tissues.

Limitations

It is important to mention that our sample had low statistical power to detect (some of) the relatively small effect sizes in the current study. At present, the large within-group variance of brain-PAD lacks utilitarian validity in a clinical context. We, therefore, emphasize the need for both methodological (i.e. brain age models) as well as epidemiological replication (i.e. other and larger samples) to test the robustness of effects. Another limitation is reflected by the lack of insights into the causal pathways implicated in advanced brain aging, given the cross-sectional nature of the study. However, a major strength is that we used a pre-established reference curve for healthy brain aging that has further potential for benchmarking, as the ENIGMA MDD working group encourages local research samples like ours to examine more detailed phenotypes that were not available within the consortium. Also important to note is that the effects of multivariate brain aging patterns (Cohen's d=0.34, between controls and all patients) was higher or comparable to other biological aging indicators (e.g. telomere length [Cohen's d=0.12][63], epigenetic aging [d=0.14])[64], biological markers (e.g. BDNF [d=0.23][65], cortisol [d=0.15-0.25][66], CRP [d=0.15][67]), and, most importantly, neuroimaging markers (e.g. hippocampal volume [d=-0.14][68]), in other or (partly) overlapping samples.

Conclusion

In summary, advanced brain aging in patients with MDD and anxiety seems to be most strongly associated with somatic health indicators such as somatic depressive symptomatology, BMI, and the number of chronic diseases under medical treatment. We also revealed that antidepressant medication use was associated with lower brain-PAD, potentially suggesting that its use may have a protective effect on the age-related structural gray matter alterations observed in patients with MDD and anxiety, an effect previously overlooked in consortium data. Our results, therefore, emphasize the importance and complementary value of smaller, yet more homogeneous, datasets with harmonized data collection and well-characterized clinical phenotyping, compared to the large-scale consortium data needed for statistical power.

Randomized clinical trials are needed to confirm whether advanced brain aging can be halted or reversed, by intervening on the cross-sectional somatic health indicators identified here, in pursuit of the characterization of a complex multifaceted process such as brain aging.

REFERENCES

1. Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA Psychiatry. 2015;72:334–341.
2. Bijl RV, Ravelli A. Current and residual functional disability associated with psychopathology: findings from the Netherlands Mental Health Survey and Incidence Study (NEMESIS). Psychol Med. 2000;30:657–668.
3. Révész D, Verhoeven JE, Milaneschi Y, Penninx BWJH. Depressive and anxiety disorders and short leukocyte telomere length: mediating effects of metabolic stress and lifestyle factors. Psychol Med. 2016;46:2337–2349.
4. Verhoeven JE, Révész D, Van Oppen P, Epel ES, Wolkowitz OM, Penninx BWJH. Anxiety disorders and accelerated cellular ageing. British Journal of Psychiatry. 2015;206:371–378.
5. Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 2013;11:129.
6. Roy-Byrne PP, Davidson KW, Kessler RC, Asmundson GJG, Goodwin RD, Kubzansky L, et al. Anxiety disorders and comorbid medical illness. Gen Hosp Psychiatry. 2008;30:208–225.
7. Ferrucci L, Gonzalez-Freire M, Fabbri E, Simonsick E, Tanaka T, Moore Z, et al. Measuring biological aging in humans: A quest. Aging Cell. 2019:e13080.
8. Jylhava J, Pedersen NL, Hagg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.
9. Hägg S, Belsky DW, Cohen AA. Developments in molecular epidemiology of aging. Emerging Topics in Life Sciences. 2019;3:411–421.
10. Cole JH, Franke K. Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. Trends Neurosci. 2017;40:681–690.
11. Gaser C, Franke K. 10 years of BrainAGE as an neuroimaging biomarker of brain aging: What insights did we gain? Front Neurol. 2019;10:789.
12. Cole J, Franke K, Cherbuin N. Quantification of the biological age of the brain using neuroimaging. Healthy ageing and longevity, Biomarkers of Human Aging; 2019.
13. Koutsouleris N, Davatzikos C, Borgwardt S, Gaser C, Bottlender R, Frodl T, et al. Accelerated brain aging in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. Schizophr Bull. 2014;40:1140–1153.
14. Besteher B, Gaser C, Nenadić I. Machine-learning based brain age estimation in major depression showing no evidence of accelerated aging. Psychiatry Research: Neuroimaging. 2019;290:1–4.
15. Kaufmann T, van der Meer D, Doan NT, Schwarz E, Lund MJ, Agartz I, et al. Common brain disorders are associated with heritable patterns of apparent aging of the brain. Nat Neurosci. 2019;22:1617–1623.
16. Han LKM, Dinga R, Hahn T, Ching C, Eyler L, Aftanas L, et al. Brain Aging in Major Depressive Disorder: Results from the ENIGMA Major Depressive Disorder working group. bioRxiv. 2019:560623.
17. Liang H, Zhang F, Niu X. Investigating systematic bias in brain age estimation with application to post-traumatic stress disorders. Hum Brain Mapp. 2019;10:1.
18. Krueger RF, Markon KE. Reinterpreting comorbidity: a model-based approach to understanding and classifying psychopathology. Annu Rev Clin Psychol. 2006;2:111–133.
19. Caspi A, Houts RM, Ambler A, Danese A, Elliott ML, Hariri A, et al. Longitudinal Assessment of Mental Health Disorders and Comorbidities Across 4 Decades Among Participants in the Dunedin Birth Cohort Study. JAMA Netw Open. 2020;3:e203221.
20. Cole JH. Multi-modality neuroimaging brain-age in UK Biobank: relationship to biomedical, lifestyle and cognitive factors.
21. Ning K, Zhao L, Matloff W, Sun F, Toga AW. Association of relative brain age with tobacco smoking, alcohol consumption, and genetic variants. Sci Rep. 2020;10:10.
22. Jonsson BA, Bjornsdottir G, Thorgeirsson TE, Ellingsen LM, Walters GB, Gudbjartsson DF, et al. Brain age prediction using deep learning uncovers associated sequence variants. Nat Commun. 2019;10:5409.
23. Wittchen HU. Reliability and validity studies of the WHO-Composite International Diagnostic Interview (CIDI): A critical review. J Psychiatr Res. 1994;28:57–84.
24. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. Psychol Med. 1996;26:477–486.
25. Wardenaar KJ, van Veen T, Giltay EJ, de Beurs E, Penninx BWJH, Zitman FG. Development and validation of a 30-item short adaptation of the Mood and Anxiety Symptoms Questionnaire (MASQ). Psychiatry Res. 2010;179:101–106.
26. Fydrich T, Dowdall D, Chambless DL. Reliability and validity of the Beck Anxiety Inventory. J Anxiety Disord. 1992;6:55–61.
27. Hovens JGFM, Wiersma JE, Giltay EJ, Van Oppen P, Spinhoven P, Penninx BWJH, et al. Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. Acta Psychiatr Scand. 2010;122:66–74.
28. Brugha TS, Cragg D. The List of Threatening Experiences: the reliability and validity of a brief life events questionnaire. Acta Psychiatr Scand. 1990;82:77–81.
29. Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT Alcohol Consumption Questions (AUDIT-C): An Effective Brief Screening Test for Problem Drinking. Arch Intern Med. 1998;158:1789–1795.
30. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381–1395.
31. Le TT, Kuplicki RT, McKinney BA, Yeh H-W, Thompson WK, Paulus MP, et al. A Nonlinear Simulation Framework Supports Adjusting for Age When Analyzing BrainAGE. Front Aging Neurosci. 2018;10:317.
32. Smith SM, Vidaurre D, Alfaro-Almagro F, Nichols TE, Miller KL. Estimation of Brain Age Delta from Brain Imaging. 2019.
33. Licht CMM, de Geus EJC, Zitman FG, Hoogendijk WJG, van Dyck R, Penninx BWJH. Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). Arch Gen Psychiatry. 2008;65:1358–1367.
34. Debonnel G, Saint-André E, Hébert C, de Montigny C, Lavoie N, Blier P. Differential physiological effects of a low dose and high doses of venlafaxine in major depression. Int J Neuropsychopharmacol. 2007;10:51–61.
35. Bas-Hoogendam JM, van Steenbergen H, Nienke Pannekoek J, Fouche J-P, Lochner C, Hattingh CJ, et al. Voxel-based morphometry multi-center mega-analysis of brain structure in social anxiety disorder. Neuroimage Clin. 2017;16:678–688.
36. Logue MW, van Rooij SJH, Dennis EL, Davis SL, Hayes JP, Stevens JS, et al. Smaller Hippocampal Volume in Posttraumatic Stress Disorder: A Multisite ENIGMA-PGC Study: Subcortical Volumetry Results From Posttraumatic Stress Disorder Consortia. Biol Psychiatry. 2018;83:244–253.

37. Santabárbara J, Lopez-Anton R, de la Cámara C, Lobo E, Gracia-García P, Villagrasa B, et al. Clinically significant anxiety as a risk factor for dementia in the elderly community. Acta Psychiatr Scand. 2019;139:6–14.
38. Brainstorm Consortium, Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J, et al. Analysis of shared heritability in common disorders of the brain. Science. 2018;360.
39. Ruscio AM, Khazanov GK. Anxiety and depression. The Oxford Handbook of Mood Disorders. 2017:313–324.
40. Van Gestel H, Franke K, Petite J, Slaney C, Garnham J, Helmick C, et al. Brain age in bipolar disorders: Effects of lithium treatment. Aust N Z J Psychiatry. 2019;53:1179–1188.
41. Le TT, Kuplicki R, Yeh HW, Aupperle RL, Khalsa SS, Simmons WK, et al. Effect of Ibuprofen on BrainAGE: A Randomized, Placebo-Controlled, Dose-Response Exploratory Study. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2018:1–8.
42. Boldrini M, Hen R, Underwood MD, Rosoklija GB, Dwork AJ, Mann JJ, et al. Hippocampal angiogenesis and progenitor cell proliferation are increased with antidepressant use in major depression. Biol Psychiatry. 2012;72:562–571.
43. Castrén E, Kojima M. Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. Neurobiol Dis. 2017;97:119–126.
44. Kraus C, Castrén E, Kasper S, Lanzenberger R. Serotonin and neuroplasticity--links between molecular, functional and structural pathophysiology in depression. Neurosci Biobehav Rev. 2017;77:317–326.
45. Cousins DA, Aribisala B, Nicol Ferrier I, Blamire AM. Lithium, gray matter, and magnetic resonance imaging signal. Biol Psychiatry. 2013;73:652–657.
46. Steffener J, Habeck C, O'Shea D, Razlighi Q, Bherer L, Stern Y. Differences between chronological and brain age are related to education and self-reported physical activity. Neurobiol Aging. 2016;40:138–144.
47. Lever-van Milligen BA, Verhoeven JE, Schmaal L, van Velzen LS, Révész D, Black CN, et al. The impact of depression and anxiety treatment on biological aging and metabolic stress: study protocol of the MOod treatment with antidepressants or running (MOTAR) study. BMC Psychiatry. 2019;19:425.
48. Aas M, Elvsåshagen T, Westlye LT, Kaufmann T, Athanasiu L, Djurovic S, et al. Telomere length is associated with childhood trauma in patients with severe mental disorders. Transl Psychiatry. 2019;9:97.
49. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A, et al. Traumatic stress and accelerated DNA methylation age: A meta-analysis. Psychoneuroendocrinology. 2018;92:123–134.
50. Franke K, Gaser C, Manor B, Novak V. Advanced BrainAGE in older adults with type 2 diabetes mellitus. Front Aging Neurosci. 2013;5:1–9.
51. Franke K, Ristow M, Gaser C. Gender-specific impact of personal health parameters on individual brain aging in cognitively unimpaired elderly subjects. Front Aging Neurosci. 2014;6:1–14.
52. Kolenic M, Franke K, Hlinka J, Matejka M, Capkova J, Pausova Z, et al. Obesity, dyslipidemia and brain age in first-episode psychosis. J Psychiatr Res. 2018;99:151–158.
53. Felger JC, Haroon E, Patel TA, Goldsmith DR, Wommack EC, Woolwine BJ, et al. What does plasma CRP tell us about peripheral and central inflammation in depression? Molecular Psychiatry. 2018.
54. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol. 2010;56:1113–1132.
55. Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. Diabetes Care. 2012;35:2402–2411.
56. Profenno LA, Porsteinsson AP, Faraone SV. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. Biol Psychiatry. 2010;67:505–512.
57. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415–1428.
58. Pan A, Keum N, Okereke OI, Sun Q, Kivimaki M, Rubin RR, et al. Bidirectional association between depression and metabolic syndrome: a systematic review and meta-analysis of epidemiological studies. Diabetes Care. 2012;35:1171–1180.
59. Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, et al. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? Am J Epidemiol. 2018;187:1220–1230.
60. Kim S, Myers L, Wyckoff J, Cherry KE, Jazwinski SM. The frailty index outperforms DNA methylation age and its derivatives as an indicator of biological age. GeroScience. 2017;39:83–92.
61. Murabito JM, Zhao Q, Larson MG, Rong J, Lin H, Benjamin EJ, et al. Measures of Biologic Age in a Community Sample Predict Mortality and Age-Related Disease: The Framingham Offspring Study. J Gerontol A Biol Sci Med Sci. 2018;73:757–762.
62. Jansen R, Verhoeven JE, Han LKM, Aberg KA, van den Oord E, Milaneschi Y, et al. An integrative study of five biological clocks in somatic and mental health. eLife. 2020. 2020.
63. Verhoeven JE, Révész D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. Mol Psychiatry. 2013:1–7.
64. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic Aging in Major Depressive Disorder. Am J Psychiatry. 2018;175:774–782.
65. Molendijk ML, Bus BAA, Spinhoven P, Penninx BWJH, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state–trait issues, clinical features and pharmacological treatment. Mol Psychiatry. 2011;16:1088–1095.
66. Vreeburg SA, Hoogendijk WJG, van Pelt J, Derijk RH, Verhagen JCM, van Dyck R, et al. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. Arch Gen Psychiatry. 2009;66:617–626.
67. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom Med. 2009;71:171–186.
68. Schmaal L, Veltman DJ, van Erp TGM, Sämann PG, Frodl T, Jahanshad N, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry. 2015:1–7.

SUPPLEMENTARY MATERIALS

Contributing Factors to Advanced Brain Aging in Depression and Anxiety Disorders

SUPPLEMENTARY METHODS

NESDA MRI substudy in- and exclusion criteria

The inclusion criteria for the MRI substudy were a DSM-IV diagnosis of major depressive disorder (MDD) and/or anxiety disorder (social anxiety disorder, panic disorder, generalized anxiety disorder) with a six month recency, but no other axis-I disorder. Control subjects were not allowed to have a history of any DSM-IV axis-I disorder. Exclusion criteria were a history of drug or alcohol abuse for both patients and controls, general MRI contraindications, and presence or history of a severe internal or neurological disorder. Additional exclusion criteria were the use of psychotropic medication with the exception of stable use of selective serotonin reuptake inhibitors (SSRIs) or infrequent benzodiazepine use for patients and use of any psychoactive medication for control subjects.

Clinical assessments

The Beck Anxiety Inventory (BAI) was used to measure the severity of anxiety symptoms as common in panic and generalized anxiety disorders, ranging from 0 (minimal) to 63 (severe)[1]. Depressive symptomatology was assessed with the Inventory Depressive Symptomatology (IDS)[2]. This 30-item questionnaire assesses the presence of all symptom domains of a major depressive episode in the past seven days on a 0-3 scale (not severe – severe), resulting in a total IDS score ranging from 0 (normal) to 84 (very severe). For both depression disorders and anxiety disorders, measures on duration (course) of psychopathology were assessed. The Life Chart Interview was used to determine the proportion of time in which symptoms relevant for the disorder were experienced in the past four years [3]. Childhood trauma before the age of 16 was assessed using the NEMESIS childhood trauma interview with personal history questions including a structured inventory of trauma exposure during childhood (emotional neglect, psychological abuse, physical abuse, sexual abuse, and important life-events in early life). In line with earlier work, a cumulative childhood trauma index (CTI) was created that

reported the sum of the categories that were scored from 0 to 2 (0: never happened, 1: sometimes, 2: happened regularly), resulting in an index score ranging from 0-8 [4]. Frequent use of antidepressants (>50% of the time) was assessed through container inspection and categorized using World Health Organization Anatomical Therapeutic Chemical (ATC) classifications: selective serotonin reuptake inhibitors (ATC code N06AB), and other antidepressants (ATC codes N06AF, N06AG, N06AX).

Depressive symptom clusters

Previous studies based on the Netherlands Study of Depression and Anxiety (NESDA) and other populations have distinguished several different clusters of symptoms within the Inventory of Depressive Symptoms (IDS) [2, 5, 6]. Here, we largely follow the two factors identified by Wardenaar et al. 2010 [7], but add a separate factor for immuno-metabolic features, thus distinguishing three symptom clusters: 1) mood/cognition symptom cluster, 2) immunometabolic symptom cluster, and 3) somatic symptom cluster (**Supplementary Table S1**). Given that the three different clusters consist of different numbers of items, we divided the total score of each cluster by the number of items in that cluster (mood/cognition: 15 items vs. immuno-metabolic: 5 items vs. somatic: 10 items) to obtain an average summary score.

Biological stress assessments

Inflammation

A previous study also described the assessment of inflammation markers [8]. Circulating plasma levels of C-Reactive Protein (CRP) (N=280), tumor necrosis level-α (TNF-α) (N=279), and Interleukin-6 (IL-6) (N=280) were assessed in duplicate. First, to measure plasma levels of CRP, an in-house enzyme-linked immunosorbent assay (ELISA) based on purified protein and polyclonal anti-CRP antibodies was used (Dako, Glostrup, Denmark). Intra- and inter-assay coefficients of variation were 5% and 10%, respectively. Second, plasma TNF-α levels were assessed using a high-sensitivity solid phase ELISA (Quantikine HS Human TNF-α Immunoassay, R&D systems, Minneapolis, MN, USA). Intra- and inter-assay coefficients of variation were 10% and 15% respectively. Finally, to measure plasma IL-6 levels a high sensitivity ELISA was used (PeliKine Compact, ELISA, Sanquin, Amsterdam, The Netherlands). Intra- and inter-assay coefficients of variation were 8% and 12%, respectively.

Supplementary Table S1. Categorization of Individual Items of the Inventory of Depressive Symptoms (IDS) into Three Symptom Clusters.

Symptoms (IDS)	Depression symptom profiles
Problems falling asleep	Somatic
Sleep during the night	Somatic
Waking-up too early	Somatic
Sleeping too much	Immuno-metabolic
Feeling sad	Mood/cognition
Feeling irritable	Mood/cognition
Feeling anxious or tense	Mood/cognition
Response of mood to good or desired events	Mood/cognition
Mood in relation to time of day	Mood/cognition
Quality of mood	Mood/cognition
Decreased appetite	Somatic
Increased appetite	Immuno-metabolic
Decreased weight	Somatic
Increased weight	Immuno-metabolic
Concentration/decision making	Mood/cognition
View of self	Mood/cognition
View of future	Mood/cognition
Thoughts of death/suicide	Mood/cognition
General interest	Mood/cognition
Energy level	Immuno-metabolic
Capacity for pleasure or enjoyment (excl. sex)	Mood/cognition
Interest in sex	Mood/cognition
Psychomotor retardation	Somatic
Psychomotor agitation	Somatic
Aches and pains	Somatic
Other bodily symptoms	Somatic
Panic/phobic symptoms	Mood/cognition
Constipation/diarrhea	Somatic
Interpersonal sensitivity	Mood/cognition
Leaden-paralysis/physical energy	Immuno-metabolic

Hypothalamic Pituitary Adrenal-axis

The assessment of hypothalamic pituitary adrenal (HPA)-axis measures have also been previously described [9]. To reliably assess the active, unbound form of cortisol participants were instructed to collect saliva samples at home (with minimal intrusiveness) on a regular (preferably working) day [10]. Salivettes were used to obtain saliva samples (Sarstedt, Nümbrecht, Germany) at six time points during a regular (work) day: at awakening (T1) and 30 (T2), 45 (T3), and 60 (T4) minutes later and at 10 PM (T5) and 11 PM (T6). The samples were stored in refrigerators and then returned by regular mail. After arrival, salivettes were centrifuged at 2000 × g for 10 min, aliquoted and stored at −80°C. Analyses of the cortisol were performed by competitive electrochemiluminescence immunoassay (Roche, Basel, Switzerland) [11]. The detection limit was 2.0 nmol/l and the intra- and inter-assay coefficients of variation were <10% [12]. Since the two evening values were highly correlated (r=0.75, p<.001), we averaged these two values.

Autonomic Nervous System

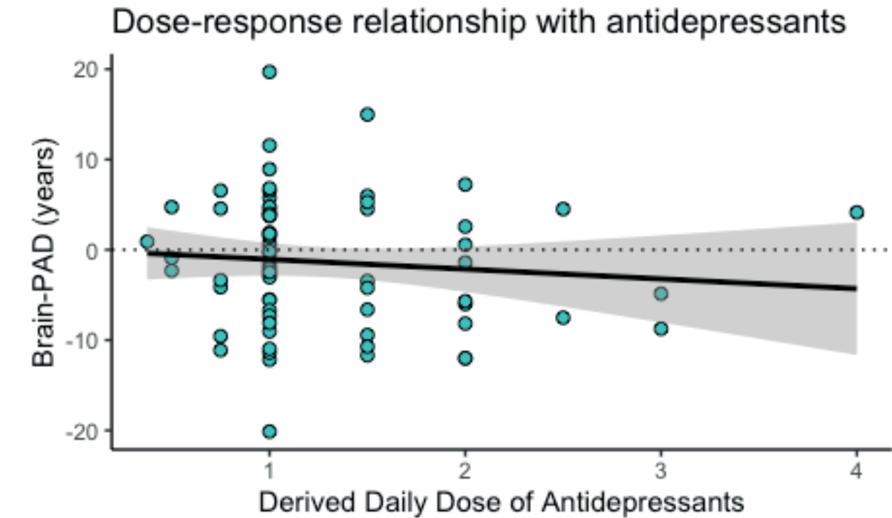
Subjects wore a so-called VU University ambulatory monitoring system (VU-ams) [13] during their interview. The VU-ams is a light-weight, unobtrusive device that records an electrocardiogram (ECG) and changes in thorax impedance (dZ) through 6 surface electrodes placed on the chest and the back [14]. The heart rate was obtained by extracting the inter-beat interval time series from the ECG signal. Respiratory sinus arrhythmia (RSA) and pre-ejection period (PEP) were extracted from the combined dZ and ECG signals [13]. RSA is a measure of cardiac parasympathetic (vagal) control, with high RSA levels reflecting high cardiac vagal control. We subtracted the shortest inter-beat interval during heart rate acceleration in the inspirational phase from the longest interbeat interval during deceleration in the expirational phase for all breaths to obtain a measure of RSA [15]. PEP is a measure of cardiac sympathetic control, as it can reliably index b-adrenergic inotropic drive to the left ventricle. Long PEP reflects low cardiac sympathetic control. PEP was defined as the interval from the beginning of the left ventricular electrical activity (ECG Q-wave onset), to the beginning of left ventricular ejection (B point in the dZ/dt signal) [16]. Given the fact that postural changes unrelated to autonomic activity affect PEP and RSA, data from periods in which participants were non-stationary (~15 min) were excluded [17]. Movement was registered through vertical accelerometry. Automated scoring of RSA and PEP was checked by visual inspection, and valid data was averaged over 98.0 ± 24 (mean ± SD) min to create single HR, PEP and RSA values.

Derived daily dose of antidepressants

We had information available on daily dose of antidepressants (n=74), with 82% of the antidepressant (AD) users using serotonin and norepinephrine reuptake inhibitors (SSRIs) and the remaining 18% using Venlafaxine on doses <150 mg/day. Of note, we excluded three subjects because they were using Venlafaxine at doses higher than 150 mg/day, acting as a dual serotonin and norepinephrine reuptake inhibitor rather than acting as a Selective Serotonin Reuptake Inhibitor (SSRI) only [18]. The derived daily dose was calculated by dividing the AD mean daily dose by the daily dose recommended by the World Health Organization [19] (also see [20]). However, brain-PAD was not significantly negatively associated with a derived daily dose of AD in (b=-0.91 year per g of AD per day, p=0.50) (**Supplementary Figure S1**).

Other biological clocks

To examine whether multivariate brain aging patterns were synchronized with telomere length (TL), and multivariate aging patterns from four omics-levels (epigenomics, proteomics, transcriptomics, metabolomics) we computed a correlation matrix between brain-PAD and these other five biological clocks. For a more detailed description of the biological clocks, please see [21]. Briefly, ridge regression was used to predict the chronological age using data from different molecular levels. Chronological age effects were regressed out of all biological age predictions to indicate biological aging. Positive correlations indicated concordant biological aging processes, whereas negative intercorrelations indicated discordant aging patterns. There was low, non-significant, agreement between brain age and four other biological age indicators. However, we found a weak but significant inverse correlation between brain age and proteomic age, while controlling for age (**Supplementary Figure S2**).



Supplementary Figure S1. Dose-response relationship between brain-PAD and antidepressants. Within the patient group, lower brain-PAD was not significantly associated with higher derived daily doses of antidepressants (AD) (SSRIs and Venlafaxine <1.5 g/day) (b=-0.94 years per g/day, p=0.50). Brain-PAD estimates (in years) were residualized for age, sex, education level (years) and two dummy variables for scanlocation.

Supplementary Figure S2. Correlation coefficients between brain-PAD and five other biological age indicators while controlling for age. Similar methods were deployed predicting age from epigenetic, transcriptomic, proteomic, and metabolomic data. Telomere length was also included as a biological age indicator. Brain-PAD was not significantly correlated with either of the other biological age indicators, except for the proteomic clock (r=-0.24, p=0.02).

REFERENCES

1. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol. 1988;56:893–897.
2. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. Psychol Med. 1996;26:477–486.
3. Lyketsos CG, Nestadt G, Cwi J, Heithoff K, Et al. The Life Chart Interview: A standardized method to describe the course of psychopathology. Int J Methods Psychiatr Res. 1994;4:143–155.
4. Hovens JGFM, Wiersma JE, Giltay EJ, Van Oppen P, Spinhoven P, Penninx BWJH, et al. Childhood life events and childhood trauma in adult patients with depressive, anxiety and comorbid disorders vs. controls. Acta Psychiatr Scand. 2010;122:66–74.
5. Schaakxs R, Comijs HC, Lamers F, Kok RM, Beekman ATF, Penninx BWJH. Associations between age and the course of major depressive disorder: a 2-year longitudinal cohort study. Lancet Psychiatry. 2018;5:581–590.
6. Hegeman JM, Wardenaar KJ, Comijs HC, de Waal MWM, Kok RM, van der Mast RC. The subscale structure of the Inventory of Depressive Symptomatology Self Report (IDS-SR) in older persons. J Psychiatr Res. 2012;46:1383–1388.
7. Wardenaar KJ, van Veen T, Giltay EJ, den Hollander-Gijsman M, Penninx BWJH, Zitman FG. The structure and dimensionality of the Inventory of Depressive Symptomatology Self Report (IDS-SR) in patients with depressive disorders and healthy controls. J Affect Disord. 2010;125:146–154.
8. Vogelzangs N, Duivis HE, Beekman ATF, Kluft C, Neuteboom J, Hoogendijk W, et al. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl Psychiatry. 2012;2:e79.
9. Vreeburg SA, Hoogendijk WJG, van Pelt J, Derijk RH, Verhagen JCM, van Dyck R, et al. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. Arch Gen Psychiatry. 2009;66:617–626.
10. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. Psychoneuroendocrinology. 1994;19:313–333.
11. van Aken MO, Romijn JA, Miltenburg JA, Lentjes EGWM. Automated measurement of salivary cortisol. Clin Chem. 2003;49:1408–1409.
12. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology. 2003;28:916–931.
13. Licht CMM, Vreeburg SA, van Reedt Dortland AKB, Giltay EJ, Hoogendijk WJG, DeRijk RH, et al. Increased sympathetic and decreased parasympathetic activity rather than changes in hypothalamic-pituitary-adrenal axis activity is associated with metabolic abnormalities. J Clin Endocrinol Metab. 2010;95:2458–2466.
14. Willemsen GH, De Geus EJ, Klaver CH, Van Doornen LJ, Carroll D. Ambulatory monitoring of the impedance cardiogram. Psychophysiology. 1996;33:184–193.
15. of Cardiology TF of TES, Others. the North American Society of Pacing and Electrophysiology (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation. 1996;93:1043–1065.
16. Berntson GG, Cacioppo JT, Binkley PF, Uchino BN, Quigley KS, Fieldstone A. Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. Psychophysiology. 1994;31:599–608.
17. Houtveen JH, Groot PFC, De Geus EJC. Effects of variation in posture and respiration on RSA and pre-ejection period. Psychophysiology. 2005;42:713–719.
18. Debonnel G, Saint-André E, Hébert C, de Montigny C, Lavoie N, Blier P. Differential physiological effects of a low dose and high doses of venlafaxine in major depression. Int J Neuropsychopharmacol. 2007;10:51–61.
19. Anatomical Therapeutic Chemical (ATC) Classification Index: Alphabetically Sorted According to Nonproprietary Drug Name ; Only ATC 5th Levels are Included. WHO Collaborating Centre for Drug Statistics and Methodology.
20. Licht CMM, de Geus EJC, Zitman FG, Hoogendijk WJG, van Dyck R, Penninx BWJH. Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). Arch Gen Psychiatry. 2008;65:1358–1367.
21. Jansen R, Verhoeven JE, Han LKM, Aberg KA, van den Oord E, Milaneschi Y, et al. An integrative study of five biological clocks in somatic and mental health. eLife. 2020.

CHAPTER 7

An Integrative Study of Five Biological Clocks in Somatic and Mental Health

Published in in *eLife* 2021, 10, e59479.

Rick Jansen*
Laura K.M. Han*
Josine E. Verhoeven
Karolina A. Aberg
Edwin C.G.J. van den Oord
Yuri Milaneschi
Brenda W.J.H. Penninx

* These authors contributed equally to this work

ABSTRACT

Biological clocks have been developed at different molecular levels and were found to be more advanced in the presence of somatic illness and mental disorders. However, it is unclear whether different biological clocks reflect similar aging processes and determinants. In ~3000 subjects, we examined whether five biological clocks (telomere length, epigenetic, transcriptomic, proteomic and metabolomic clocks) were interrelated and associated to somatic and mental health determinants. Correlations between biological clocks residualized for age were small (all r<0.2), indicating little overlap. The most consistent associations of advanced biological aging were found for male sex, higher BMI, metabolic syndrome, smoking and depression. As compared to the individual biological aging indicators, a composite index of all five biological aging indicators showed most pronounced associations with health determinants. The large effect sizes of the composite index and the low correlation between biological aging indicators suggest that one's biological aging is best reflected by combining measures from multiple cellular levels.

INTRODUCTION

Aging can be conceptualized in different ways. While chronological age is measured by date of birth, biological age reflects the relative aging of an individual's physiological condition. Biological aging can be estimated by various cellular indices[1]. Commonly used indices are based on telomere length, DNA methylation patterns (epigenetic age), variation in transcription (transcriptomic age) as well as alterations in the metabolome (metabolomic age) and in the proteome (proteomic age) (see Han et al.[2], Xia et al.[3] and Jylhava et al.[4] for recent reviews). Biological aging is defined as the residuals of regressing predicted biological age on chronological age: a positive value indicates that the biological age is larger than the chronological age. Advanced biological aging (i.e., an increased biological clock) has been associated to poor somatic health, including the onset of aging-related somatic diseases such as cardiovascular disease, diabetes and cognitive decline[3]. Advanced biological aging has also been correlated to mental health: childhood trauma[5], psychological stress and psychiatric disorders[6, 7]. Specifically, telomere length has been most extensively researched and was found to be shorter in various somatic conditions[8], all-cause mortality[9, 10] and a range of psychiatric disorders[11]. Advanced epigenetic aging has also been linked to worse somatic health, mortality[12], depressive disorder[7, 13] and post-traumatic stress disorder[14], although some studies have found associations with the opposite direction of effect[15, 16]. Advanced transcriptomic aging was found in those with higher blood pressure, cholesterol levels, fasting glucose, and body mass index (BMI)[17]. Advanced metabolomic aging increases risk on future cardiovascular disease, mortality, and functionality[18].

While all biological clocks aim to measure the biological aging process, there is limited evidence for cross-correlations among different clocks. Belsky and colleagues[19] recently showed low agreement between eleven quantifications of biological aging including telomere length, epigenetic aging and biomarker-composites. In contrast, Hasting and colleagues[20] showed relatively strong correlations (r>.50) between three physiological composite biological clocks (i.e. homeostatic dysregulation, Klemer and Doubal's method and Levine's method), but not with telomere length. Other studies showed that telomere length was not correlated with epigenetic aging[7, 21], although cell type composition adjustments revealed a modest association[22]. Further, both Hannum and Horvath epigenetic clocks[23, 24] showed modest correlations to a transcriptomic clock[17]but the molecular characteristics of ageing that lead to increased disease susceptibility remain inadequately understood. Here we

perform a whole-blood gene expression meta-analysis in 14,983 individuals of European ancestry (including replication.

Most previous studies, however, have separately considered the relation between a single biological clock and different somatic and mental health conditions. To date, extensive integrated analyses across multiple cellular and molecular aging markers in one study are lacking and it remains unknown to what extent different biological clocks are similarly associated to different health determinants. In addition, most studies did not examine health in its full range and, consequently, whether both somatic and mental health are associated with biological aging remains elusive. As it is unlikely that a single biological clock can fully capture the complexity of the aging process[25], a composite index, that integrates the different biological clocks and thereby aging at several molecular levels, may reveal the strongest health impact. Therefore, there is an additional need to integrate different biological clocks and test whether such a "composite clock" outperforms single biological blocks in its association with health determinants.

To develop a better understanding of the mechanisms underlying biological aging, this study aimed to examine 1) the intercorrelations between biological clocks based on different molecular levels ranging from DNA to metabolites, namely telomere length, epigenetic, transcriptomic, proteomic and metabolomic clocks; 2) the relationships between different biological clocks with both somatic and mental health determinants; and 3) whether a composite biological clock outperforms single biological clocks in its association with health. For the five biological clocks and the composite clock, associations were computed with a wide panel of lifestyle (e.g. alcohol use, physical activity, smoking), somatic health (functional indicators, BMI, metabolic syndrome, chronic diseases) and mental health (childhood trauma, depression status) determinants. All biological clock outcomes were residualized for chronological age.

METHODS

Study design and participants

Data used were from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study examining course and consequences of depressive and anxiety disorders. The NESDA sample consists of 2,981 persons between 18 and 65 years including persons with a current or remitted diagnosis of a depressive and/or anxiety disorder (74%) and healthy controls

(26%). Individuals were recruited from mental health care settings, general practitioners, and the general population in the period from September 2004 to February 2007. Persons with insufficient command of the Dutch language or a primary clinical diagnosis of other severe mental disorders, such as severe substance use disorder or a psychotic disorder were excluded. Participants were assessed during a 4-hour clinical visit, consisting of the collection of all somatic and mental health determinants in the current study, as well as a fasting blood draw. All omics data was obtained from the same blood sample, drawn at the same time point as the health determinant examination during the face-to-face visit. The study was approved by the Ethical Review Boards of participating centers, and all participants signed informed consent. More than 94% of the NESDA participants were from North European origin. The population and methods of the NESDA study have been described in more detail elsewhere[26]and (2.

Data to derive different biological clocks was available for different subsamples and all based on the same fasting blood draw from participants in the morning between 8:30 and 9:30 after which samples were stored in a -80°C freezer or – for RNA - transferred into PAXgene tubes (Qiagen, Valencia, California, USA) and stored at −20°C. To create biological clocks, we used telomere length (N=2936), DNA methylation (N=1130, MBD-seq, 28M CpGs), gene expression (N=1990, Affymetrix U219 micro arrays, >20K genes), proteins (N=1837, Myriad RBM DiscoveryMAP 250+, 171 proteins) and metabolites (N=2910, Nightingale platform, 231 metabolites), see Table 1 and details in the following sections.

Biological clock assessments

Telomere length. Leukocyte telomere length was determined at the laboratory of Telomere Diagnostics, Inc. (Menlo Park, CA, USA), using quantitative polymerase chain reaction (qPCR), adapted from the published original method by Cawthon et al.[27]. Telomere sequence copy number in each patient's sample (T) was compared to a single-copy gene copy number (S), relative to a reference sample. The resulting T/S ratio is proportional to mean leukocyte telomere length. The detailed method is described elsewhere[28] diabetes, obesity and cancer. This suggests mechanisms of accelerated biological aging among the depressed, which can be indicated by a shorter length of telomeres. We examine whether MDD is associated with accelerated biological aging, and whether depression characteristics such as severity, duration, and psychoactive medication do further impact on biological aging. Data are from the Netherlands Study of Depression and Anxiety, including

1095 current MDD patients, 802 remitted MDD patients and 510 control subjects. Telomere length (TL. The reliability of the assay was adequate: eight included quality control DNA samples on each PCR run illustrated a small intra-assay coefficient of variation (CV=5.1%), and inter-assay CV was also sufficiently low (CV=4.6%).

DNA methylation (Epigenetic clock). To assay the methylation levels of the approximately 28 million common CpG sites in the human genome, we used an optimized protocol for MBD-seq[7, 29]we assay methylation in MDD cases and controls from both blood (N = 1132. With this method, genomic DNA is first fragmented and the methylated fragments are then bound to the MBD2 protein that has high affinity for methylated DNA. The non-methylated fraction is washed away and only the methylation-enriched fraction is sequenced. This optimized protocol assesses about 94% of the CpGs in the methylome. The sequenced reads were aligned to the reference genome (build hg19/GRCh37) with Bowtie2 [30] using local and gapped alignment. Aligned reads were further processed using the RaMWAS Bioconductor package33) to perform quality control and calculate methylation scores for each CpG.

Gene expression (Transcriptomic clock). RNA processing and assaying -done at Rutgers University Cell and DNA repository- have been described previously [31–33]. Samples were hybridized to Affymetrix U219 arrays (Affymetrix, Santa Clara, CA). Array hybridization, washing, staining, and scanning were carried out in an Affymetrix GeneTitan System per the manufacturer's protocol. Gene expression data were required to pass standard Affymetrix QC metrics (Affymetrix expression console) before further analysis. We excluded from further analysis probes that did not map uniquely to the hg19 (Genome Reference Consortium Human Build 37) reference genome sequence, as well as probes targeting a messenger RNA (mRNA) molecule resulting from transcription of a DNA sequence containing a single nucleotide polymorphism (based on the dbSNP137 common database). After this filtering step, data for analysis remained for 423,201 probes, which was summarized into 44,241 probe sets targeting 18,238 genes. Normalized probe set expression values were obtained using Robust Multiarray Average (RMA) normalization as implemented in the Affymetrix Power Tools software (APT, version 1.12.0, Affymetrix). Data for samples that displayed a low average Pearson correlation with the probe set expression values of other samples, and samples with incorrect sex-chromosome expression were removed.

Proteins (Proteomic clock). As described previously[34], a panel of 243 analytes (Myriad RBM DiscoveryMAP 250+) involved in various hormonal, immunological, and metabolic pathways was assessed in serum using multiplexed immunoassays in a Clinical Laboratory Improvement Amendments

(CLIA)-certified laboratory (Myriad RBM; Austin, TX, USA;). After excluding analytes with more than 30% missing data (mostly due to values outside the ranges of detection), 171 of the 243 analytes remained for analysis (with values below and above detection limits imputed with the detection limit values).

Metabolites (Metabolomic clock). Metabolite measurements have been described in detail previously[18, 35]which adversely impact cardiometabolic health. Here, a comprehensive set of metabolic markers, predominantly lipids, was compared between depressed and nondepressed persons. METHODS Nine Dutch cohorts were included, comprising 10,145 control subjects and 5283 persons with depression, established with diagnostic interviews or questionnaires. A proton nuclear magnetic resonance metabolomics platform provided 230 metabolite measures: 51 lipids, fatty acids, and low-molecular-weight metabolites; 98 lipid composition and particle concentration measures of lipoprotein subclasses; and 81 lipid and fatty acids ratios. For each metabolite measure, logistic regression analyses adjusted for gender, age, smoking, fasting status, and lipid-modifying medication were performed within cohort, followed by random-effects meta-analyses. RESULTS Of the 51 lipids, fatty acids, and low-molecular-weight metabolites, 21 were significantly related to depression (false discovery rate q < .05. In short, a total of 232 metabolites or metabolite ratios were reliably quantified from Ethylenediaminetetraacetic acid plasma samples using targeted high-throughput proton Nuclear Magnetic Resonance (1H-NMR) metabolomics (Nightingale Health Ltd, Helsinki, Finland) [36]. Metabolites measures provided by the platform include 1) lipids, fatty acids and low-molecular-weight metabolites (N=51); 2) lipid composition and particle concentration measures of lipoprotein subclasses (N=98); 3) metabolite ratios (N=81). This metabolomics platform has been extensively used in large-scaled epidemiological studies in the field of diabetes, cardiovascular disease, mortality and alcohol intake[18, 37–40]yet alcohol is associated with both favourable and adverse effects on cardiometabolic risk markers. We aimed to characterize the associations of usual alcohol consumption with a comprehensive systemic metabolite profile in young adults. METHODS Cross-sectional associations of alcohol intake with 86 metabolic measures were assessed for 9778 individuals from three population-based cohorts from Finland (age 24-45 years, 52% women. The data contained missing values due to detection limits. Samples with more than 25 missings were removed (N=71), metabolites with more than 250 missings were removed (N=1). Other missing values were replaced with the median value per metabolite. In total 231 metabolites in 2910 samples remained for analysis.

Building biological clocks for multiple omics domains

Telomere length was multiplied by -1 to be able to compare directions of effects consistent with that of other biological clocks. For each of the other four omics domains (epigenetic, transcriptomic, metabolomic and proteomic data) the same approach was used to compute biological clocks. First, the omics data were residualized with respect to technical covariates (batch, lab). Second, data per omics marker were normalized using a quantile-normal transformation. Finally, biological age was computed using cross-validation by splitting the sample in 10 equal parts. For each of the ten groups, 9 parts were used as training set and the 10th as test set. In the training set the biological age estimator was computed using ridge regression (R library glmnet), with chronological age as the outcome, and the omics data as predictors. Only for methylation and gene expression a selection of predictors (CpGs for methylation based models and genes for gene expression based models) was made for each cross validation step: we increased the number of sites included in the elastic net in steps (steps for CpGs: 0, 100, 1000, 10 000, 80 000, 100 000, steps for gene expression 100, 500, 1000, 1200, 1400). CpGs/genes were selected in the order of their ranks derived from the association with age in the training sample. We selected the number of CpGs/genes where the cumulative association signal reached a stable plateau. This approach is based on the rationale that adding more markers should theoretically never decrease predictive power. We previously performed tests where the number of CpGs/genes was included in the loop over the k-folds. However, as it produced very similar results but is much more computer intensive[41]overlapped with genes found in GWAS of MDD disease status, autoimmune disease and inflammation, and co-localized with eQTLS and (genic enhancers of, this latter approach was not used. This approach resulted in 80,000 CpGs (mapping to 2,976 genes) for the epigenetic clock, and 1,200 probes (mapping to 767 genes) for the transcriptomic clock. For the proteomic and metabolomic data, all markers were used to predict age, since leaving markers out decreased the prediction accuracy. The predictor was then used in the test set to create an unbiased omics-based biological age. For each omics domain, biological aging was defined as the residuals of regressing biological age on chronological age[7, 17]but the molecular characteristics of ageing that lead to increased disease susceptibility remain inadequately understood. Here we perform a whole-blood gene expression meta-analysis in 14,983 individuals of European ancestry (including replication. Thus, in the terminology we use here, the biological aging indicators represent the biological age acceleration: a positive value means that the biological age is

larger than the chronological age. A composite index of biological aging was made by scaling each of the five biological indicators and taking the sum, in the 653 samples that had data for all five omics levels.

Health determinants

Lifestyle. Alcohol consumption was assessed as units per week by using the AUDIT[42]alcoholic (N = 65. Smoking status was assessed by pack years (smoking duration * cigarettes per day/20). Physical activity[43] was assessed using the International Physical Activity Questionnaire (IPAQ)[44]but diverse physical activity measures in use prevent international comparisons. The International Physical Activity Questionnaire (IPAQ and expressed as overall energy expenditure in Metabolic Equivalent Total (MET) minutes per week (MET level * minutes of activity * events per week).

Somatic health. Body mass index (BMI) was calculated as measured weight divided by height-squared. Functional status is one of the most potent health status indicators in predicting adverse outcomes in aging populations[45]but it is less well recognized that mortality declines at older ages have also played a substantial role in prolonging expectation of life. A person reaching age 65 in 1900 could expect to live an additional 11.9 years. Life expectancy at age 65 rose to 14.4 years by 1960 and then increased by about three years in the next three decades, reaching 17.5 years in 1992 (56, 70, including depression[46]in turn, physical disability results in increased depressive symptoms. Moreover, depression affects also the earlier stages of the disablement process (including functional limitation in mobility. Assessment of functional status includes measures of physical impairments and disability, reflecting how individuals' limitations interact with the demands of the environment. Two measures of physical impairments were available: Lung capacity was determined by measuring the peak expiratory flow (PEF in liter/minute) using a mini Wright peak flow meter. Hand grip strength was measured with a Jamar hand held dynamometer in kilograms of force and was assessed for the dominant hand. Furthermore, physical disability was measured with the World Health Organization Disability Assessment Schedule II (WHODAS-II)s the sum of scale 2 (mobility) and scale 3 (self-care). The number of self-reported current somatic diseases for which participants received medical treatment was counted. We used somatic disease categories as categorized previously[43, 47]: cardiometabolic, respiratory, musculoskeletal, digestive, neurological and endocrine diseases, and cancer. Metabolic syndrome components included waist circumference, systolic blood pressure, HDL cholesterol, triglycerides and glucose levels, which measurement methods are described elsewhere[48].

Mental health. Presence of current (6-month recency) major depressive disorder was assessed by the DSM-IV Composite International Diagnostic Interview (CIDI) version 2.1. Depressive severity levels in the week prior to assessment were measured with the 28-item Inventory of Depressive Symptomatology (IDS) self-report[49]. Childhood trauma was assessed with the Childhood Trauma Interview (CTI)[50]. In this interview, participants were asked whether they were emotionally neglected, psychologically abused, physically abused or sexually abused before the age of 16. The CTI reports the sum of the categories that were scored from 0 to 2 (0: never happened; 1: sometimes; 2: happened regularly), which was categorized into five categories.

Statistical analyses

For each of the five biological aging indicators we computed associations with demographic (sex, education), lifestyle (physical activity, smoking, alcohol use), somatic health (BMI, hand grip strength, lung function, physical disability, chronic diseases) and mental health (current depression, depression severity, childhood trauma) determinants using linear models with health determinants as predictors and biological aging as outcome (for each health determinant separately). All models included a covariate for sex, except for when sex was the outcome. For telomere length, chronological age was used as covariate in the models, for the other biological aging indicators age was not used as covariate since they are independent of chronological age by design. Standardized betas from these models are reported (by scaling predictor and outcome). Correction for multiple testing was done using permutation based FDR[51]genetic variants have been found associated. However, it is still mostly unclear through which downstream mechanism these variants cause these phenotypes. Knowledge of these intermediate steps is crucial to understand pathogenesis, while also providing leads for potential pharmacological intervention. Here we relied upon natural human genetic variation to identify effects of these variants on trans-gene expression (expression quantitative trait locus mapping, eQTL. Subject labels were permuted 1000 times and associations were computed using the permuted data (all biological aging indicators vs all health determinants). For each of the observed P-values (p) the FDR was computed as the average number of permuted P-values smaller than p, divided by the amount of real P-values smaller than p, resulting in a P-value threshold of 2e-2 for a FDR of 5% for all tests. In the 653 overlapping samples with data in each biological clock domain, we scaled (mean 0, standard deviation 1) and summed up the five biological aging indicators in order to create a composite index of biological aging.

Longitudinal analysis of mortality and chronic disease onset

As NESDA is a longitudinal study, with several follow-up measurement waves, we conducted post-hoc analyses on the relationship between the biological aging indicators and subsequent outcomes after six years of follow-up duration. The average chronological age of our cohort (mean=41 years, sd=13, range=18-65 years) is rather young, so high rates of mortality and morbidity were not expected. Mortality data was gathered at each measurement wave. Also, at each wave self-reported somatic diseases for which participants received medical treatment were assessed. Based on this, we created somatic disease categories as categorized previously[43, 47]: cardiometabolic, respiratory, musculoskeletal, digestive, neurological and endocrine diseases, and cancer. For these categories we computed chronic disease onset defined as the disease not being present at baseline (time of biological aging assessment) and present at the latest wave (six years after baseline). For each biological clock we computed longitudinal analyses, using a linear model with mortality or chronic disease onset as outcome, and the biological clock residualized for chronological age as predictor, while correcting for sex.

RESULTS

Sample characteristics

To create indicators for biological aging we used whole blood derived measurements from the Netherlands Study of Depression and Anxiety (NESDA) baseline assessment: telomere length (N=2936), epigenetics (DNA methylation, N=1130, MBD-seq, 28M CpGs), gene expression (N= 1990, Affymetrix U219 micro arrays, >20K genes), proteomics (N=1837, Myriad RBM DiscoveryMAP 250+, 171 proteins) and metabolites (N=2910, Nightingale Health platform, 231 metabolites), with 653 overlapping samples (see Table 1 for sample characteristics). Each subsample included around 66% female, with mean age of around 42 years.

Table 1. Sample description.

		Telomere Length	Epigenetic Aging	Transcriptomic Aging	Proteomic Aging	Metabolomic Aging	Composite Index
	# Subjects	2936	1130	1990	1837	2910	653
Demographic	Sex (%female)	66.00	65.00	67.00	67.00	66.00	66.00
	Education years (mean)	12.15	11.93	12.07	12.07	12.15	11.71
	Age (mean)	41.81	41.53	38.71	41.37	41.94	41.23
Lifestyle	Alcohol use (units per week, mean)	6.24	6.54	6.38	6.39	6.29	6.48
	Smoking (pack years, mean))	11.00	11.43	11.84	10.37	11.12	10.90
	Physical activity (MET minutes per week, mean)	3679.72	3638.54	3729.20	3741.00	3668.13	3525.05
Somatic Health	BMI (mean)	25.60	25.67	25.68	25.66	25.60	25.82
	Physical disability (score, mean)	24.40	29.45	26.00	23.22	24.45	30.27
	Lung capacity (PEF in liter/minute, mean)	477.74	479.75	478.42	477.19	477.23	475.23
	Hand grip strength (kg, mean)	37.06	37.77	37.08	37.46	37.05	37.74
	Cardiometabolic disease (%cases)	18	18	18	18	18	17
	Respiratory disease (%cases)	9	9	9	9	9	10
	Musculoskeletal disease (%cases)	10	10	10	9	10	9
	Digestive disease (%cases)	9	9	9	8	9	8
	Neurological disease (%cases)	3	2	3	3	3	2
	Endocrine disease (%cases)	3	3	3	3	3	4
	Cancer (%cases)	7	8	7	7	7	8
	Metabolic syndrome (# components, mean)	1.36	1.39	1.37	1.33	1.36	1.41
	# Chronic diseases (mean)	0.61	0.62	0.62	0.58	0.61	0.63
Mental Health	Current MDD (%cases)	27	72	34	26	27	76
	Depression severity (IDS, mean)	21.46	25.80	22.91	20.96	21.48	26.67
	Childhood Trauma (score from 0-4, mean)	0.91	0.97	1.00	0.87	0.92	1.01

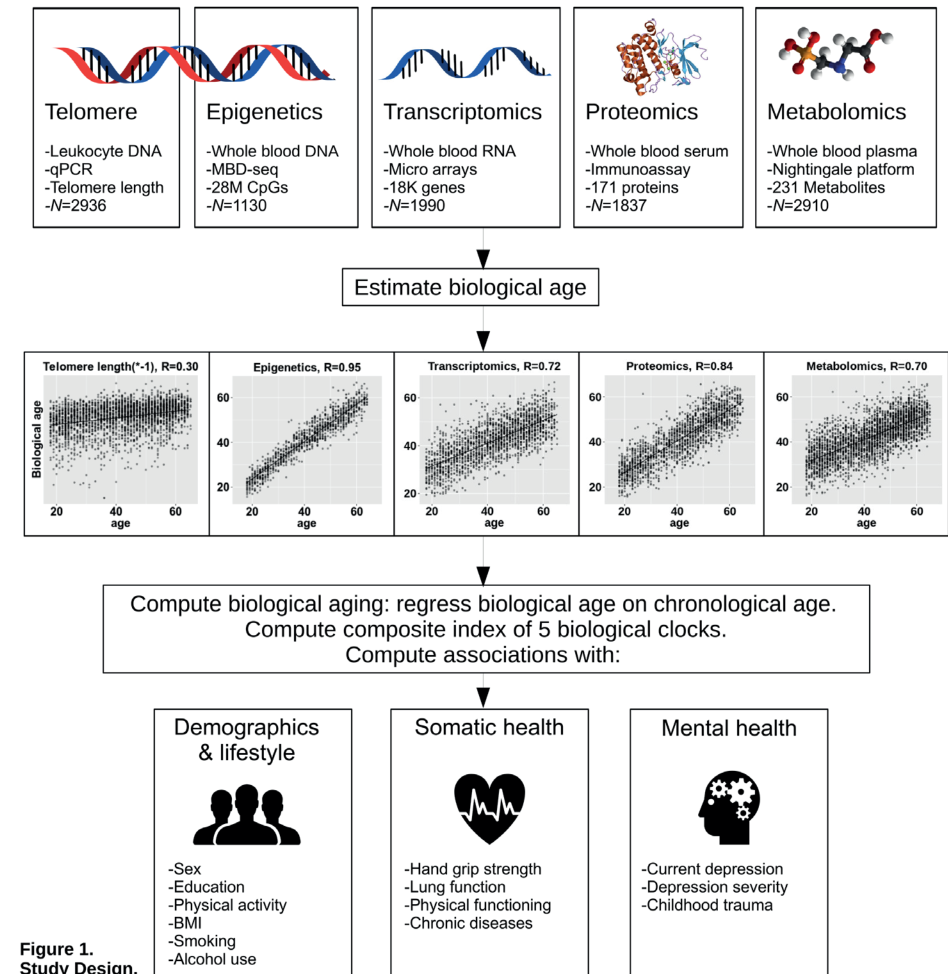


Figure 1. Study Design. The upper part of the figure shows the five biological layers. From each of the four omics layers (epigenetic, transcriptomic, proteomic and metabolomic data), biological age was estimated, and biological age was regressed on age to obtain measures of biological aging. Only telomere length was not age-regressed. The five biological aging indicators were associated with multiple demographic, lifestyle, somatic health and mental health determinants.

Computing biological clocks

The methods for creating the biological clocks are described in detail in the methods section. In brief, for each of the four omics measures (epigenetic, transcriptomic, metabolomic and proteomic) we estimated biological age using ridge regression and cross validation (see Figure 1 for study design).

As telomere length values usually decline with increasing chronological age, this indicator was multiplied by -1 to be able to compare directions of effects consistent with the other biological clocks. Correlations between chronological age and predicted biological age were 0.30 for telomere length, 0.95 for epigenetic age, 0.72 for transcriptomic age, 0.85 for proteomic age, and 0.70 for metabolomic age (Figure 1). For each omics-based biological clock, biological aging is defined as the residuals of regressing predicted biological age on chronological age: a positive value means that the biological age is larger than the chronological age. The individual clocks residualized for chronological age are also referred to as biological aging indicators. Correlations between biological aging indicators, corrected for sex, are presented in Figure 2. Correlations were significant for 3 out of 10 pairs; proteomic vs metabolomic aging (r=0.19, P=2e-16), transcriptomic vs epigenetic aging (r=0.15, P=3e-06) and transcriptomic vs proteomic aging (r=0.08, P=2e-06).

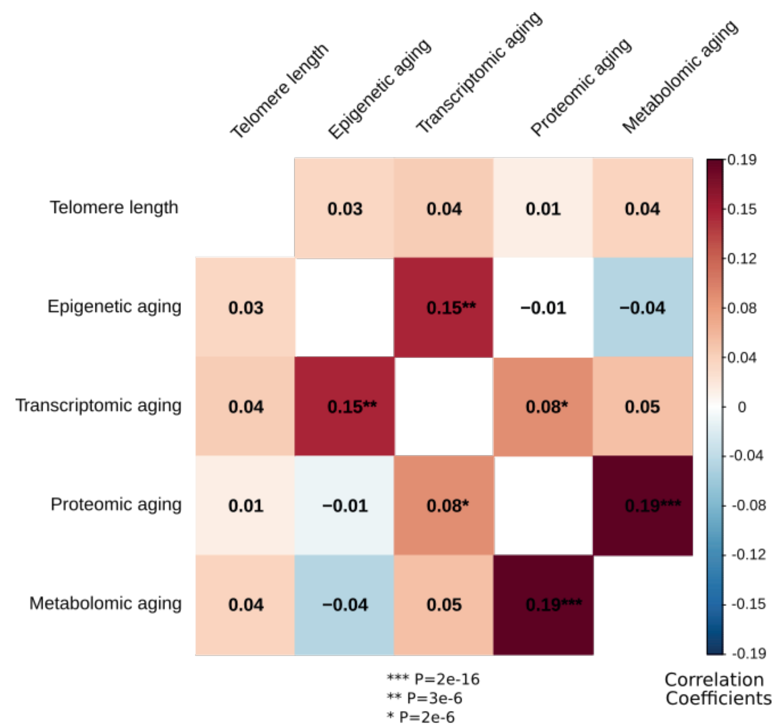


Figure 2. Correlations between the biological aging indicators. The heatmap represents Spearman rank correlations between the five biological aging indicators, all corrected for sex. Out of ten pairs, three are significant: transcriptomic vs epigenetic aging, metabolomic vs proteomic aging and proteomic vs transcriptomic aging. All biological aging indicators were age-regressed, only telomere length was not.

Associations between individual biological aging indicators and health determinants

For each of the five biological aging indicators we computed associations with several demographic (sex, education), lifestyle (physical activity, smoking, alcohol use), somatic health (BMI, hand grip strength, lung function, physical disability, chronic diseases) and mental health (current depression, depression severity, childhood trauma) determinants. Except for proteomic aging, sex was associated with all biological aging indicators: women were biologically younger than men (P=3e-4 for telomere length, P=5e-4 for epigenetic aging, P=4e-11 for transcriptomic aging, P=1e-5 for metabolomic aging). Education was not associated with any biological aging indicator. We controlled for sex by using it as a covariate in all following models (except for in the model where sex was the outcome). Table 2 and Figure 3 give an overview of all associations. Correction for multiple testing was done using permutation-based FDR (Methods), resulting in a P-value threshold of 2e-2 for an FDR of 5% for all tests.

Among the lifestyle determinants, alcohol use was associated with advanced proteomic aging (P=3e-3) and smoking (packs per year) was associated with shorter telomere length (P=3e-3), and advanced transcriptomic (P=2e-2), proteomic (P=1e-5) and metabolomic aging (P=5e-3). Physical activity was not associated with any biological aging indicator.

From the somatic health determinants, high BMI was strongly associated with advanced biological aging of all indicators (P=2e-2 for telomere length, P=4e-3 for epigenetic aging, P=6e-10 for transcriptomic aging, P=1e-7 for proteomic aging, and P=2e-35 for metabolomic aging). Physical disability was associated with advanced epigenetic aging (P=1e-4). Within the domain of chronic diseases, the presence of digestive diseases and endocrine diseases were associated with advanced proteomic aging (P=2e-2 and P=1e-2, respectively). Subjects with cardiometabolic disease showed advanced metabolomic aging (P=4e-3) and subjects with digestive disease exhibited advanced transcriptomic aging (P=1e-2). Those with metabolic syndrome showed advanced biological aging across four indicators (P=6e-4 for telomere length, P=1e-8 for transcriptomic aging, P=5e-9 for proteomic aging, P=5e-29 for metabolomic aging).

The presence of current depression and depression severity were associated advanced epigenetic (P=2e-3 and P=9e-5) and proteomic aging (P=8e-3 and P=6e-3 respectively). Current depression was also associated with advanced transcriptomic aging (P=2e-2) and those with childhood trauma showed advanced epigenetic aging (P=8e-5). To verify if the results

Table 2. Associations between five biological aging indicators and multiple health determinants.

		Telomere Length	N=2936	Epigenetic clock	N=1130	Transcriptomic Clock	N= 1990	Proteomic Clock	N=1837	Metabolom-ic Clock	N=2910	Composite Index (sum)	N=653	Composite Index (PC1)	N=653
		Beta*	P	Beta	P	Beta	P	Beta	P	Beta	P	Beta	P	Beta	P
Demographic	Sex (male/female)	-0.06	**2.89E-04**	-0.10	**4.65E-04**	-0.15	**3.64E-11**	-0.03	1.46E-01	-0.08	**1.25E-05**	-0.18	**2.33E-06**	-0.11	**3.59E-03**
	Education (# years)	-0.03	1.12E-01	-0.02	5.21E-01	-0.01	6.37E-01	-0.05	3.43E-02	-0.03	8.22E-02	-0.04	3.11E-01	-0.05	2.27E-01
Lifestyle	Alcohol use (units per week)	0.03	1.05E-01	-0.05	1.40E-01	0.00	9.21E-01	0.07	**2.89E-03**	0.04	4.57E-02	0.07	6.05E-02	0.09	**1.50E-02**
	Smoking (pack years)	0.06	**3.11E-03**	0.02	6.22E-01	0.05	**1.55E-02**	0.10	**1.33E-05**	0.05	**5.09E-03**	0.10	**1.15E-02**	0.12	**2.85E-03**
	Physical activity	0.02	2.75E-01	-0.06	3.88E-02	-0.04	6.42E-02	0.03	1.51E-01	0.01	5.18E-01	-0.04	3.62E-01	0.01	7.38E-01
Somatic Health	BMI	0.04	**1.80E-02**	0.09	**3.94E-03**	0.14	**6.02E-10**	0.12	**9.82E-08**	0.23	**2.07E-35**	0.24	**2.32E-10**	0.22	**2.18E-09**
	Physical disability	0.03	9.11E-02	0.11	**1.41E-04**	0.04	8.61E-02	0.04	7.42E-02	-0.01	4.24E-01	0.10	**7.38E-03**	0.03	4.01E-01
	Lung capacity	0.02	4.19E-01	0.03	4.65E-01	0.04	2.13E-01	-0.04	1.51E-01	0.03	2.37E-01	0.03	5.34E-01	-0.02	6.57E-01
	Hand grip strength	-0.02	3.33E-01	-0.06	1.71E-01	0.03	3.52E-01	0.01	7.30E-01	0.03	2.24E-01	-0.03	6.14E-01	0.03	6.20E-01
	Cardiometabolic disease (no/yes)	0.02	3.37E-01	0.04	1.56E-01	0.03	1.44E-01	0.03	1.35E-01	0.05	**3.94E-03**	0.10	**1.37E-02**	0.08	3.19E-02
	Respiratory disease (no/yes)	-0.02	2.12E-01	-0.01	6.34E-01	0.02	2.85E-01	0.03	1.27E-01	0.01	4.67E-01	-0.03	4.70E-01	0.01	7.17E-01
	Musculoskeletal disease (no/yes)	0.00	8.11E-01	-0.01	7.37E-01	0.04	1.04E-01	0.02	4.36E-01	0.02	2.23E-01	0.09	2.27E-02	0.11	**4.96E-03**
	Digestive disease (no/yes)	0.03	5.77E-02	-0.02	5.71E-01	0.06	**9.76E-03**	0.06	**1.21E-02**	0.02	2.81E-01	0.05	2.01E-01	0.04	2.86E-01
	Neurological disease (no/yes)	-0.02	2.58E-01	0.02	5.60E-01	0.01	5.44E-01	0.02	2.84E-01	0.02	1.93E-01	-0.04	2.64E-01	-0.02	5.09E-01
	Endocrine disease (no/yes)	-0.01	4.45E-01	0.01	8.13E-01	-0.01	5.75E-01	0.06	**1.03E-02**	0.03	1.23E-01	0.06	1.18E-01	0.09	1.64E-02
	Cancer (no/yes)	0.00	9.66E-01	0.02	5.65E-01	0.02	4.88E-01	0.03	1.81E-01	0.02	2.01E-01	0.08	3.22E-02	0.07	5.00E-02
	Metabolic syndrome (# components)	0.06	**6.35E-04**	0.04	1.46E-01	0.13	9.98E-09	0.13	**5.34E-09**	0.21	**4.53E-29**	0.28	**9.10E-13**	0.26	**6.41E-12**
	# Chronic diseases	0.00	7.99E-01	0.03	3.63E-01	0.05	3.20E-02	0.09	**1.24E-04**	0.03	1.39E-01	0.06	1.26E-01	0.07	8.43E-02
Mental Health	Current MDD (no/yes)	0.03	1.59E-01	0.09	**1.99E-03**	0.07	**1.68E-02**	0.08	**7.62E-03**	-0.03	1.61E-01	0.11	**6.05E-03**	-0.12	2.29E-01
	Depression severity	0.04	2.40E-02	0.12	**8.67E-05**	0.03	2.76E-01	0.07	**5.99E-03**	-0.02	3.74E-01	0.13	**7.61E-04**	0.05	1.87E-01
	Childhood Trauma	0.01	4.54E-01	0.12	**7.99E-05**	0.03	2.06E-01	0.04	8.96E-02	0.04	2.46E-02	0.09	**1.96E-02**	0.07	7.19E-02

For each biological aging indicator linear models were fit with the health determinant as predictor, while controlling for sex. Beta's and P-values from these models are presented here. In the 653 samples with all five data layers available, a composite index was constructed which was significantly associated with more variables than any of the five individual biological aging indicators. All biological aging indicators were age-regressed, only telomere length was not. Telomere length models were corrected for age instead. * Beta for telomere length was multiplied by -1 to compare with other biological aging indicators. All measures are coded such that higher values indicate advanced biological aging. Bold indicates FDR<5%.

were confounded by medication use, we computed associations between antidepressant medication (SSRIs, TCAs, or other antidepressants), metabolic syndrome related medication ('metabolic medication': anti-diabetic, fibrates, or anti-hypertensives) and biological aging (Table S1). After FDR correction, we found that metabolomic aging was associated with the use of metabolic medication (P=2.35e-3), and antidepressant use with proteomic (P=7.16e-5) and transcriptomic aging (P=8.1e-3). The design of the current observational study cannot conclusively prove whether this is a direct medication effect or confounding by indication.

Associations between the biological aging composite index and all health determinants

The composite index was computed as the sum of the five scaled biological aging indicators in the 653 samples with data of all five biological levels. Correlations between the five biological aging indicators and the composite index were between 0.43 and 0.51. We found more and stronger associations for the composite index than for any of the individual biological aging indicators: including sex (P=2e-6), BMI (P=2e-10), smoking (P=2e-2), metabolic syndrome (P=9e-13), current MDD (P=6e-3), depression severity (P=7e-4) and childhood trauma (P=2e-2). As an alternative approach, Principal Component Analysis (PCA) was used to compute an alternative composite index. We used the first principle component (PC) of this analysis, which was a weighted sum of the biological aging indicators (for telomere length the weight (w)=0.042, epigenetic aging w=0.094, transcriptomic aging w=0.220, proteomic aging w=0.707, metabolomic aging w=0.664), reflecting the highest correlations between the biological aging indicators, which is between metabolomic and proteomic aging. Compared to the composite index that was based on the sum and thus gives equal weight to all five biological aging indicators, the PC-based index had less significant associations with sex, smoking, BMI, and metabolic syndrome. The PC-based index was not significantly associated with physical disability, or mental health outcomes, as opposed to the summed index. The five PC's each explain more than 15% of variance (the first 2 PC's more than 25% each), indicating the multidimensionality and non-redundancy of the five biological clocks.

To allow for direct effect size comparisons between the composite (summed) index and the individual clocks, we compared the findings for the composite index to those of each individual biological aging indicator with the same subsample. In this analysis, P-values and effect sizes were often more pronounced for the composite index (Figure 4, Table S2). For example,

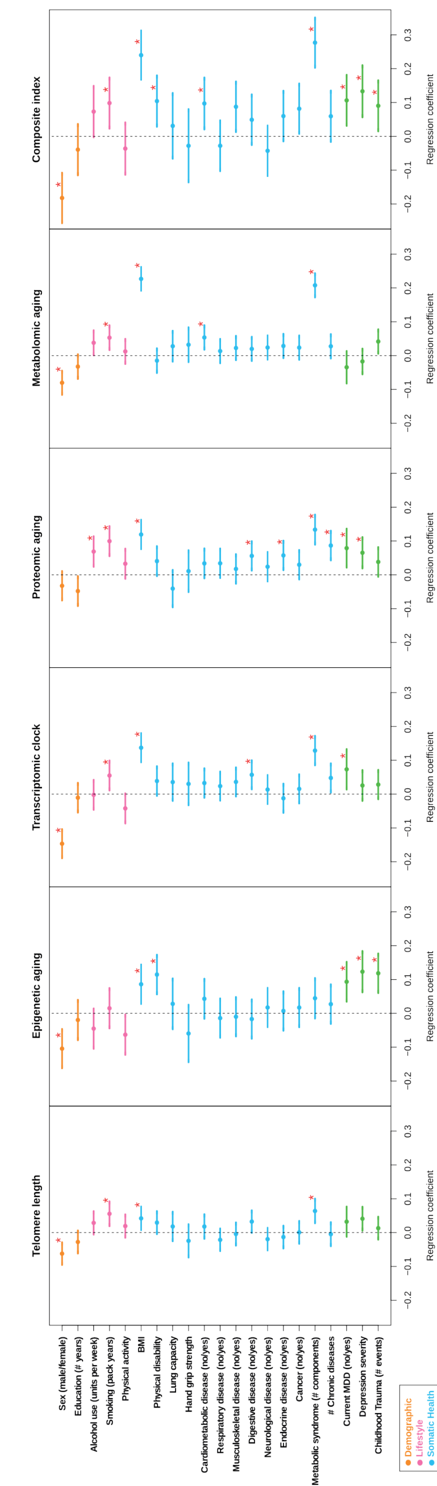


Figure 3. Forest plot of associations between biological aging and health determinants. For each of the associations between biological aging indicators and health determinants, the standardized beta and standard deviation derived from linear models were plotted. The significant associations (P<2e-2, FDR<5%) are shown with red stars. The composite index, which is the scaled sum of the five biological aging indicators, clearly shows most associations and often largest effect sizes. Biological aging was used as outcome in the linear models. Beta for telomere length was multiplied by -1 to compare with other biological clocks. Red stars indicate FDR<5%.

sex, BMI, metabolic syndrome and current MDD, were significantly associated with the composite index, but the betas for the composite index were larger than the betas from any individual indicator. For the other five variables significantly associated with the composite index (smoking, physical disability, cardiometabolic disease, depression severity and childhood trauma) the betas for the composite index were larger than 4 out of 5 betas from the individual clocks.

DISCUSSION

In this study, we examined five biological clocks based on telomere length and four omics levels from a large, clinically well-characterized cohort. We demonstrated significant intercorrelations between three pairs of biological aging indicators, illustrating the complex and multifactorial processes of biological aging. Furthermore, we observed both overlapping and unique associations between the individual clocks and different lifestyle, somatic and mental health determinants. Separate linear regressions showed that male sex, high BMI, smoking, and metabolic syndrome were consistently associated with more advanced levels of biological aging across at least four of the biological clocks. Strikingly, depression was associated to more advanced levels of epigenetic, transcriptomic and proteomic aging, signifying that both somatic and mental health is associated with the biological clocks. Finally, by integrating a composite index of all biological aging indicators we were able to obtain larger effect sizes with e.g. physical disability and childhood trauma exposure, underscoring the broad impact of determinants on cumulative multi-system biological aging.

The range of correlations among the biological aging indicators considered in this study indicates that the correlates of chronological age in different molecular layers were not strongly correlated, suggesting that biological aging may be differently manifested at certain cellular levels. Consistent with prior studies we showed weak correlations between different biological clocks[52] and we confirm the absent relationship between telomere length and epigenetic aging[21, 53, 54], but also show lack of associations with transcriptomic, proteomic or metabolomic aging. However, we do confirm an earlier finding showing a significant but modest correlation between epigenetic and transcriptomic aging[55]. The correlation between metabolomic and proteomic aging may partly be explained by the fact that both data were obtained from platforms that were aimed at probing central inflammation lipid processes, rather than the full proteome or metabolome. Nevertheless, we

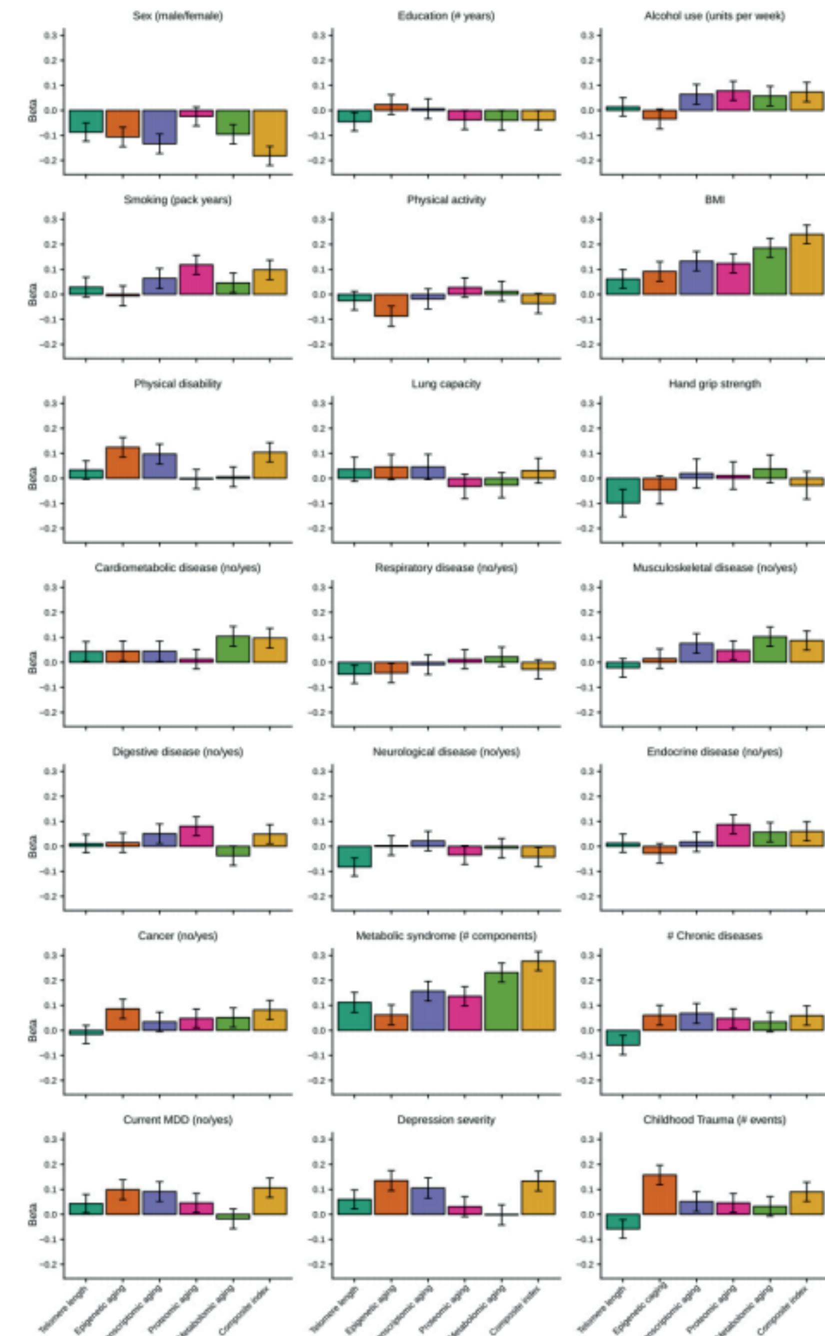


Figure 4. Barplots of betas from associations between biological aging and health determinants. For each of the associations between biological aging and health determinants, the standardized beta and standard deviation derived from linear models were plotted. Only samples that had data for all five biological clocks (N=653) were used.

can infer that only some biological clocks show overlap, while most of them seem to be tracking distinctive parts of the aging process, even if they are associated with the same somatic or health determinants.

Our study showed that several of the determinants considered exhibited consistent associations with different biological aging indicators. First, male sex was associated with shorter telomere length and advanced epigenetic, transcriptomic and metabolomic aging, in line with a large body of literature that shows advanced biological aging and earlier mortality in males compared to females[56]. Second, high BMI was consistently related to all biological clocks, showing that the more overweight or obese, the higher the biological age[57], also after controlling for sex. Earlier studies showed similar associations between high BMI and shorter telomere length[57], and older epigenetic[58] and transcriptomic aging signatures[17]. Third, our analyses showed similarly consistent associations between the prevalence of metabolic syndrome and advanced levels of aging. Further, all but epigenetic aging was advanced with respect to cigarette smoking.

Major depressive disorder (MDD) status was consistently related to advanced aging in three (epigenetic, transcriptomic, proteomic) out of the five biological clocks. In contrast, a recent study (N>1000) in young adults (20-39 years) did not show associations between mental health (as measured by the CIDI) and biological aging (indicated by telomere length, homeostatic dysregulation, Klemer and Doubal's method and Levine's method)[20], but it seems possible that this sample was too young to fully develop aging-related manifestations of mental health problems, or lacked age variation. It is likely that our data (obtained from participants 18-64 years) may have been more sensitive in picking up associations with mental health due to increased variation in both chronological age (i.e. inclusion of older persons), as well as symptom severity. To further examine whether the results were consistent across participants with and without depressive psychopathology, we repeated all models in post-hoc analyses and added an interaction term between current depression status and health determinants. There was an overall consistent pattern of non-significant interaction terms for most health determinants and biological aging, although only higher BMI was significantly associated to advanced epigenetic aging in the psychopathology group. However, taken together, the results suggest that findings are not different in persons with and without mental disorders. We observed some significant associations between biological aging and medication use. The design of the current observational study cannot conclusively prove whether this is a direct medication effect or confounding by indication: the patient group using antidepressant medication is also the group that is more chronically

and severely depressed. This is similar for the metabolic syndrome related medication. Future studies using randomized clinical trial designs are needed to investigate the mechanism of action of direct pharmacological effects of medication on biological aging.

Furthermore, we computed a composite index by summing up the five biological aging indicators studied here. In other words, this integrative metric contains cumulative independent signal from the individual markers and dependent shared signal - with possible reduced noise due to the summation - between them. Given that this composite index demonstrates larger effect sizes for BMI, sex, smoking, depression severity, and metabolic syndrome than the individual aging indicators, it is suggested that being biologically old at multiple cellular levels has a cumulative multi-systemic effect. When integrated, the composite index reveals stronger (i.e. greater cumulative betas for the composite index than individual clocks) converging associations with sex, BMI, metabolic syndrome and current MDD. This provides further support for the hypothesis that not one biological clock sufficiently captures the biological aging process and that not all clocks are under the control of one unitary aging process. There is abundant room for further progress in determining whether biological aging can be modified by intervening on these determinants.

Nonetheless, the question remains which biological mechanism could plausibly link the current quantification of biological aging and its lifestyle, somatic, and mental health determinants. Part of this answer requires discussion on the features used to build the different clocks: the proteomic and metabolomic clocks mostly measure inflammatory or metabolic factors, two highly integrated processes in aging and aging-related diseases[59]. Previous studies suggest immune-mediated mechanisms (specifically inflammatory signaling) connecting metabolic syndrome[60], mental health disorders[61], and aging[62]. Moreover, MDD is a condition in which inflammation, obesity, and premature or advanced aging co-occur and converge. It might therefore be speculated that immunity and "inflammaging"[63] may tie together the currently observed associations.

This study did not include existing biological clocks. While the application of established algorithms would increase generalizability of our findings, there are several reasons why it was not optimal to implement previously published algorithms in the NESDA data. First and foremost, generated omics data are platform-dependent and the existing epigenetic[64] and gene expression[55] clocks rely on arrays with different coverage of probes as was used in NESDA, that also target different parts of genes. Second, a subsample of NESDA was part of the previously published metabolomic clock[65], thus application of this

model to the current dataset would result in overfitting. The current proteomic platform has not been used before to train a biological clock. Moreover, there is currently no validated gold standard for calculating transcriptomic, proteomic, or metabolomic clocks. Importantly, in spite of these limitations, we have followed an alternative but consistent methodological approach for training our omics-based biological clocks, leveraging the advantage to compare, combine, and integrate these clocks within the same population. However, we emphasize the need for epidemiological replication of these determinants in other datasets (e.g. those including different ethnicities) and we recognize that data harmonization and pooling are important strategies on the scientific research agenda that may overcome this limitation in the future.

Since no previously published algorithms were used, we trained our own clocks using ridge regression with cross-validation. This approach relies on the assumption that the determined cross-sectional correlation between the omics patterns and chronological age arise mainly as a consequence of biological aging, and is independent from potential secular trends[66–68]. As common to cross-sectional studies, it is, however, impossible to completely rule out potential cohort effects or uncontrolled individual differences and results should be interpreted in light of this limitation. Future longitudinal studies are needed to identify patterns of biological changes that go beyond their ability to predict age at the time of sampling. While the current study only used chronological age as criterion endpoint, it is important to mention that other epigenetic clocks exist that are trained to predict other potential criteria such as phenotypic markers of age (DNAm PhenoAge)[69] or a composite biomarker that was derived from DNAm surrogates and smoking in pack-years (GrimAge)[70]. Such clocks were developed to lead to improved predictions of risk of mortality.

More research is needed to elucidate whether: 1) physiological disturbances, such as loss of inflammatory control associated with somatic and psychopathology, accelerate biological aging over time, 2) advanced biological aging precedes and constitutes a vulnerability factor that causes somatic and psychopathology, or 3) somatic and psychopathology and biological aging processes are not causally linked, but share underlying etiological roots (e.g. shared genetic risks or environmental factors)[2]. Yet, it could conceivably be hypothesized that dysregulation of immunoinflammatory control may be related to metabolic outcomes, aging, and depression[71], providing scope as to why some of these determinants converge across different platforms and multiple biological levels.

Here, we used a large cohort that was well-characterized in terms of demographics, lifestyle, and both somatic and mental health assessments,

to study and integrate five biological clocks across multiple levels of analysis. This is particularly important as we show that the determinants of biological aging encompass several different domains. Moreover, our sample was adequately powered to detect statistically significant associations, limiting the possibility for chance findings and increasing probability for identifying robust biological age determinants. On the other hand, an obvious limitation is the cross-sectional nature of this study that prevents us from drawing any conclusions on whether the determinants accelerate the aging trajectory over time, the other way around, or whether “third” variables effect this association.

Another aspect that limits the interpretability of our findings in the context of increased risk of developing aging-related diseases and mortality was the relatively young age of the current sample. To illustrate, we were unable to predict future incidence of chronic disease or mortality from baseline biological aging, likely due to the low numbers of mortality and disease onset (Table S3, e.g. the number of deceased cases ranged from 64 (TL) to 27 (proteomic clock). Previous studies that have associated biological aging with mortality risk commonly include aging cohorts (Danish longitudinal twin study with mean age of 86.1 years; Framingham Offspring Study with mean age 61.0 years; Swedish population cohort SATSA with mean age 63.6 years; German population cohort ESTHER with mean age 62.5 years; Lothian Birth Cohorts with mean age >69.5 years; Normative Aging Study with mean age 71.7 years)[21, 52, 72–75]. Before definitively interpreting a “clock” as a measure of biological aging, further independent studies are needed to establish that the clock changes with advancing age and forecasts disease, disability and mortality.

Conclusions

In conclusion, this study examined the overlap between five biological clocks and their shared and unique associations with somatic and mental health. Our findings indicate that they largely track distinct, but also partially overlapping aspects of this aging process. Further, we demonstrated that male sex, smoking, higher BMI and metabolic syndrome were consistently related to advanced aging at multiple biological levels. Remarkably, our study also converges evidence of depression and childhood trauma associations across multiple platforms, cellular levels, and sample sizes, highlighting the important link between mental health and biological aging. Taken together, our findings contribute to the understanding and identification of biological age determinants, important to the development of end points for clinical and epidemiological research.

REFERENCES

1. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–1217.
2. Han LK, E Verhoeven J, Tyrka AR, Penninx BW, Wolkowitz OM, Månsson KN, et al. Accelerating Research on Biological Aging and Mental Health: Current Challenges and Future Directions. Psychoneuroendocrinology. 2019;106:293–311.
3. Xia X, Chen W, McDermott J, Han J-DJ. Molecular and phenotypic biomarkers of aging. F1000Research. 2017;6:860.
4. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.
5. Li Z, He Y, Wang D, Tang J, Chen X. Association between childhood trauma and accelerated telomere erosion in adulthood: A meta-analytic study. J Psychiatr Res. 2017;93:64–71.
6. Darrow SM, Verhoeven JE, RÈvÈsz Dû, Lindqvist D, Penninx BWJH, Delucchi KL, et al. The Association Between Psychiatric Disorders and Telomere Length. Psychosom Med. 2016;78:776–787.
7. Han LKM, Aghajani M, Clark SL, Chan RF, Hattab MW, Shabalin AA, et al. Epigenetic Aging in Major Depressive Disorder. Am J Psychiatry. 2018:appi.ajp.2018.1.
8. Jin X, Pan B, Dang X, Wu H, Xu D. Relationship between short telomere length and stroke: A meta-analysis. Medicine (Baltimore). 2018;97:e12489.
9. Mons U, M¸ezzinler A, Schˆttker B, Dieffenbach AK, Butterbach K, Schick M, et al. Leukocyte Telomere Length and All-Cause, Cardiovascular Disease, and Cancer Mortality: Results From Individual-Participant-Data Meta-Analysis of 2 Large Prospective Cohort Studies. Am J Epidemiol. 2017;185:1317–1326.
10. Wang Q, Zhan Y, Pedersen NL, Fang F, Hägg S. Telomere Length and All-Cause Mortality: A Meta-analysis. Ageing Res Rev. 2018;48:11–20.
11. Lindqvist D, Epel ES, Mellon SH, Penninx BW, RÈvÈsz D, Verhoeven JE, et al. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neurosci Biobehav Rev. 2015;55:333–364.
12. Marioni RE, Shah S, McRae AF, Chen BH, Colicino E, Harris SE, et al. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 2015;16:25.
13. Whalley HC, Gibson J, Marioni R, Walker RM, Clarke T-K, Howard DM, et al. Accelerated Epigenetic Ageing in Major Depressive Disorder. BioRxiv. 2017:210666.
14. Wolf EJ, Maniates H, Nugent N, Maihofer AX, Armstrong D, Ratanatharathorn A, et al. Traumatic stress and accelerated DNA methylation age: A meta-analysis. Psychoneuroendocrinology. 2018;92:123–134.
15. Verhoeven JE, Yang R, Wolkowitz OM, Bersani FS, Lindqvist D, Mellon SH, et al. Epigenetic Age in Male Combat-Exposed War Veterans: Associations with Posttraumatic Stress Disorder Status. Mol Neuropsychiatry. 2018;4:90–99.
16. Boks MP, Mierlo HC van, Rutten BPF, Radstake TRDJ, De Witte L, Geuze E, et al. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. Psychoneuroendocrinology. 2015;51:506–512.
17. Peters MJ, Joehanes R, Pilling LC, Schurmann C, Conneely KN, Powell J, et al. The transcriptional landscape of age in human peripheral blood. Nat Commun. 2015;6:8570.
18. Akker EB van den, Trompet S, Wolf JJHB, Beekman M, Suchiman HED, Deelen J, et al. Predicting biological age based on the BBMRI-NL 1H-NMR metabolomics repository. BioRxiv. 2019:632919.

19. Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, et al. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? Am J Epidemiol. 2017. November 2017. https://doi.org/10.1093/aje/kwx346.
20. Hastings WJ, Shalev I, Belsky DW. Psychoneuroendocrinology Comparability of biological aging measures in the National Health and Nutrition Examination Study , 1999 – 2002. Psychoneuroendocrinology. 2019;106:171–178.
21. Marioni RE, Harris SE, Shah S, Mcrae AF, Zglinicki T Von, Martin-ruiz C, et al. The epigenetic clock and telomere length are independently associated with chronological age and mortality. Int J Epidemiol. 2016:1–9.
22. Chen BH, Carty CL, Kimura M, Kark JD, Chen W, Li S, et al. Leukocyte telomere length , T cell composition and DNA methylation age. 2017;9:1983–1995.
23. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 2013;49:359–367.
24. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
25. Cole JH, Marioni RE, Harris SE, Deary IJ. Brain age and other bodily 'ages': implications for neuropsychiatry. Mol Psychiatry. 2018. June 2018. https://doi.org/10.1038/s41380-018-0098-1.
26. Penninx BWJH, Beekman ATF, Smit JH, Zitman FG, Nolen WA, Spinhoven P, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res. 2008;17:121–140.
27. Cawthon RM. Telomere measurement by quantitative PCR. 2002;30:1–6.
28. Verhoeven JE, RÈvÈsz D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH, et al. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. MolPsychiatry. 2014;19:895–901.
29. Aberg KA, Dean B, Shabalin AA, Chan RF, Han LKMM, Zhao M, et al. Methylome-wide association findings for major depressive disorder overlap in blood and brain and replicate in independent brain samples. Mol Psychiatry. 2018:1.
30. Langmead. Bowtie2. 2013;9:357–359.
31. Wright FA, Sullivan PF, Brooks AI, Zou F, Sun W, Xia K, et al. Heritability and genomics of gene expression in peripheral blood. Nat Genet. 2014;46:430–437.
32. Hattab MW, Shabalin AA, Clark SL, Zhao M, Kumar G, Chan RF, et al. Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. Genome Biol. 2017;18.
33. Jansen R, Batista S, Brooks AI, Tischfield JA, Willemsen G, van Grootheest G, et al. Sex differences in the human peripheral blood transcriptome. BMC Genomics. 2014;15:33.
34. Lamers F, Bot M, Jansen R, Chan MK, Cooper JD, Bahn S, et al. Serum proteomic profiles of depressive subtypes. Transl Psychiatry. 2016;6:e851–e851.
35. Bot M, Milaneschi Y, Al-Shehri T, Amin N, Garmaeva S, Onderwater GLJ, et al. Metabolomics Profile in Depression: A Pooled Analysis of 230 Metabolic Markers in 5283 Cases With Depression and 10,145 Controls. Biol Psychiatry. 2019. August 2019. https://doi.org/10.1016/j.biopsych.2019.08.016.
36. Soininen P, Kangas AJ, W¸rtz P, Suna T, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. Circ Cardiovasc Genet. 2015;8:192–206.

37. Würtz P, Cook S, Wang Q, Tiainen M, Tynkkynen T, Kangas AJ, et al. Metabolic profiling of alcohol consumption in 9778 young adults. Int J Epidemiol. 2016;45:1493–1506.
38. Wurtz P, Makinen V-P, Soininen P, Kangas AJ, Tukiainen T, Kettunen J, et al. Metabolic Signatures of Insulin Resistance in 7,098 Young Adults. Diabetes. 2012;61:1372–1380.
39. Würtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite Profiling and Cardiovascular Event Risk. Circulation. 2015;131:774–785.
40. Fischer K, Kettunen J, Würtz P, Haller T, Havulinna AS, Kangas AJ, et al. Biomarker Profiling by Nuclear Magnetic Resonance Spectroscopy for the Prediction of All-Cause Mortality: An Observational Study of 17,345 Persons. PLoS Med. 2014;11:e1001606.
41. Clark SL, Hattab MW, Chan RF, Shabalin AA, Han LKM, Zhao M, et al. A methylation study of long-term depression risk. Mol Psychiatry. 2020. 2020. https://doi.org/10.1038/s41380-019-0516-z.
42. Babor TF, Kranzler HR, Lauerman RJ. Early detection of harmful alcohol consumption: comparison of clinical, laboratory, and self-report screening procedures. Addict Behav. 1989;14:139–157.
43. Gerrits MM, van Oppen P, van Marwijk HW, van der Horst H, Penninx BW. The impact of chronic somatic diseases on the course of depressive and anxiety disorders. Psychother Psychosom. 2013;82:64–66.
44. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35:1381–1395.
45. Guralnik JM, Fried LP, Salive ME. Disability as a Public Health Outcome in the Aging Population. Annu Rev Public Health. 1996;17:25–46.
46. Milaneschi Y, Penninx B. Depression in Older Persons with Mobility Limitations. Curr Pharm Des. 2014;20:3114–3118.
47. Gaspersz R, Lamers F, Beekman ATF, van Hemert AM, Schoevers RA, Penninx BWJH. The Impact of Depressive Disorder Symptoms and Subtypes on 6-Year Incidence of Somatic Diseases. Psychother Psychosom. 2018;87:308–310.
48. RÈvÈsz D, Milaneschi Y, Verhoeven JE, Penninx BW. Telomere length as a marker of cellular ageing is associated with prevalence and progression of metabolic syndrome. JClinEndocrinolMetab. 2014:jc20141851.
49. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. Psychol Med. 1996;26:477–486.
50. De Graaf R, Bijl R V., Smit F, Vollebergh WAM, Spijker J. Risk factors for 12-month comorbidity of mood, anxiety, and substance use disorders: Findings from the Netherlands Mental Health Survey and Incidence Study. Am J Psychiatry. 2002;159:620–629.
51. Fehrmann RSN, Jansen RC, Veldink JH, Westra HJ, Arends D, Bonder MJ, et al. Trans-eqtls reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the hla. PLoS Genet. 2011;7.

SUPPLEMENTARY MATERIALS

An Integrative Study of Five Biological Clocks in Somatic and Mental Health

SUPPLEMENTARY TABLES

Table S1. Associations between biological aging and medication use.

	Telomere Length			Epigenetic Aging			Transcriptomic Aging			Proteomic Aging			Metabolomic Aging		
	Beta	SD	P	Beta	SD	P	Beta	SD	P	Beta	SD	P	Beta	SD	P
Metabolic medication (no/yes)	0.049	0.050	3.29E-01	0.139	0.082	9.09E-02	0.075	0.061	2.18E-01	0.097	0.062	1.16E-01	0.153	0.050	***2.35E-03***
Antidepressants (no/yes)	0.083	0.040	3.87E-02	0.106	0.064	9.96E-02	0.129	0.049	***8.10E-03***	0.208	0.052	***7.16E-05***	0.086	0.043	4.46E-02

In bold the P-values significant at FDR 5%
SD=standard deviation

Table S2. **Associations between biological aging (individual indicators and composite index) and health determinants in 653 overlapping samples.**

		Telomere Length	N=653	Epigenetic Aging	N=653	Transcriptomic Aging	N=653	Proteomic Aging	N=653	Metabolomic Aging	N=653	Composite Index	N=653
		Beta*	P	Beta	P	Beta	P	Beta	P	Beta	P	Beta	P
Demographic	Sex (male/female)	-0.09	**1.77E-02**	-0.11	**6.69E-03**	-0.13	**6.55E-04**	-0.02	5.21E-01	-0.10	**1.39E-02**	-0.18	**2.33E-06**
	Education (# years)	-0.05	2.20E-01	0.02	5.62E-01	0.01	8.73E-01	-0.04	3.19E-01	-0.04	3.13E-01	-0.04	3.11E-01
Lifestyle	Alcohol use (units per week)	0.01	7.06E-01	-0.03	3.88E-01	0.06	1.05E-01	0.08	4.21E-02	0.06	1.46E-01	0.07	6.05E-02
	Smoking (pack years)	0.03	4.67E-01	-0.01	8.83E-01	0.06	1.08E-01	0.12	**2.09E-03**	0.05	2.42E-01	0.10	**1.15E-02**
	Physical activity	-0.03	5.03E-01	-0.09	3.11E-02	-0.02	6.57E-01	0.03	4.77E-01	0.01	7.51E-01	-0.04	3.62E-01
Somatic Health	BMI	0.06	1.03E-01	0.09	**1.92E-02**	0.13	**6.52E-04**	0.12	**1.08E-03**	0.19	**1.49E-06**	0.24	**2.32E-10**
	Physical disability	0.03	3.69E-01	0.12	**1.65E-03**	0.10	**1.42E-02**	0.00	9.40E-01	0.01	8.82E-01	0.10	**7.38E-03**
	Lung capacity	0.04	4.52E-01	0.05	3.70E-01	0.05	3.66E-01	-0.03	5.10E-01	-0.03	5.91E-01	0.03	5.34E-01
	Hand grip strength	-0.10	6.93E-02	-0.05	4.17E-01	0.02	7.37E-01	0.01	8.38E-01	0.04	4.99E-01	-0.03	6.14E-01
	Cardiometabolic disease (no/yes)	0.04	2.80E-01	0.05	2.59E-01	0.04	2.69E-01	0.01	7.42E-01	0.10	**9.07E-03**	0.10	**1.37E-02**
	Respiratory disease (no/yes)	-0.05	1.93E-01	-0.04	2.80E-01	-0.01	8.12E-01	0.01	7.42E-01	0.02	5.65E-01	-0.03	4.70E-01
	Musculoskeletal disease (no/yes)	-0.02	5.58E-01	0.02	7.02E-01	0.08	5.27E-02	0.05	2.05E-01	0.10	**8.27E-03**	0.09	2.27E-02
	Digestive disease (no/yes)	0.01	7.59E-01	0.01	7.05E-01	0.05	1.94E-01	0.08	3.32E-02	-0.04	3.35E-01	0.05	2.01E-01
	Neurological disease (no/yes)	-0.08	2.28E-02	0.00	9.29E-01	0.02	5.80E-01	-0.03	3.60E-01	-0.01	8.51E-01	-0.04	2.64E-01
	Endocrine disease (no/yes)	0.01	7.23E-01	-0.03	4.79E-01	0.02	6.42E-01	0.09	2.02E-02	0.06	1.45E-01	0.06	1.18E-01
	Cancer (no/yes)	-0.02	6.53E-01	0.09	2.69E-02	0.03	3.80E-01	0.05	2.03E-01	0.05	1.87E-01	0.08	3.22E-02
	Metabolic syndrome (# components)	0.11	**5.08E-03**	0.06	1.23E-01	0.16	**7.74E-05**	0.14	**4.47E-04**	0.23	**3.07E-09**	0.28	**9.10E-13**
	# Chronic diseases	-0.06	1.30E-01	0.06	1.26E-01	0.07	8.66E-02	0.05	2.14E-01	0.03	3.89E-01	0.06	1.26E-01
Mental Health	Current MDD (no/yes)	0.04	2.43E-01	0.10	**1.26E-02**	0.09	2.14E-02	0.05	2.34E-01	-0.02	6.53E-01	0.11	**6.05E-03**
	Depression severity	0.06	1.16E-01	0.14	**8.43E-04**	0.11	**9.96E-03**	0.03	4.43E-01	0.00	9.58E-01	0.13	**7.61E-04**
	Childhood Trauma	-0.06	1.17E-01	0.16	**6.12E-05**	0.05	1.94E-01	0.05	2.32E-01	0.03	4.15E-01	0.09	**1.96E-02**

For each biological aging indicator linear models were fit with the health determinant as predictor, while controlling for sex. The analysis was limited to the 653 samples with all five data layers available. Beta's and P-values from these models are presented here. * Beta for telomere length was multiplied by -1 to compare with other biological aging indicators. All measures are coded such that higher values indicate advanced biological aging. Bold indicates FDR<5%.

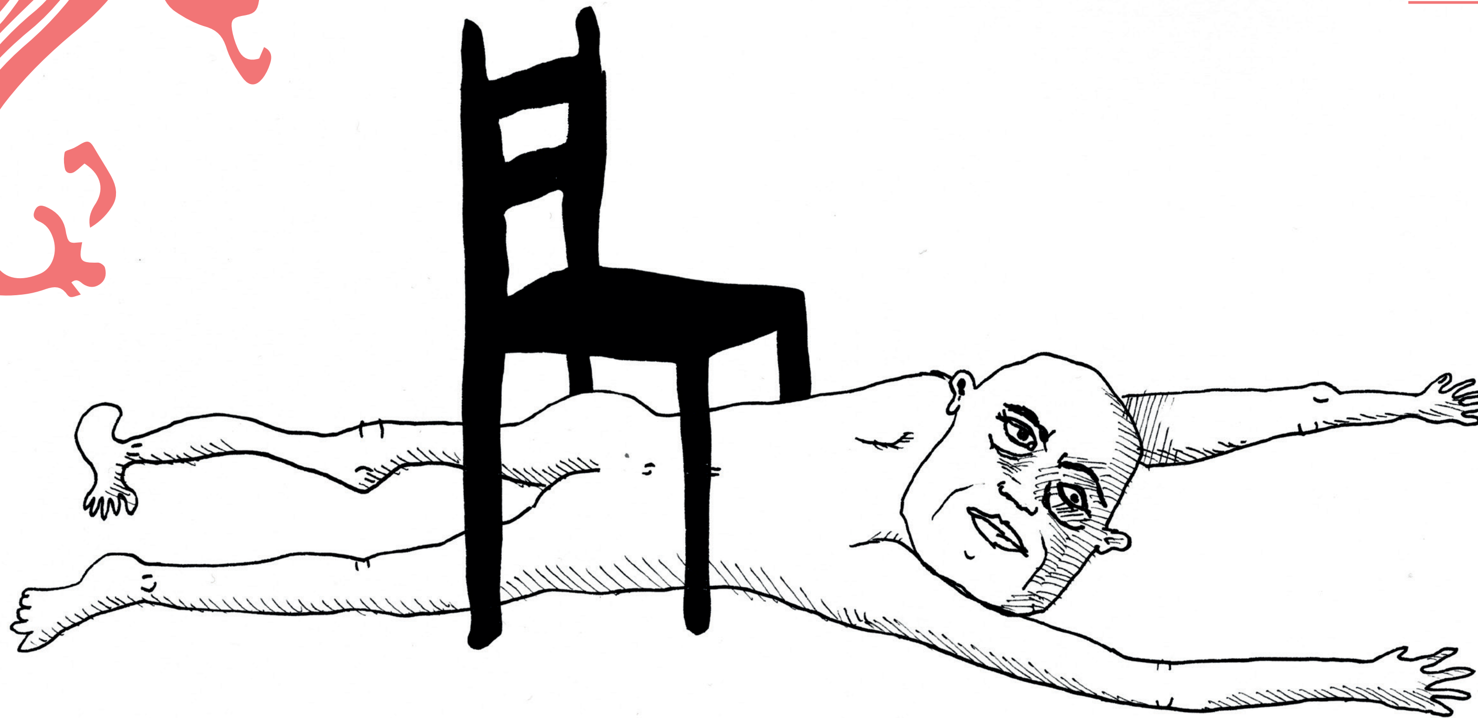
Table S3. Longitudinal analysis of biological aging and mortality and chronic disease onset.

	Telomere Length	N=2936		Epigenetic Aging	N=1130		Transcriptomic Aging	N= 1990		Proteomic Aging	N=1837		Metabolomic Aging	N=2910	
	Beta*	P	# cases	Beta	P	# cases	Beta	P	# cases	Beta	P	# cases	Beta	P	# cases
Mortality (no/yes)	0.124	2.92E-01	64	0.116	5.36E-01	29	0.005	9.72E-01	42	0.337	6.88E-02	27	-0.031	8.04E-01	63
Metabolic syndrome onset (no/yes)	-0.101	4.24E-01	55	0.085	6.91E-01	22	-0.049	7.65E-01	37	0.108	4.61E-01	44	0.237	7.90E-02	55
Cardiometabolic disease onset (no/yes)	0.020	7.96E-01	164	-0.127	2.98E-01	71	0.078	4.06E-01	118	0.200	2.70E-02	119	0.217	6.38E-03	165
Respiratory disease onset (no/yes)	0.045	6.64E-01	82	-0.200	2.42E-01	35	0.186	1.83E-01	51	0.217	8.55E-02	59	-0.122	2.67E-01	83
Musculoskeletal disease onset (no/yes)	-0.009	9.19E-01	128	0.014	9.17E-01	58	-0.007	9.43E-01	98	0.086	4.02E-01	92	-0.058	5.17E-01	130
Digestive disease onset (no/yes)	0.026	8.02E-01	82	0.267	1.31E-01	33	-0.134	2.88E-01	63	-0.033	7.93E-01	61	-0.126	2.61E-01	80
Endocrine disease onset (no/yes)	0.054	7.13E-01	41	0.094	7.00E-01	17	-0.195	2.96E-01	28	0.041	8.19E-01	29	-0.367	1.99E-02	40
Cancer onset (no/yes)	-0.086	4.84E-01	59	-0.274	2.47E-01	18	-0.013	9.31E-01	42	-0.059	6.83E-01	45	-0.040	7.57E-01	61

For all P: FDR>5%

CHAPTER 8

Summary and general discussion



SUMMARY OF MAIN FINDINGS

The aim of the present research was to study multisystem quantifications of the biological age in major depressive disorder (MDD), in pursuance of a better understanding of the complex links between mental health and biological aging. To this aim, a literature review and four studies using empirical data were carried out to examine whether patients with MDD exhibit older appearing brains and bodies, as measured through age-related patterns derived from peripheral and brain biology. This section summarizes the main findings of the studies described in the different chapters. In this thesis, the terms "**biological clock**" or "**biological age predictor**" are used to refer to the method that generates "**biological age**" predictions that correlate highly with chronological age. The terms **"biological aging"** and "**biological aging indicator**" are used interchangeably and indicate metrics that are independent from chronological age effects (i.e., either by representing the difference between predicted biological age and chronological age, or as the residuals from a linear regression model of predicted biological age on chronological age).

Chapter 2 described that aging does not occur at one biological level or in a single cell-type, but is multi-systemic and can manifest across multiple organs and tissues across several biological levels. To accelerate progress in the field of biological aging research, six current challenges, as well as recommendations to overcome them were outlined. First, caution is warranted against interpreting cross-sectional correlations as causation and it is recommended to collect longitudinal data, consider experimentation, and choose prediction over explanation. Second, single biological age indicators tend to be uncorrelated, and may be better integrated. It is therefore recommended to create composite indices and panels of biological age indicators relevant to mental health. Third, biological aging may be tissue- and cell-type specific, and it is therefore recommended to purify cell types using established molecular markers. Fourth, within-group variances may be larger than between-group variances, and it is recommended to visualize data, carefully assess known influences, move beyond group-based analyses, and use within-person modeling approaches. Fifth, large sample sizes are needed to detect small effect sizes, and it is recommended to collaborate and harmonize data collection and analysis protocols to facilitate data pooling worldwide. Lastly, cellular measures of biological aging are often obtained from non-uniform laboratory assays and storage conditions, and it is recommended for future studies to harmonize measurements and storage conditions. For now, it is suggested that the current field would benefit from

adherence to a minimum standard of reporting to facilitate harmonization of datasets across laboratories and cohort studies. Future studies capitalizing on these opportunities will hopefully enhance our understanding of the psychobiological factors that influence the aging process.

In **Chapter 3**, it was examined whether depression was associated with advanced biological aging as measured by DNA methylation patterns obtained from blood. Patients with depression were, on average, +0.64 years older compared to controls, and this effect was more pronounced (+1.06 years) in a subgroup of patients that experienced both depression and childhood trauma. Exploratory analyses showed that advanced epigenetic aging was further significantly associated to higher depression severity, male sex, higher BMI, and low physical activity in all subjects, and to higher cumulative childhood trauma but not depression severity in MDD patients only. There were no significant associations between epigenetic aging and smoking/alcohol use or the duration/age of onset of illness, nor with antidepressant use (i.e., tricyclic antidepressants, selective serotonin reuptake inhibitors, other antidepressants). To test the robustness of findings, depression was also associated with biological aging as measured by DNA methylation patterns obtained from post-mortem brain tissue, and it was found that patients with depression showed an average difference of +1.11 years compared to controls. Enrichment testing showed that both the degree of overlap between the CpG sites associated with age and epigenetic aging, and the CpG sites included in the blood and brain epigenetic aging indicators were highly significant. Finally, to examine biological pathways underlying epigenetic aging in both tissues, gene ontology (GO) analyses were performed on the 1,094 overlapping CpG sites associated with epigenetic aging, resulting in 330 genes that were present in at least one GO category. The top significantly enriched GO terms included neuronal processes such as neurogenesis, neuron differentiation, and regulation of neuron death, indicating that several depression-relevant pathways were enriched in the cross-tissue epigenetic aging indicators.

The main goal of **Chapter 4** was to determine whether previously reported cross-sectional correlates of epigenetic aging also accelerate the metric over time. Using longitudinal data, it was found that 45% of the total variance in epigenetic aging can be attributed to differences between subjects, while the remaining 55% can be attributed to changes over time. These findings suggest that epigenetic aging is both relatively stable but also shows dynamic potential. However, when decomposing the covariance between a wide variety of correlates and epigenetic aging, higher subject- compared to wave-level contributions were found. Thus, although several weak but significant wave-level contributions were observed, a change in the epigenetic correlate

at a particular wave was often unaccompanied by a parallel change in epigenetic aging. The main findings of this chapter therefore caution against the causal interpretation of correlates of epigenetic aging, and emphasize that the commonly used term "accelerated" epigenetic aging in cross-sectional studies may often be incorrect.

The purpose of the study in **Chapter 5** was to investigate whether depression is associated with advanced biological aging, as measured by structural MRI of the brain. To that aim, multi-site brain age prediction models were developed in 2,188 healthy controls (separately in males and females) from 19 different cohorts worldwide. The models were further validated in 23 truly independent cohorts and scanners. The learned model coefficients were applied to 2,126 independent controls and 2,675 patients with MDD to calculate brain aging differences between the diagnostic groups. On average, depressed patients showed +1.08 years of added brain aging compared to control, but post hoc comparisons between depression subgroups did not show any significant differences (i.e., first vs. recurrent episode status, early vs. adult vs. late onset of depression, antidepressant medication-free vs. antidepressant users, remitted vs. currently depressed patients).

Chapter 6 extends the work described in **Chapter 5** by exploring which symptom clusters (mood/cognition, immunometabolic, somatic) of MDD are associated with brain aging, and whether patients with anxiety disorders also show older appearing brains. After correcting for antidepressant use, both patients with MDD (+2.78 years) as well as anxiety disorders (+2.91 years) showed significantly higher brain aging than controls. This study further indicated unique contributions of higher severity of somatic depression symptoms to advanced brain aging, and a potential protective effect of antidepressant medication (-2.53 years) in patients with depression and/or anxiety disorders. There were no significant associations with lifestyle (i.e., alcohol, smoking, physical exercise) or biological stress systems (i.e., inflammatory markers, autonomic nervous system, hypothalamic–pituitary–adrenal axis).

Finally, the study described in **Chapter 7** combined one "traditional" biological age indicator (i.e. telomere length) with four modern omics-based biological clocks (i.e., epigenetics, transcriptomics, proteomics, metabolomics), to examine whether the different biological clocks measure the same or different aspects of biological aging. The five biological clocks were integrated and residualized for age, and correlations between them were calculated. This showed that intercorrelations were small (all r's<0.2), indicating little overlap. To examine whether the different biological aging indicators were associated with similar determinants, all were associated with a wide variety

of somatic and mental health variables. Consistent associations between advanced biological aging across multiple biological levels were found for male sex, higher BMI, metabolic syndrome, smoking, and depression. As compared to the individual biological aging indicators, a composite index of all five biological aging indicators was computed and showed the strongest associations with health determinants. Taken together, the larger effect sizes of the composite index and the low correlations between the different biological aging indicators suggest that biological aging is best reflected by combining measures from multiple biological levels.

GENERAL DISCUSSION OF MAIN FINDINGS

The main findings of the current thesis can be contextualized against theoretical and practical research dimensions and themes. This chapter also reflects on the extent to which the current findings may address clinical implications, provide perspective on methodological challenges, and outline recommendations for future research. Finally, this chapter ties together the various theoretical and empirical strands in order to end with an overall conclusion.

The Biological Age Paradigm

The overview in **Table 1** displays the different biological age prediction models included in the studies of the current thesis, and their performance accuracy. In terms of the ability to predict age, the most accurate model was based on epigenetics, followed by proteomics, brain structure, transcriptomics, and finally metabolomics. However, important to note, the different platforms and their coverage, samples and sample sizes, their properties, and number and type of features make it difficult to draw any conclusions as to why some models predict better than others. For example, the studies in **Chapter 3 and 4** had near complete coverage of the epigenetics level, whereas the metabolomic platform used in **Chapter 7** covered only a selection of probes at that biological level. **Table 1** should therefore not be interpreted as a prioritization of which biological level shows most relevant age-related changes, but, rather, conveys that all biological levels carry important age-related information that can be leveraged to generate unbiased biological age estimates. Importantly, the biological age estimates vary across subjects with the same chronological age, highlighting its appropriateness to study the basis for inter-individual differences in the rate of biological aging.

Machine learning methods reliably capture age-related patterns from epigenetics, transcriptomics, proteomics, metabolomics, and structural brain features, and are able to predict an individual's chronological age with moderate to high accuracy.

Older Biological Age Predictions in Depression

Previous studies have convincingly shown robust associations between depression and stress-related psychopathology and cellular measures of aging (e.g., telomere length, mitochondrial DNA)[1–3]. The current thesis adds to the existing literature by showing that depression is also associated with an older appearing biological state as indicated by biological aging based on epigenetics, transcriptomics, proteomics, and brain structure. An overview of the convergent patterns of advanced or premature biological aging across the multiple biological systems in patients with depression are shown in **Table 2**. Depressed patients were consistently predicted to be older by the algorithms presented in **Table 1**, but it is important to emphasize that the biological aging effects are small, often less than one year on average. This suggests that while the age-related biological changes in depression are robust across multiple platforms and methods, they also reflect rather subtle differences.

The current thesis shows that depression is associated with subtle but robust age-related biological changes, as measured by epigenetics, transcriptomics, proteomics, and brain structure.

Table 1. Biological age prediction models and performance accuracy.

Biological age indicator	Age range	# Features	Type of features	Correlation with age	R^2	MAE	Validation testing	Platform/ Method used	Chapter
Telomere length	18-64 years	N/A	N/A	-0.30	N/A	N/A	N/A	qPCR	7
Epigenetic	9-35 years	25,000	CpG sites	0.93	0.85	1.85	Cross-validation	MBD-seq	4
Epigenetic	18-64 years	80,000	CpG sites	0.95	0.90	3.33	Cross-validation	MBD-seq	3
Transcriptomic	18-64 years	1,200	RNA	0.72	0.52	7.19	Cross-validation	Micro arrays	7
Proteomic	18-64 years	171	Proteins	0.85	0.72	5.67	Cross-validation	Immunoassay	7
Metabolomic	18-64 years	231	Metabolites	0.69	0.47	7.66	Cross-validation	Nightingale	7
Brain structure	18-57 years	77	ROIs	0.73	0.41	7.70	Independent validation	FreeSurfer	6
Brain structure	18-75 years	77	ROIs	0.81	0.67	6.69	Independent validation	FreeSurfer	5
Post-mortem brain tissue epigenetics	20-100 years	100,000	CpG sites	0.69	0.44	11.88	Cross-validation	MBD-seq	3

Note that N/A = not applicable. Abbreviations: qPCR, quantitative Polymerase Chain Reaction; CpG sites, regions of DNA where a cytosine and guanine nucleotide are separated by one phosphate group, and where methylation can occur; MBD-seq, methyl-binding domain sequencing; RNA, ribonucleic acid; ROIs, regions of interest. For the brain age predictions separate models for males and females were used, but the averaged metrics across both sexes and diagnostic groups (control and MDD) are displayed here. All biological aging indicators except for telomere length were estimates using ridge regression.

Table 2. Biological aging in depression.

Biological aging indicator	Added years of aging	Cohen's *d*	N Controls/MDD	Covariates	Contributing factors	Data	Chapter
Telomere length	+0.02	0.06	644/1,137	Age, sex, lab	Smoking, BMI, metabolic syndrome	NESDA	7
Epigenetic	+0.64**	0.18	319/811	Age, sex, education level, BMI, smoking, alcohol use, physical activity, number of chronic diseases	Depression severity, childhood trauma, BMI, physical disability	NESDA	3
Transcriptomic	+0.87*	0.12	367/948	Age, sex, lab	Smoking, BMI, digestive disease	NESDA	7
Proteomic	+0.93*	0.14	426/712	Age, sex, lab	Alcohol use, smoking, BMI, digestive disease, metabolic syndrome, chronic diseases, depression severity	NESDA	7
Metabolomic	-0.44	-0.06	633/1,135	Age, sex, lab	Smoking, BMI, cardiometabolic disease, metabolic syndrome	NESDA	7
Composite	+0.25**	0.25	158/495	Age, sex, lab	Smoking, BMI, physical disability, cardiometabolic disease, metabolic syndrome, current depression, depression severity, childhood trauma	NESDA	7
Brain structure	+1.75	0.24	65/220	Age, sex, scanner, education level	Depression severity, anxiety severity, somatic depressive symptoms, BMI, antidepressant medication use	NESDA	6
Brain structure	+1.08***	0.14	2,126/2,675	Age, age^2, sex, scanner	None	ENIGMA	5
Post-mortem brain tissue epigenetics	+1.11*	0.25	64/74	Age, sex, brain collection	N/A	Four brain banks	3

Notes: The composite indicator is a scaled sum of the telomere length, epigenetic, transcriptomic, proteomic, and metabolomic aging indicators. NESDA brain aging cases included both depression as well as anxiety disorders. Post-mortem brain samples were obtained from the Victorian Brain Bank, the Stanley Medical Research Institute, the Netherlands Brain Bank and the Harvard Brain Tissue Resource Center. Post-mortem brain tissue includes methylation data obtained from neurons and glial tissue. Post-mortem P-value was derived by a hypothesis driven one-sided likelihood ratio test. * indicates significance at the P<0.05 level. **indicates significance at the P<0.01 level. *** indicates significance at the P<0.0001 level.

Current Findings Against Findings From Other Studies

Of note, telomere length was only trending towards significance (i.e., shorter in the presence of current depression) in the current thesis, compared to previously reported significance in NESDA using largely overlapping samples, but with a slightly different selection of covariates (i.e., lab covariates) [4]. Other biological clocks with stronger correlations with chronological age, tended to show stronger associations with depression. However, the field of biological age prediction is still relatively novel, and although it is rapidly expanding,

studies investigating these types of biological clocks and associating them specifically to depression are still relatively scarce. While current findings are in line with some prior studies [5, 6], other, often smaller studies, did not reliably detect a *significant* association between depression or depressive symptoms and epigenetic aging of blood [7] or brain tissue [8], or even brain aging [9, 10], although effect sizes were compatible [11]. While there are studies showing proteomic [12, 13] and transcriptomic signatures [14, 15] associated with depression, to the best of our knowledge, no studies have specifically investigated machine learning biological age quantifications of these omics levels in depressed persons yet. However, metabolomic aging (predicted from 1,311 metabolomic features there vs. 231 in the current thesis) has been associated with depressive symptoms as measured by the Patient Health Questionnaire in a UK cohort [16], highlighting the need for methodological replication using the same platform and prediction model. Important to remark is that despite the clinically well-characterized samples from NESDA (**Chapter 3, 6, 7**) and the largest pooled sample of depressed patients (**Chapter 5**), effects remain subtle, and it might therefore not be surprising that smaller studies might not be sufficiently powered to detect these small biological aging effects.

The considered age range also seems to play a role in these findings, as prior studies did not find associations with depressive psychopathology and brain aging in youth samples (8-21 years old)[17], or in midlife depression, but did find significant associations in older depressed patients [18]. Inconsistent findings might also be due to the type of features and/or biological age prediction model used, heterogeneity of depression, inadequate statistical power to detect such effects, or otherwise. Moreover, the diversity of depression ascertainment, ranging from self-report depression rating scales or clinician-based psychiatric diagnoses of depression, might have also contributed to the different findings. All studies in the current thesis, except the one described in **Chapter 3** included patients with a clinical diagnosis of depression. Coincidentally, the study in **Chapter 3** also did not find associations with longitudinal epigenetic aging and depressive symptoms, although weak but significant evidence was found for anxiety symptoms, likely due to increased symptom variation. The inconsistencies between biological aging findings in depressed persons might be due to any (combination) of the sources of variation in the aforementioned studies and hampers direct comparisons between existing studies. Yet, the current thesis found significant associations between depression severity and epigenetics, proteomics, brain, and the composite index. Thus, these findings indicate that each additional depressive symptom is associated with even more advanced biological aging for four out of seven biological clocks

(excluding the post-mortem brain epigenetic clock), bolstering confidence in the observed advanced biological aging in depression. Nevertheless, more research is needed to verify the current results, and data harmonization and pooling are important strategies on the scientific research agenda to formally test the size, robustness, and generalization of these findings.

The heterogeneity of studies investigating machine learning quantifications of the biological age in depression using different platforms, prediction models, ascertainment of depression, sample sizes, and age ranges hamper direct comparisons between studies.

Small but Robust Effects

Small effect sizes are more the rule than the exception in the field of biological psychiatry [19–22], and the same holds true for biological aging indicators in depression. However, this does not mean that these findings should be dismissed or that they are less valid. For instance, with respect to the age-related structural brain changes in depressed individuals, not only the brain-based studies considered in this thesis, but also previous studies including well-powered neuroimaging research [23–26], show that variability in structural brain alterations only accounts for a small percentage of the total depression phenotype [27]. These findings may have important implications for our theoretical understanding of psychiatric disorders such as depression, as small effect sizes make it unlikely that psychiatric disorders can be explained by a generic disease process [28]. It is more likely that many different biological and psychosocial mechanisms, all with small individual effect sizes, contribute to the total depression phenotype. This may also suggest that the findings of older appearing biology in depressed persons observed in this thesis may not be clinically useful in isolation at this point. However, rather than interpreting this as a negative statement, it may also be an encouraging and hopeful message for most people who suffer from depression, because the biological aging effects tend to be small for most patients.

Given the small effect sizes, it is currently unlikely that the individualized biological aging scores can be used as a biomarker in clinical practice.

Convergent Evidence of Contributing Factors

When taking a closer look at the contributing factors to advanced biological aging, regardless of depression diagnosis, several consistent associations can be observed. **Table 3** presents an overview of psychological, somatic, and lifestyle variables. Important to note from this table is the following:

(1) Biological aging is sensitive to psychological stress, but not specific to depression and is also related to anxiety symptoms;

(2) Higher BMI is a consistent contributing factor to advanced biological aging, generally for both control and patient groups.

(3) Smoking is consistently associated with advanced biological aging on four out of six biological levels, only showing non-significant findings for the epigenetic and brain aging indicators.

> Depressive and anxiety symptoms, high BMI, and smoking are consistent risk factors for more advanced biological aging, also in the non-clinical group.

Table 3. Convergent evidence of contributing factors across controls and depressed patients.

Biological aging indicator	Childhood trauma	Depression severity	Anxiety severity	BMI	Smoking	Alcohol	Physical activity
Telomere length	n.s.	n.s.	N/A	+	+	n.s.	n.s.
Epigenetic	+	+	+	+/-	n.s.	n.s.	n.s.
Transcriptomic	n.s.	n.s.	N/A	+	+	n.s.	n.s.
Proteomic	n.s.	+	N/A	+	+	+	n.s.
Metabolomic	n.s.	n.s.	N/A	+	+	n.s.	n.s.
Composite	+	+	N/A	+	+	n.s.	n.s.
Brain structure	n.s.	+	+	+	n.s.	n.s.	n.s.

Notes: the composite indicator is a scaled sum of the telomere length, epigenetic, transcriptomic, proteomic, and metabolomic aging indicators. Brain epigenetics was excluded from this table due to the lack of phenotypic information. n.s. = not significant. + indicates positive association. - indicates negative association. +/- indicates that BMI was associated to advanced biological aging in the depression but not control group. N/A indicates the association was not tested.

Aging is Complex, Multifactorial and Not Under Unitary Control

The many diverse biological changes that occur as we age, the hallmarks of aging, as described by Lopez-Otin et al. (2013)[29], signify that aging is a complex process. It may therefore be considered rather unlikely that there would be a single underlying factor driving the multifactorial biological aging manifestations [30]. In **Chapter 7** it is shown that a composite index that summed up five biological aging indicators demonstrated larger effect sizes for BMI, sex, smoking, depression (severity), and metabolomic syndrome than the individual aging indicators. The same chapter also describes that the different biological aging markers are weakly correlated, and thus complementary, a finding further supported by other existing integrative studies on biological aging [31–37]. Together, these findings provide further support for the hypothesis that no single biological clock sufficiently captures the biological aging process and that it is unlikely that the clocks are under control of one unitary aging process.

> Not one biological clock can fully capture the complex and multifactorial aging process, and being biologically old at multiple biological levels has a cumulative multi-systemic effect.

Advanced Aging vs. Accelerated Aging

All studies but the one described in **Chapter 4** were cross-sectional and can therefore not distinguish advanced or premature from accelerated biological aging. However, longitudinal studies are needed to shed light on the potential causal link between depression and biological aging. To date, existing longitudinal studies have mainly focused on mapping aging trajectories over time rather than studying causality [32, 38]. The study in **Chapter 4** examined whether cross-sectionally identified correlates such as psychiatric problems, lifestyle variables, and adversities potentially play a causative role in epigenetic aging. Such examination is needed to obtain insight into whether epigenetic aging drives the identified correlates, or whether epigenetic aging is a consequence of these correlates [39]. However, a change in these correlates at a particular wave was often unaccompanied by a change in epigenetic aging, while such covariance is at least a necessary, but not sufficient, condition for causality. Thus, caution is warranted in interpreting cross-sectional correlations as causal factors.

More work is needed to establish whether:
(1) Depression accelerates biological aging
(2) Premature biological aging is a vulnerability factor that may cause depression
(3) Depression and biological aging processes share underlying etiological roots, such as genetic risks, but are not necessarily causally linked such that dynamic changes in depression coincide with responsive changes in biological aging throughout the course of life.

Forecasting Disease, Disability, and Mortality

Related to the previous paragraph, before conclusively interpreting a biological aging indicator as a measure of "true" biological aging, more independent work is needed to establish that the metric changes with advancing age and forecasts disease, disability, and mortality. The currently built machine learning algorithms rely on the assumption that the cross-sectional age-related biological patterns and chronological age primarily arise as a consequence of biological aging, and not because of other secular trends [40–42]. However, due to the cross-sectional nature of most of the studies considered in this thesis, it might be possible that there was uncontrolled residual confounding, either due to cohort effects or other individual differences. The study in **Chapter 3** specifically tested the degree of biological overlap between the CpG sites associated with epigenetic aging from blood and brain, and the CpG sites associated with chronological age, and found that this was highly significant. In other words, biological aging overlapped with the same epigenetic processes that underlie the chronological aging process. The other biological aging indicators considered in **Chapter 7** were assessed at baseline and did not predict future aging-related outcomes six years later, although this was likely due to the relatively young age of the sample (mean age ~41 years) and the low numbers disease onset and mortality (<64 deceased cases). However, NESDA is an ongoing study for which data with a 13-year follow-up duration is currently being collected. These studies with longer follow-up durations are expected to cast more light on this matter, and the validity of the models in terms of forecasting future health and disease therefore remains to be established.

The predictive power of the biological aging indicators studied in this thesis in relation to future health outcomes remains to be established.

What Are the Biological Mechanisms Underlying Biological Aging?

The big question remains which biological mechanisms could plausibly link quantifications of biological aging and depression. However, potential underlying biological mechanisms are dependent on the platform and features used to develop the biological clocks, and existing literature largely depends on differently developed algorithms. All but the brain-based biological clock in the current thesis have not been validated in other external samples, and it should be noted that this section should therefore be interpreted in light of this limitation. Nevertheless, for the epigenetic aging indicator in **Chapter 3** the biological pathways are discussed and for the other indicators some epidemiological replications and comparisons are discussed here.

With respect to the epigenetic aging indicator, the almost full methylome coverage of the platform used in this thesis (i.e., interrogation of 94% of all 28 million common CpG sites in blood) allowed for thorough exploration of the biological pathways that seemingly underlie epigenetic aging. This is particularly unique, as other epigenetic clocks frequently rely on arrays with 2-4% of methylome coverage, potentially missing important biological information from other parts of the methylome. The study in **Chapter 3** performed biological pathway analyses and found significantly enriched gene ontology terms including neurogenesis, neuron differentiation, and regulation of neuron death. Epigenetic mechanisms are essential during brain maturation and development, adult neurogenesis, and late-stage brain maturation [43], and these processes seem disturbed in patients with depression [44]. It is interesting to speculate whether these findings might also explain the age-related structural brain differences in **Chapter 5,** but this seems unlikely as an independent study by Cole et al. (2017) (N=620, mean age ~69.3 years), as well as the study in **Chapter 6** (N=98, mean age ~38.4 years), showed that brain aging and epigenetic aging were uncorrelated [45]. Regarding dynamic potential, previous studies suggest that epigenetic aging is under strong genetic control [46, 47], when corrected for blood cell-type proportions (i.e., intrinsic epigenetic age acceleration measured by the Horvath epigenetic clock)[48], potentially offering an explanation as to why external stress factors only have a weak effect on blood epigenetic aging [49]. The findings from the study described in **Chapter 4** are in line with this suggestion, given both

the stability of epigenetic aging over time (i.e., 45% of the total epigenetic variance was attributed to the subject-level), as well as that a wide range of psychiatric problems, lifestyle variables, and adversities also had no or weak effects on epigenetic aging rates. However, studies using the same epigenetic and brain aging algorithms are clearly needed to draw definitive conclusions.

With respect to the other omics-based biological aging indicators in **Chapter 7**, part of the answer requires discussion on the features used to build the different clocks. The narrow selection of proteomic and metabolomic probes considered in this study only comprise a small fraction of the full proteomic and metabolomic landscape, and explanations should therefore be held against this limitation. The proteomic and metabolomic probes that contribute to the age prediction mostly target inflammatory or metabolic factors. Inflammation and metabolic dysregulations are highly integrated in aging and aging-related diseases [50], and it may be possible that immune-mediated mechanisms tie together metabolic syndrome [51], depression [52], and aging [53]. Part of this hypothesis is further supported by studies showing shared genetic pathways between depression and metabolic syndrome [54] and cardiometabolic diseases [55]. Overall, studies investigating the complete proteomic and metabolomic landscape are needed, but in general depression is a medical disorder in which inflammation, metabolic traits, and premature aging co-occur and converge. The brain aging indicator in **Chapter 6**, however, was not associated with inflammatory markers (i.e., C-Reactive Protein, Tumor Necrosis Factor-α, Interleukin-6). Similarly, no significant associations were found between brain aging and autonomic nervous system dysregulations (i.e., resting heart rate, respiratory sinus arrhythmia, pre-injection period) or the hypothalamic-pituitary-adrenal axis (i.e., cortisol awakening response, evening cortisol). One previous study showed a small correlation (r=0.29) between brain aging and Tumor Necrosis Factor-α in older adults (>64 years) using a different brain age prediction model [6], but more work is needed to identify robust potential genetic, biological, and other early-factors that contribute to brain aging. As a follow-up study to the one described in **Chapter 5,** such an endeavor is currently being undertaken within the ENIGMA MDD consortium. This study examines which genetic (i.e., polygenic risk scores of depression, C-Reactive Protein, BMI, and epigenetic clocks) and environmental risk factors (i.e., BMI, smoking, childhood trauma) may potentially underlie brain aging.

Important to emphasize is that the omics-platforms used by individual studies often include different probes and features, hampering side-by-side methodological comparisons and the development of robust biological age prediction models. To fully dissect which biological mechanisms underlie biological aging, the field is in dire need of robust models, but to date, no gold standard exists for transcriptomic, proteomic, metabolomic, or brain-based models. The epigenetic data in the current thesis is inherently different from the epigenetic data used to build previously established and validated clocks, unfortunately not allowing direct comparison. Next to developing robust models, experimental approaches using cellular or animal models may also help aid omics-based biological aging interpretations. To illustrate, if depression-like stressors can be modeled accurately in animals or in vitro, experimental studies allow for direct manipulation, potentially providing causal evidence. A previous study has shown that e.g. epigenetic clocks can also be meaningful in vitro [56], demonstrating promise for such experimentation. In conclusion, the biological mechanisms underlying biological aging remain elusive, and several steps need to be undertaken to gain more important biological insights. First, robust models are needed. Second, more preclinical and in-vitro work needs to be performed to shed light on the biological processes [57]. It is therefore of utmost importance for scientists to enter in dialogues and collaborate with other disciplines outside of their own field, as it seems unlikely that there are only a few biological mechanisms of aging, and far more probable that aging is poly-mechanistic involving many biological pathways.

> A two-step approach is needed to answer the big question of which most important biological mechanisms underlie biological aging. First, uniform use of platforms and robust biological age models are needed. Second, dialogues between clinicians, psychologists, epidemiologists, behavioral scientists, and experimental biologists are needed to generate innovative methods in order to reveal poly-mechanistic pathways.

CLINICAL IMPLICATIONS

General Indicators of Somatic and Mental Health

Within the field of psychiatry, studying aging has been instrumental in understanding the somatic consequences and comorbidities of depression. Yet, the biological aging indicators considered in the current thesis cannot distinguish depressed patients from healthy control subjects, and are thus not useful for group inference. The current literature suggests that the biological aging indicators studied here are non-specific, but abnormal in unfavorable or unhealthy conditions. Then, in what way may biological age indicators play a

role in psychiatric research? This question will be discussed in more detail in the sections below.

To overcome the limitation of comparing the mean biological aging levels of cases (i.e., depressed patients) with the means of biological aging levels of controls, transdiagnostic dimensional approaches are needed that associate the individual predictions with continuous measures of psychopathology. Studying individualized biological aging scores is important to faithfully capture individual differences, rather than examining "the average patient" in case-control designs [58], and may provide better understanding of the age-related abnormalities in mental disorders. Yet, the biological age predictions in the current thesis are not meaningful on an individual-level, and whether improved accuracy of individualized predictions will lead to improved clinical value, remains an open question. Longitudinal studies with more timepoints will be instrumental in trying to answer this question, because it will provide insight in the test-retest reliability and validity of the metrics. It is expected and hoped, but not determined that more precise methods will allow for individual-level stratification, ultimately leading towards precision medicine. Meanwhile, a "dose-response" relationship was generally found, such that those with more severe depression showed more advanced biological aging. Clinicians may therefore potentially consider paying extra attention or adhere to a closer monitoring of the somatic problems in severely depressed persons.

> Biological aging indicators are not disease-specific nor suitable as diagnostic tools, but rather, transdiagnostic, general indicators impacted by somatic and mental health.

Biological Aging and Treatment

The findings from the current thesis have little direct implications for clinical practice, however, the brain-based biological aging study in **Chapter 6** showed that patients with depression and/or anxiety disorders using antidepressant medication showed similar brain aging to controls, but not to antidepressant-free patients. Interestingly, the antidepressants-using group constituted a more severely depressed and anxious group as measured by higher symptom severity scores. However, interpretative caution is warranted as the study was cross-sectional and a dose-response association with antidepressant medication not statistically significant. Nonetheless, this promising finding will be followed-up using data from the MOod Therapy using Antidepressant or Running (MOTAR, www.motar.nl) study, a randomized clinical trial comparing antidepressant medication and running therapy in the treatment of depression and anxiety disorders [59]. Previous studies investigating brain-based biological aging measures have suggested potential protective effects of mindfulness meditation [60], music performance [61], and physical activity [62], and while these should also be further addressed in future research, evidence from clinical trials is currently lacking. To date, only one randomized, placebo-controlled, exploratory study showed that acute oral administration of ibuprofen temporarily reduced brain aging by one year [63]. With respect to the limited evidence from clinical trials and epigenetic aging, protective or rejuvenating associations have been found in vivo for vitamin D [64] and dietary factors (i.e., folic acid and vitamin B12) but only in females with the MTHFR 677CC genotype [65]. One study reported epigenetic aging reversal through thymus regeneration in males [66], suggesting dynamic potential, but obviously more studies are needed to confirm these findings. Previous examples also demonstrate that participation in family-centered prevention training [67], and supportive family environments [68], mitigated accelerated epigenetic aging effects in spite of parental depressive symptoms or exposure to higher levels of racial discrimination in African American Youth, respectively. Epigenetic mechanisms of psychotherapy may potentially provide opportunities to modify aging patterns in anxiety, affective, and stress-related disorders [69].

Overall, some evidence seems to be emerging that epigenetic aging and brain aging can be targeted with clinical interventions. While still in its infancy, randomized clinical trials may identify individuals with abnormal age-related biological patterns who carry quantifiable risks for poorer functioning, disease course, and treatment response. To determine to which extent biological age indicators can be of potential clinical value, more research is needed associating biological aging with clinical characteristics cross-sectionally and particularly longitudinally. Importantly, more work is needed to examine whether biological aging can predict treatment response or be halted/reversed by clinical interventions.

> 1. More longitudinal work is needed to evaluate whether biological aging can predict treatment outcomes or response.
> 2. Randomized controlled intervention studies are needed to develop an understanding of how reversible or modifiable biological aging is in response to pharmacological and nonpharmacological treatments.

Potential Clinical Value

Assuming that the longitudinal and randomized controlled intervention studies may point out that baseline or pre-treatment biological aging predicts future health or treatment outcomes, several examples for practical application can potentially be outlined:

(1) Disease monitoring
(2) Assessment of current age-related biological health and prognosis
(3) Risk and population stratification

With respect to (1), longitudinal studies could track whether biological aging during the start of the study is advanced in patients with depression, and whether this metric progresses, normalizes, or maybe even reverses at later collected timepoints. In (2), baseline assessment of biological aging may predict treatment response, as has previously been described for telomere length and antidepressant [70, 71] or lithium [72, 73] treatment. Another important aspect is outlined in (3), in which it might be possible to screen and identify individuals with high biological aging, and stratify enrolment of these individuals for clinical trials increasing the likelihood for functional decline over a shorter period of time as has previously also been suggested by Cole and colleagues (2018)[30].

At the heart of the above avenues lies that biological aging indicators indeed show predictive and prognostic value, which is currently not robustly established. However, a recent longitudinal study showed that the brain-age paradigm was sensitive to multiple sclerosis-related atrophy, that baseline levels of brain aging predicted clinical progression and higher disability, and that the rate of increasing change of the aging metric paralleled worsening disability [74]. Together these findings emphasize that biological aging indicators may provide exciting new research avenues and clinical applications.

For now, biological aging research seems to hold great promise for both clinical and non-clinical populations, but it remains to be seen whether changes in age-related biological patterns also parallel improvements in functioning and quality of life, and reduction of depressive symptoms. It will be interesting to see whether normalizing or reversing biological aging will also result in lower subjective age, or one's own perception of how old one feels [75].

> Biological aging indicators hold promise for future disease monitoring, assessing current somatic and mental health and prognosis, and risk stratification.

METHODOLOGICAL CHALLENGES

A common theme that ties the discussion section together is that the heterogeneity of studies examining biological aging in depressed persons limit the direct comparisons and obscure generalization of findings. In this section, several challenges are outlined that need to be overcome in the future:

(1) The current dataset does not contain the same features that were used to train existing biological age prediction models (or other biological age prediction models simply do not exist yet)
(2) The existing biological age prediction models do not generalize to the current dataset
(3) The contributing factors to biological aging are different in each study

The challenge in (1) describes a problem that is often encountered, namely, that data collection is heterogeneous, involving different methods and platforms that do not probe the same features. For example, the study in **Chapter 3** was the first to develop an epigenetic clock using optimized Methyl-CpG binding domain sequencing (MBD-seq) data. However, **Chapter 4** is also an example of the challenge outlined in (2) as the epigenetic age indicator developed in **Chapter 3** did not generalize to this study, likely due to largely non-overlapping age ranges of the samples (18-64 years vs. 9-35 years) used to train the different models (i.e., the age of the training dataset was not representative of the dataset it was applied to). Not all studies should have to study the same algorithms, but if the challenge in (3) presents itself it is difficult to discern whether this is due to the different features used to build a prediction model (e.g., targeting different parts of the methylome), properties of the group used to train the model (e.g., age differences), or other uncontrolled sources of variation. One of the main challenges and current limitations can thus be found in the lack of generalization of models to unseen data and independent cohorts. Harmonization of data collection, handling, and analysis are needed to aid the development of robust, reliable, and valid models that may serve as a gold standard.

Harmonization of data collection, preprocessing, and analyses should be high priorities on the research agenda.

Local vs. Global Datasets

As mentioned before, large datasets are needed to detect small effects in depression. In addition, larger sample sizes may facilitate findings that reflect more "true" effects. For example, Miller and colleagues (2016) illustrate using UK Biobank data that imaging effect sizes show noticeable instability up to as many as ~2000 subjects, before stabilizing around the "true value" at ~5000 subjects [76]. However, while consortium work such as that described in **Chapter 5** might have greater statistical power, it also has its limitations due to less in-depth phenotyping. Individual cohorts such as those described in **Chapter 6** have more detailed information on clinical characteristics, lifestyle variables, and biological markers, emphasizing the complementary value of local and global datasets. Together, local and global datasets may be used to answer different research questions.

Large multisite studies often generate robust findings and hypotheses, whereas local datasets with in-depth clinical phenotyping may answer more specific research questions.

Multivariate Biological Aging As A Single Summarized Outcome

All biological aging indicators but telomere length are by definition a measure of residual error, because they depend on the difference between predicted biological age and actual chronological age. It should be mentioned that this also slightly complicates the interpretation of this metric, because it remains unknown how much of the residual variance is attributed to biological aging, and how much of it is due to noise or measurement error. A previous study showed that a near-perfect epigenetic age predictor can be developed with a sufficiently large training sample size, but that by increasing the prediction accuracy the association between epigenetic aging and mortality subsequently attenuates and eventually becomes non-significant [77]. The

trade-off between improving performance accuracy of age prediction models and its utility as a biomarker of aging therefore seems to be an important topic that should be disentangled by further research. As the machine learning methods to quantify the biological age are rapidly evolving, it is also important to mention that other paradigms also exist that predict age-related phenotypes other than chronological age itself [78, 79]. These seem very promising and often show stronger associations with future negative health outcomes and mortality. Models may also incorporate and integrate multiple feature sources (e.g., both voxel wise data and cortical thickness) or multimodal information, often showing that performance can be gained by combining (multimodal) information in, for instance, brain age prediction [80, 81]. It should also be mentioned that some criticisms towards biological age paradigms have also been expressed, for example with respect to brain aging. By modelling age-related brain patterns in healthy controls, and testing whether disease populations deviate from this normative pattern, one assumes that disease-related brain patterns and aging-related brain patterns overlap [80]. Thus, alternative growth chart approaches may also be used and provide an answer to a fundamentally different question [82], namely, how much does my brain deviate from what is normally expected at this age? vs. the currently used paradigm, how old am I based on my brain? Such normative models thus allow persons to deviate from normative biological patterns, instead of only being estimated to be "younger" or "older". Finally, other methods also exist where multiple biological age predictions are made using multiple different "modes" that track different parts of the brain aging process and are presumed to be under differential genetic control [83]. Such techniques reduce complexity, but still take different modes of brain aging into account, limiting the dilution of relevant aging-related information [84].

The methods and ways of quantifying human biological aging are rapidly evolving, emphasizing this novel and promising avenue of research.

FUTURE RECOMMENDATIONS

Towards Generalizable Biological Age Prediction Models

The field of biological aging is rapidly expanding, and studies investigating omics- or brain-based predictions of age are dramatically increasing [30, 85,

86]. One of the most extensively used and validated biological age prediction models is the epigenetic clock developed by Dr. Steve Horvath [48]. An online calculator exists that researchers may use to calculate epigenetic age based on data measured using the Illumina Infinium platform (https://dnamage.genetics.ucla.edu/). A web-based metabolomic age calculator based on the Nightingale platform also exists (https://metaboage.researchlumc.nl/), and, similarly, the developed algorithm in **Chapter 5** is also available to the research community as a web-based tool (www.photon-ai.com/enigma_brainage) to which researchers may upload their FreeSurfer ROI data to obtain individual brain age predictions using output from standardized ENIGMA imaging protocols (http://enigma.ini.usc.edu/protocols/imaging-protocols/). Open science and code sharing practices are becoming much more standard, and examples of other developed brain age models can be found on https://github.com/james-cole/brainageR and https://github.com/BIDS-Apps/baracus. Model sharing allows for external validation testing, and will accelerate research with the goal of improving global biological and mental health, as we can then collaboratively study protective and harmful effects, screen those at risk for poorer mental and somatic health, and examine intervention effects and treatment response.

To facilitate the development of robust biological age prediction models it would therefore be helpful to have one integrated, clear, and convenient repository for all existing models, categorized per biological level. The minimum standard of information should contain the age range and distribution used to train the algorithm, the feature-type and platforms or processing method used, and participant characteristics (e.g., % females). If independent researchers apply a model to their data, they could report back the properties and performance metrics for their samples. By also filtering on specific characteristics, one would be able to find models that potentially fit the properties of their own dataset more easily, and opportunities for data harmonization, pooling, and collaborations will become more apparent. Other benefits include that smaller (clinical) studies that do not allow for training their own models due to the limited sample size, can calculate unbiased individualized biological aging scores and associate these with often in-depth phenotypic information collected. In addition, scientists from low- and middle-income countries with less resources (e.g., computational power, data infrastructure, digital facilities) might also benefit from more model sharing opportunities. In closing, to combat and comprehensively study biological aging and mental illness, collaboration across countries, cultures, and disciplines is needed to create robust biological age prediction models.

A clear and convenient repository with a vast collection of existing biological clocks would promote the generalizability, deployability, and shareability of models, to mature the development of robust biological age algorithms.

Towards Enhanced Clinical Value

The current thesis suggests that if a biological aging-informed management of depression is to be outlined, important aspects to be included would be smoking cessation, lowering of BMI, and somatic depressive symptoms. Thus, clinical efforts would likely include a broader, lifestyle-focused strategy that may reduce both depressive symptoms and biological aging. The MOTAR study mentioned in section 3.4. will be helpful in this regard, because it can investigate the effects of both antidepressant medication use (i.e., potential neuroprotective effect on brain aging), against the metabolic improvements (i.e., lower BMI) that are expected to occur during running therapy only. Moreover, such randomized clinical trials may potentially unveil which treatments have a higher likelihood of clinical success for patients with high or low biological aging. Finally, these worthwhile efforts will be more able to challenge the causal links between exercise and/or antidepressant therapy and biological aging.

Clinical efforts are needed that integrate the management of both mental as well as somatic health symptoms to reduce the personal disease burden of depression.

Towards Early Intervention And Prevention

One important aspect of studying biological aging is to evaluate whether the process may contribute to somatic and mental health disparities later on in life. Ideally, one wishes to detect those at increased health risk early on, and target accelerated aging processes through intervention, before the onset of medical (both somatic and mental) or chronic diseases [87]. A recent Swedish population-based cohort study of nearly 1.5 million young individuals showed

that youth depression (5-19 years) was associated with increased relative and absolute risks of a wide range of medical conditions and premature mortality compared to the general population [88].

Adolescence is the developmental period of peak onset of depression and anxiety disorders. More than 55% of the burden of these diseases is observed in individuals aged 15-24 years [89], significantly contributing to global disability, with pessimistic projections into 2030 [90]. Youth depression has serious disruptive and limiting consequences for a young person's potential and quality of life [91, 92]. At worst, it may lead to suicide, and, sadly, this is one of the most common causes of death in young individuals 15-29 years worldwide [93]. Importantly, adolescence is an important window for physical, intellectual, and social development. A mental disorder in this critical period may have damaging consequences and if untreated, may lead to chronic disability, suggesting that full recovery may become the exception rather than the rule [94, 95]. Also, presence of one disorder often increases the risk of developing another, and individuals often accumulate several mental disorders over the course of life [96]. These disturbing statistics and long-term negative effects emphasize the importance of prevention and early-intervention of depression.

Future studies should test the clinical value of studying biological age estimations in youth samples, by calculating individualized scores that, contrary to aging in adults, signify *precocious* or *delayed* development. Abnormal age-related biological patterns in youth depression and anxiety disorders can then also be associated to functioning, disease severity, and treatment response. Investigating whether developmental or maturational biological changes can be normalized, may hold great promise for reducing future comorbidities, so that they become less disabling [97].

> The importance of prevention and early-intervention of depression is emphasized to reduce future somatic comorbidities.

CONCLUSIONS

The aim of this thesis was to investigate multisystem quantifications of the biological age in depression in order to better understand the complex interplay between mental health and biological aging. The most important finding to emerge from this thesis is that there is convergent support across multiple biological systems that depression is associated with an older appearing biological state of the brain and body, as measured by epigenetics, transcriptomics, proteomics, and brain-based biological clocks. This potentially offers an explanation as to why depressed persons have an increased risk of developing age-related diseases earlier in life than non-depressed peers. Several factors contribute to the observed biological aging, but specifically BMI was consistently associated with advanced aging across six biological levels and studies. Whilst this thesis did not establish ***accelerated*** biological aging in depression, it did partially substantiate that, at least at the epigenetic level, most considered correlates were unlikely to have causal accelerating effects on biological aging, because many of the wave-level changes in correlates were unaccompanied by a change in epigenetic aging. Although it remains to be elucidated if the different biological aging indicators considered in this thesis may represent potential targets for intervention, it strongly emphasizes, yet again, that depression has health consequences that go beyond psychological disturbances. A promising lead that requires follow-up investigation is the finding that antidepressant medication use may have protective effects on brain aging. Future longitudinal studies including multiple assessments are needed to further characterize the complex interplay between psychological, biological, and social factors and aging.

REFERENCES

1. Darrow SM, Verhoeven JE, Révész D, Lindqvist D, Penninx BWJH, Delucchi KL, et al. The Association Between Psychiatric Disorders and Telomere Length: A Meta-Analysis Involving 14,827 Persons. Psychosom Med. 2016;78:776–787.
2. Cai N, Chang S, Li Y, Li Q, Hu J, Liang J, et al. Molecular signatures of major depression. Curr Biol. 2015;25:1146–1156.
3. Tyrka AR, Carpenter LL, Kao H-T, Porton B, Philip NS, Ridout SJ, et al. Association of telomere length and mitochondrial DNA copy number in a community sample of healthy adults. Exp Gerontol. 2015;66:17–20.
4. Verhoeven JE, Révész D, Epel ES, Lin J, Wolkowitz OM, Penninx BWJH. Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study. Mol Psychiatry. 2013:1–7.
5. Koutsouleris N, Davatzikos C, Borgwardt S, Gaser C, Bottlender R, Frodl T, et al. Accelerated brain aging in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. Schizophr Bull. 2014;40:1140–1153.
6. Franke K, Gaser C, Manor B, Novak V. Advanced BrainAGE in older adults with type 2 diabetes mellitus. Front Aging Neurosci. 2013;5:1–9.
7. Vyas CM, Hazra A, Chang S-C, Qiu W, Reynolds CF 3rd, Mischoulon D, et al. Pilot study of DNA methylation, molecular aging markers and measures of health and well-being in aging. Transl Psychiatry. 2019;9:118.
8. Li Z, He Y, Ma X, Chen X. Epigenetic age analysis of brain in major depressive disorder. Psychiatry Res. 2018;269:621–624.
9. Besteher B, Gaser C, Nenadić I. Machine-learning based brain age estimation in major depression showing no evidence of accelerated aging. Psychiatry Research: Neuroimaging. 2019;290:1–4.
10. Hatton SN, Franz CE, Elman JA, Panizzon MS, Hagler DJ Jr, Fennema-Notestine C, et al. Negative fateful life events in midlife and advanced predicted brain aging. Neurobiol Aging. 2018;67:1–9.
11. Kaufmann T, van der Meer D, Doan NT, Schwarz E, Lund MJ, Agartz I, et al. Common brain disorders are associated with heritable patterns of apparent aging of the brain. Nat Neurosci. 2019;22:1617–1623.
12. Martins-de-Souza D, Guest PC, Harris LW, Vanattou-Saifoudine N, Webster MJ, Rahmoune H, et al. Identification of proteomic signatures associated with depression and psychotic depression in post-mortem brains from major depression patients. Transl Psychiatry. 2012;2:e87.
13. Frye MA, Nassan M, Jenkins GD, Kung S, Veldic M, Palmer BA, et al. Feasibility of investigating differential proteomic expression in depression: implications for biomarker development in mood disorders. Transl Psychiatry. 2015;5:e689.
14. Grzenda A, Siddarth P, Laird KT, Yeargin J, Lavretsky H. Transcriptomic signatures of treatment response to the combination of escitalopram and memantine or placebo in late-life depression. Mol Psychiatry. 2020. 7 May 2020. https://doi.org/10.1038/s41380-020-0752-2.
15. Cho JH-J, Irwin MR, Eisenberger NI, Lamkin DM, Cole SW. Transcriptomic predictors of inflammation-induced depressed mood. Neuropsychopharmacology. 2019;44:923–929.
16. Robinson O, Chadeau Hyam M, Karaman I, Climaco Pinto R, Ala-Korpela M, Handakas E, et al. Determinants of accelerated metabolomic and epigenetic aging in a UK cohort. Aging Cell. 2020;19:e13149.
17. Cropley VL, Tian Y, Fernando K, Mansour L S, Pantelis C, Cocchi L, et al. Brain-Predicted Age Associates With Psychopathology Dimensions in Youths. Biol Psychiatry Cogn Neurosci Neuroimaging. 2020. 1 August 2020. https://doi.org/10.1016/j.bpsc.2020.07.014.
18. Christman S, Bermudez C, Hao L, Landman BA, Boyd B, Albert K, et al. Accelerated brain aging predicts impaired cognitive performance and greater disability in geriatric but not midlife adult depression. Transl Psychiatry. 2020;10:317.
19. Black CN, Bot M, Scheffer PG, Cuijpers P, Penninx BWJH. Is depression associated with increased oxidative stress? A systematic review and meta-analysis. Psychoneuroendocrinology. 2015;51:164–175.
20. Vreeburg S a., Hoogendijk WJG, van Pelt J, Derijk RH, Verhagen JCM, van Dyck R, et al. Major Depressive Disorder and Hypothalamic-Pituitary-Adrenal Axis Activity. Arch Gen Psychiatry. 2009;66:617–626.
21. Molendijk ML, Bus BAA, Spinhoven P, Penninx BWJH, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state–trait issues, clinical features and pharmacological treatment. Mol Psychiatry. 2011;16:1088–1095.
22. Penninx BWJH, Milaneschi Y, Lamers F, Vogelzangs N. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 2013;11:129.
23. Koolschijn PCMP, van Haren NEM, Lensvelt-Mulders GJ, Hulshoff Pol HE, Kahn RS. Brain volume abnormalities in major depressive disorder: A meta-analysis of magnetic resonance imaging studies. Hum Brain Mapp. 2009;30:3719–3735.
24. Bora E, Fornito A, Pantelis C, Yücel M. Gray matter abnormalities in Major Depressive Disorder: a meta-analysis of voxel based morphometry studies. J Affect Disord. 2012;138:9–18.
25. Schmaal L, Veltman DJ, van Erp TGM, Sämann PG, Frodl T, Jahanshad N, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry. 2015:1–7.
26. Schmaal L, Hibar DP, Sämann PG, Hall GB, Baune BT, Jahanshad N, et al. Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group. Mol Psychiatry. 2016;22:900.
27. Boyle EA, Li YI, Pritchard JK. An Expanded View of Complex Traits: From Polygenic to Omnigenic. Cell. 2017;169:1177–1186.
28. Paulus MP, Thompson WK. The Challenges and Opportunities of Small Effects: The New Normal in Academic Psychiatry. JAMA Psychiatry. 2019. 27 February 2019. https://doi.org/10.1001/jamapsychiatry.2018.4540.
29. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–1217.
30. Cole JH, Marioni RE, Harris SE, Deary IJ, Cole JH. Brain age and other bodily ' ages ' : implications for neuropsychiatry. Mol Psychiatry. 2018. 2018. https://doi.org/10.1038/s41380-018-0098-1.
31. Hastings WJ, Shalev I, Belsky DW. Comparability of biological aging measures in the National Health and Nutrition Examination Study, 1999–2002. Psychoneuroendocrinology. 2019;106:171–178.

32. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. Elife. 2020;9.
33. Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, et al. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? Am J Epidemiol. 2018;187:1220–1230.
34. Kim S, Myers L, Wyckoff J, Cherry KE, Jazwinski SM. The frailty index outperforms DNA methylation age and its derivatives as an indicator of biological age. GeroScience. 2017;39:83–92.
35. Vetter VM, Antje M, Karbasiyan M, Steinhagen-Thiessen E, Hopfenmüller W, Demuth I. Epigenetic clock and relative telomere length represent largely different aspects of aging in the Berlin Aging Study II (BASE-II). J Gerontol A Biol Sci Med Sci. 2018. 18 August 2018. https://doi.org/10.1093/gerona/gly184.
36. Murabito JM, Zhao Q, Larson MG, Rong J, Lin H, Benjamin EJ, et al. Measures of Biologic Age in a Community Sample Predict Mortality and Age-Related Disease: The Framingham Offspring Study. J Gerontol A Biol Sci Med Sci. 2018;73:757–762.
37. Zhang Y, Saum K-U, Schöttker B, Holleczek B, Brenner H. Methylomic survival predictors, frailty, and mortality. Aging . 2018;10:339–357.
38. Marioni RE, Suderman M, Chen BH, Horvath S, Bandinelli S, Morris T, et al. Tracking the Epigenetic Clock Across the Human Life Course: A Meta-analysis of Longitudinal Cohort Data. J Gerontol A Biol Sci Med Sci. 2019;74:57–61.
39. Fransquet PD, Wrigglesworth J, Woods RL, Ernst ME, Ryan J. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Clin Epigenetics. 2019;11:62.
40. Belsky DW, Caspi A, Arseneault L, Baccarelli A, Corcoran DL, Gao X, et al. Quantification of the pace of biological aging in humans through a blood test, the DunedinPoAm DNA methylation algorithm. Elife. 2020;9.
41. Nelson PG, Promislow DEL, Masel J. Biomarkers for Aging Identified in Cross-sectional Studies Tend to Be Non-causative. J Gerontol A Biol Sci Med Sci. 2020;75:466–472.
42. Belsky DW. Reply to Newman: Quantification of biological aging in young adults is not the same thing as the onset of obesity. Proc Natl Acad Sci U S A. 2015;112:E7164–E7165.
43. Matt S, Roth ED, Roth TL. Role of epigenetics in the brain. Epigenetics in Psychiatry, Elsevier; 2014. p. 79–99.
44. Balu DT, Lucki I. Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci Biobehav Rev. 2009;33:232–252.
45. Cole JH, Ritchie SJ, Bastin ME, Valdés Hernández MC, Muñoz Maniega S, Royle N, et al. Brain age predicts mortality. Mol Psychiatry. 2018;23:1385–1392.
46. Lu AT, Xue L, Salfati EL, Chen BH, Ferrucci L, Levy D, et al. GWAS of epigenetic aging rates in blood reveals a critical role for TERT. Nat Commun. 2018;9:387.
47. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. 2018;19:371–384.
48. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14:R115.
49. Quach A, Levine ME, Tanaka T, Lu AT, Chen BH, Ferrucci L, et al. Epigenetic clock analysis of diet, exercise, education, and lifestyle factors. Aging . 2017;9:419–446.
50. Frasca D, Blomberg BB, Paganelli R. Aging, Obesity, and Inflammatory Age-Related Diseases. Front Immunol. 2017;8:1745.
51. Révész D, Milaneschi Y, Verhoeven JE, Lin J, Penninx BWJH. Longitudinal Associations Between Metabolic Syndrome Components and Telomere Shortening. J Clin Endocrinol Metab. 2015;100:3050–3059.
52. Wohleb ES, Franklin T, Iwata M, Duman RS. Integrating neuroimmune systems in the neurobiology of depression. Nat Rev Neurosci. 2016;17:497–511.
53. Révész D, Verhoeven JE, Picard M, Lin J, Sidney S, Epel ES, et al. Associations Between Cellular Aging Markers and Metabolic Syndrome: Findings From the CARDIA Study. The Journal of Clinical Endocrinology & Metabolism. 2018;103:148–157.
54. Postolache TT, del Bosque-Plata L, Jabbour S, Vergare M, Wu R, Gragnoli C. Co-shared genetics and possible risk gene pathway partially explain the comorbidity of schizophrenia, major depressive disorder, type 2 diabetes, and metabolic syndrome. Am J Med Genet B Neuropsychiatr Genet. 2019;180:186–203.
55. Amare AT, Schubert KO, Klingler-Hoffmann M, Cohen-Woods S, Baune BT. The genetic overlap between mood disorders and cardiometabolic diseases: a systematic review of genome wide and candidate gene studies. Transl Psychiatry. 2017;7:e1007.
56. Horvath S, Oshima J, Martin GM, Lu AT, Quach A, Cohen H, et al. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. Aging . 2018;10:1758–1775.
57. Brunet A. Old and new models for the study of human ageing. Nat Rev Mol Cell Biol. 2020;21:491–493.
58. Wolfers T, Doan NT, Kaufmann T, Alnæs D, Moberget T, Agartz I, et al. Mapping the Heterogeneous Phenotype of Schizophrenia and Bipolar Disorder Using Normative Models. JAMA Psychiatry. 2018;75:1146–1155.
59. Lever-van Milligen BA, Verhoeven JE, Schmaal L, van Velzen LS, Révész D, Black CN, et al. The impact of depression and anxiety treatment on biological aging and metabolic stress: study protocol of the MOod treatment with antidepressants or running (MOTAR) study. BMC Psychiatry. 2019;19:425.
60. Luders E, Cherbuin N, Gaser C. Estimating brain age using high-resolution pattern recognition: Younger brains in long-term meditation practitioners. Neuroimage. 2016;134:508–513.
61. Rogenmoser L, Kernbach J, Schlaug G, Gaser C. Keeping brains young with making music. Brain Struct Funct. 2018;223:297–305.
62. Steffener J, Habeck C, O'Shea D, Razlighi Q, Bherer L, Stern Y. Differences between chronological and brain age are related to education and self-reported physical activity. Neurobiol Aging. 2016;40:138–144.
63. Le TT, Kuplicki R, Yeh HW, Aupperle RL, Khalsa SS, Simmons WK, et al. Effect of Ibuprofen on BrainAGE: A Randomized, Placebo-Controlled, Dose-Response Exploratory Study. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2018:1–8.
64. Chen L, Dong Y, Bhagatwala J, Raed A, Huang Y, Zhu H. Effects of Vitamin D3 supplementation on epigenetic aging in overweight and obese African Americans with suboptimal vitamin D status: a randomized clinical trial. J Gerontol A Biol Sci Med Sci. 2018. 25 September 2018. https://doi.org/10.1093/gerona/gly223.
65. Sae-Lee C, Corsi S, Barrow TM, Kuhnle GGC, Bollati V, Mathers JC, et al. Dietary intervention modifies DNA methylation age assessed by the epigenetic clock. Mol Nutr Food Res. 2018;62:1800092.
66. Fahy GM, Brooke RT, Watson JP, Good Z, Vasanawala SS, Maecker H, et al. Reversal of epigenetic aging and immunosenescent trends in humans. Aging Cell. 2019;18:e13028.

67. Brody GH, Yu T, Chen E, Beach SRH, Miller GE. Family-centered prevention ameliorates the longitudinal association between risky family processes and epigenetic aging. J Child Psychol Psychiatry. 2016;57:566–574.
68. Brody GH, Miller GE, Yu T, Beach SRH, Chen E. Supportive Family Environments Ameliorate the Link Between Racial Discrimination and Epigenetic Aging: A Replication Across Two Longitudinal Cohorts. Psychol Sci. 2016;27:530–541.
69. Schiele MA, Gottschalk MG, Domschke K. The applied implications of epigenetics in anxiety, affective and stress-related disorders - A review and synthesis on psychosocial stress, psychotherapy and prevention. Clin Psychol Rev. 2020;77:101830.
70. Wolkowitz OM, Mellon SH, Epel ES, Lin J, Reus VI, Rosser R, et al. Resting leukocyte telomerase activity is elevated in major depression and predicts treatment response. Mol Psychiatry. 2012;17:164–172.
71. Hough CM, Bersani FS, Mellon SH, Epel ES, Reus VI, Lindqvist D, et al. Leukocyte telomere length predicts SSRI response in major depressive disorder: A preliminary report. Mol Neuropsychiatry. 2016;2:88–96.
72. Li Z, Hu M, Zong X, He Y, Wang D, Dai L, et al. Association of telomere length and mitochondrial DNA copy number with risperidone treatment response in first-episode antipsychotic-naïve schizophrenia. Sci Rep. 2015;5:18553.
73. Yu W-Y, Chang H-W, Lin C-H, Cho C-L. Short telomeres in patients with chronic schizophrenia who show a poor response to treatment. J Psychiatry Neurosci. 2008;33:244–247.
74. Cole JH, Raffel J, Friede T, Eshaghi A, Brownlee WJ, Chard D, et al. Longitudinal Assessment of Multiple Sclerosis with the Brain-Age Paradigm. Ann Neurol. 2020;88:93–105.
75. Mitina M, Young S, Zhavoronkov A. Psychological aging, depression, and well-being. Aging . 2020;12:18765–18777.
76. Miller KL, Alfaro-Almagro F, Bangerter NK, Thomas DL, Yacoub E, Xu J, et al. Multimodal population brain imaging in the UK Biobank prospective epidemiological study. Nat Neurosci. 2016;19:1523–1536.
77. Zhang Q, Vallerga CL, Walker RM, Lin T, Henders AK, Montgomery GW, et al. Improved precision of epigenetic clock estimates across tissues and its implication for biological ageing. Genome Med. 2019;11:54.
78. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging . 2019;11:303.
79. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging . 2018;10:573–591.
80. Gutierrez Becker B, Klein T, Wachinger C. Gaussian process uncertainty in age estimation as a measure of brain abnormality. Neuroimage. 2018;175:246–258.
81. Valizadeh SA, Hänggi J, Mérillat S, Jäncke L. Age prediction on the basis of brain anatomical measures. Hum Brain Mapp. 2017;38:997–1008.
82. Marquand AF, Rezek I, Buitelaar J, Beckmann CF. Understanding Heterogeneity in Clinical Cohorts Using Normative Models: Beyond Case-Control Studies. Biol Psychiatry. 2016;80:552–561.
83. Smith SM, Elliott LT, Alfaro-Almagro F, McCarthy P, Nichols TE, Douaud G, et al. Brain aging comprises many modes of structural and functional change with distinct genetic and biophysical associations. Elife. 2020;9.
84. Nyberg L, Wåhlin A. Imaging: The many facets of brain aging. Elife. 2020;9:e56640.
85. Hägg S, Belsky DW, Cohen AA. Developments in molecular epidemiology of aging. Emerging Topics in Life Sciences. 2019;3:411–421.
86. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. EBioMedicine. 2017;21:29–36.
87. Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, et al. Quantification of biological aging in young adults. Proc Natl Acad Sci U S A. 2015;112:E4104–E4110.
88. Leone M, Kuja-Halkola R, Leval A, D'Onofrio BM, Larsson H, Lichtenstein P, et al. Association of Youth Depression With Subsequent Somatic Diseases and Premature Death. JAMA Psychiatry. 2020. 9 December 2020. https://doi.org/10.1001/jamapsychiatry.2020.3786.
89. Yung AR, Cotter J, McGorry PD. Youth Mental Health: Approaches to Emerging Mental Ill-Health in Young People. Routledge; 2020.
90. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:e442.
91. McGorry PD, Purcell R, Hickie IB, Jorm AF. Investing in youth mental health is a best buy. Medical Journal of. 2007. 2007.
92. Insel TR, Fenton WS. Psychiatric epidemiology: it's not just about counting anymore. Arch Gen Psychiatry. 2005;62:590–592.
93. Cha CB, Franz PJ, M. Guzmán E, Glenn CR, Kleiman EM, Nock MK. Annual Research Review: Suicide among youth--epidemiology,(potential) etiology, and treatment. J Child Psychol Psychiatry. 2018;59:460–482.
94. Verhoeven J, Verduijn J, Milaneschi Y, Beekman A, Penninx B. The clinical course of depression: Chronicity is the rule rather than the exception. Eur Psychiatry. 2017;41:S144–S145.
95. Verduijn J, Verhoeven JE, Milaneschi Y, Schoevers RA, van Hemert AM, Beekman ATF, et al. Reconsidering the prognosis of major depressive disorder across diagnostic boundaries: full recovery is the exception rather than the rule. BMC Med. 2017;15:215.
96. Caspi A, Houts RM, Ambler A, Danese A, Elliott ML, Hariri A, et al. Longitudinal Assessment of Mental Health Disorders and Comorbidities Across 4 Decades Among Participants in the Dunedin Birth Cohort Study. JAMA Netw Open. 2020;3:e203221.
97. Christensen K, Doblhammer G, Rau R, Vaupel JW. Ageing populations: the challenges ahead. Lancet. 2009;374:1196–1208.

NEDERLANDSE SAMENVATTING (SUMMARY IN DUTCH)

DEPRESSIE

Depressie is een veelvoorkomende psychische aandoening die wereldwijd meer dan 264 miljoen mensen van alle leeftijden raakt. De aandoening komt vaker voor bij vrouwen dan bij mannen, is een van de hoofdoorzaken van gezondheidsverlies en gaat gepaard met een hoge ziektelast. Dit zal nog toenemen; de vooruitzichten tot 2030 zijn zorgelijk. In het ergste geval kan depressie leiden tot zelfmoord, momenteel helaas een van de meest voorkomende doodsoorzaken bij jongeren van 15 tot 29 jaar oud. Depressie wordt gekenmerkt door somberheid, verlies van interesse of onvermogen om ergens van te genieten voor een periode van tenminste twee aaneengesloten weken, gedurende het grootste deel van de dag en bijna elke dag. In totaal zijn er negen symptomen, dit zijn naast een sombere stemming en interesseverlies, problemen met gewicht, eetlust, slaap, concentratie, psychomotorische traagheid, vermoeidheid en gedachten aan de dood. Het Handboek voor psychiatrische stoornissen (DSM-5) stelt dat een individu, om te voldoen aan de voorwaarden van een klinische depressie, vijf (of meer) van de negen symptomen moet hebben, waaronder ten minste een van de twee kernsymptomen van somberheid en/of onvermogen om ergens van te genieten. Uit verschillende onderzoeken is duidelijk gebleken dat een depressie vaak gepaard gaat met angstsymptomen. Tot wel 75% van de mensen met een depressie heeft ook een angststoornis (sociale fobie, paniekstoornis, agorafobie, gegeneraliseerde angststoornis). Er is niet alleen een overlap in symptomen, er zijn ook andere overeenkomsten tussen depressie- en angststoornissen in termen van genetica, neurobiologie, gedeelde risicofactoren en fysiologische ontregelingen. Dit toont aan dat beide psychische stoornissen nauw met elkaar verbonden zijn en verklaart waarom depressie en angststoornissen vaak samen bestudeerd worden.

VEROUDERING VAN DE MENS

Het verouderingsproces is al lange tijd onderwerp van wetenschappelijk onderzoek wellicht omdat veroudering een bijna universele eigenschap is van de meeste soorten op aarde, met uitzondering van enkele bacteriën, planten of hele simpele dieren. Bij mensen hebben vooral verbeteringen van de

volksgezondheid, zoals een betere hygiëne en bestrijding van infectieziekten, wereldwijd bijgedragen aan een langere levensduur. Mede hierdoor is de levensverwachting van de mens in de afgelopen 100 jaar met meer dan 35 jaar gestegen. Hierbij moet wel worden aangetekend dat deze stijging van de levensverwachting niet over alle bevolkingsgroepen gelijk verdeeld is. Er bestaan nog steeds omvangrijke sociaaleconomische gezondheidsverschillen, zelfs in een welvarend land als Nederland. Desalniettemin is er in onze huidige samenleving een sterke toename van het aantal mensen dat een hoge leeftijd bereikt. Hoewel sommige leeftijd gerelateerde veranderingen, zoals het grijs worden van haar, als relatief goedaardig kunnen worden beschouwd, zijn andere kenmerken objectief gezien nadeliger. Ouder worden is bijvoorbeeld ook een sterke risicofactor voor chronische ziekten zoals hart- en vaatziekten, diabetes mellitus type 2, kanker en de kans op overlijden. Naast de stijging van de levensverwachting van de mens, zien we dus eveneens een toename van chronische ziekten en het aantal jaren dat men moet leven met de nadelige gevolgen daarvan. Dit wordt gedeeltelijk verklaard door het feit dat veel ziekten eerder ontdekt worden en nu beter behandeld kunnen worden, met als resultaat een afname in mortaliteit. Chronische ziekten hebben echter nog steeds een negatieve invloed op de kwaliteit van leven. Over de vraag waarom we verouderen en welke biologische mechanismen hieraan ten grondslag liggen, is nog veel onbekend. Als we de verouderingsbiologie en de impact ervan op ziekten beter begrijpen, kunnen we mogelijk een manier vinden om succesvoller ouder te worden. Succesvol oud worden betekent dat mensen op hogere leeftijd meer gezonde en gelukkige jaren kennen. Binnen de psychiatrie heeft het bestuderen van veroudering een belangrijke rol gespeeld bij het begrijpen van de somatische gevolgen en medische comorbiditeiten van een depressie.

SAMENHANG TUSSEN DEPRESSIE EN VEROUDERING

Somatische symptomen van depressie

Interessant is dat chronische ziekten die regelmatig optreden op oudere leeftijd ook vaak voorkomen bij depressie. Eerder onderzoek heeft aangetoond dat de impact van depressie veel verder reikt dan alleen de aanwezigheid van psychische symptomen. De Inventory of Depressive Symptoms (IDS) is een betrouwbaar instrument om symptomen van depressie te meten en geeft een indicatie van de ernst van de ziekte. Het instrument omvat voor de helft items die betrekking hebben op stemming/cognitie en voor de andere helft

somatische symptomen zoals slaapproblemen, veranderingen in gewicht en/of eetlust, fysieke energie, psychomotorische agitatie/retardatie en pijn. Dit suggereert dat depressie per definitie niet alleen als een "probleem van de geest" wordt beschouwd, maar ook als een "probleem van het lichaam".

Leeftijd gerelateerde comorbiditeiten

Een groeiend aantal studies laat zien dat depressie het risico op hart- en vaatziekten verhoogt, maar andersom lijken cardiovasculaire problemen ook het risico op depressie te vergroten. Depressie wordt niet alleen met hart- en vaatziekten geassocieerd; ook andere klachten of symptomen van een slechtere somatische gezondheid komen vaker voor bij mensen met een depressie. Er is consistent bewijs dat bij depressie meer chronische ziekten voorkomen, zoals diabetes mellitus type 2, obesitas, herseninfarct, hypertensie en stofwisselingsstoornissen. Bovendien blijkt uit longitudinaal onderzoek dat depressie ook het risico vergroot op ouderdom gerelateerde aandoeningen zoals dementie, de ziekte van Alzheimer en in sommige gevallen zelfs kanker. Al met al is er overtuigend bewijs dat depressie een grote nadelige invloed heeft op de lichamelijke gezondheid.

Een andere verklaring voor de relatie tussen depressie en gezondheid kan zijn dat een ongezonde leefstijl en slechte zelfzorg leidt tot een slechtere somatische gezondheid bij mensen met een depressie. Onderzoek naar dit thema wordt beperkt door het feit dat gedragsmatige, psychiatrische en somatische aandoeningen vaak samen voorkomen en dus verstorende effecten kunnen hebben. Zo lijken psychiatrische patiënten langer, vaker en meer te roken en komt bij hen meer problematisch alcohol- en middelengebruik voor in vergelijking met controlegroepen. Bovendien zijn overgewicht, slechte voeding en lichamelijke inactiviteit veel voorkomende kenmerken van mensen met een depressie. Andere onderzoeken tonen echter aan dat een slechte leefstijl het verband tussen depressie en nadelige gevolgen voor de gezondheid niet volledig kan verklaren, statistische modellen die corrigeren voor deze verschillen in leefstijl laten nog steeds significante effecten zien van depressie op bijv. hart- en vaatziekten.

De literatuur laat dus zien dat depressie wordt geassocieerd met oversterfte en verloren levensjaren door directe en/of indirecte effecten van de ziekte. Het verhoogde risico op het ontwikkelen van ouderdom gerelateerde aandoeningen is een belangrijk effect van depressie die de ziektelast verder verhoogt door een verminderde kwaliteit van leven en een verhoogd gebruik van de gezondheidszorg. Er zijn dus twee mondiale uitdagingen waarmee we momenteel worden geconfronteerd, aangezien psychiatrische ziekten

en vergrijzende populaties beide wereldwijd toenemen. Dit onderstreept de noodzaak om beide uitdagingen tegelijkertijd in dezelfde onderzoeken te bestuderen.

Cellulaire veroudering bij depressie

Om de invloed van leeftijd gerelateerde somatische aandoeningen bij depressie te onderzoeken, hebben eerdere studies zich voornamelijk gericht op het meten van cellulaire veroudering. Bij de start van het onderzoek voor dit proefschrift (april 2016) was de meeste literatuur over biologische veroudering bij depressie gericht op telomeerlengte, een marker die in toenemende mate korter wordt naarmate de leeftijd toeneemt. Er zijn robuuste associaties gevonden tussen een kortere telomeerlengte en depressie, maar ook bij angststoornissen wordt dit gezien. Sindsdien hebben verschillende technologische ontwikkelingen geleid tot "moderne" machine learning benaderingen van biologische veroudering die in de volgende paragrafen zullen worden besproken. De meest populaire algoritmes voor biologische veroudering zijn gebaseerd op epigenetica en worden "epigenetische klokken" genoemd. Er bestaan verschillende epigenetische klokken die de leeftijd heel nauwkeurig kunnen voorspellen.

KWANTIFICERING VAN DE BIOLOGISCHE LEEFTIJD

Concepten van chronologische veroudering en biologische veroudering

Zoals eerdergenoemd, is veroudering een van de sterkste "risicofactoren" voor chronische ziekten, verlies van functionele capaciteit en dus ook de kans op sterfte. Over de hele levensduur kunnen we twee verschillende concepten van veroudering onderscheiden, namelijk chronologische veroudering enerzijds en biologische veroudering anderzijds, hoewel er geen overeenstemming bestaat over wat biologische veroudering precies inhoudt. Terwijl onze chronologische leeftijd in feite alleen een antwoord is op de vraag hoeveel kaarsjes we op onze verjaardagstaart mogen zetten, kan onze biologische leeftijd op veel verschillende manieren worden gekwantificeerd en is deze daarmee meer ongrijpbaar. De chronologische leeftijd is onveranderlijk en is louter gebaseerd op het verstrijken van de tijd, terwijl de biologische leeftijd de functionele en biologische toestand van ons lichaam weerspiegelt, en voor of achter kan lopen op de chronologische leeftijd. Het concept van de biologische leeftijd is

dus bedoeld om de mate van veroudering tussen twee mensen van dezelfde chronologische leeftijd te verklaren, of, om het botweg te zeggen, om te duiden waarom twee mensen die op dezelfde datum zijn geboren, op uiteenlopende momenten in de tijd kunnen overlijden (door 'natuurlijke' oorzaken).

Omdat het niet mogelijk is om een volledig beeld te krijgen van de biologische toestand van een individu, kunnen biologische leeftijdsindicatoren een benadering geven. Biologische leeftijdsindicatoren kunnen worden afgeleid uit functionele, neuro-anatomische, cellulaire of moleculaire metingen die gecorreleerd zijn met leeftijd of "kenmerken van veroudering". Biologische leeftijdsindicatoren weerspiegelen vaak functieverlies, verhoogd risico op medische aandoeningen en ziekten, en de nabijheid van de dood. Belangrijk is dat een biologische leeftijdsindicator een betere voorspelling hiervoor zou moeten geven dan de chronologische leeftijd. Dit proefschrift is voornamelijk gericht op epigenetische en op de hersenen gebaseerde proxy's (*"voorspellers"*) van biologische veroudering.

Moderne instrumenten om biologische veroudering te kwantificeren

Normale veroudering gaat gepaard met hersenatrofie, afname van de corticale dikte en vergroting van de ventrikels. Evenzo treden stochastische, omgevings- en individueel-specifieke methylatie veranderingen in het DNA op tijdens gezond ouder worden. Door gebruik te maken van de natuurlijke, aan veroudering gerelateerde biologische veranderingen die voor individuen redelijk consistent zijn, kunnen we statistische hulpmiddelen gebruiken om deze specifieke patronen vast te leggen en voorspellende algoritmen te ontwikkelen die in staat zijn om de chronologische leeftijd van een persoon op basis van deze patronen nauwkeurig te voorspellen. Centraal in deze aanpak staat het gebruik van een grote steekproef van deelnemers waarvan de chronologische leeftijd bekend is, zodat een "supervised machine learning"-methode kan worden gebruikt. Over het algemeen verwijst supervised machine learning naar een methode waarbij het ontwikkelde algoritme gecorreleerde patronen leert te herkennen in de data van individuen waarvan de chronologische leeftijd bekend is ("training data"), voordat de juiste chronologische leeftijd wordt voorspeld voor nieuw gepresenteerde data ("test data"). Dit proefschrift richt zich voornamelijk op epigenetische (d.w.z. methylatie niveaus van CpG-sites) en hersenstructuur gegevens (d.w.z. grijze stof, corticale dikte, oppervlak en subcorticale volumes) om schattingen van de biologische leeftijd te verkrijgen. Belangrijk is dat door de voorspelde biologische leeftijd van een individu te vergelijken met zijn of haar chronologische leeftijd, kan worden onderzocht of deze biologisch jonger of ouder is dan verwacht op

basis van hun chronologische leeftijd. Ter illustratie: als een persoon van 40 jaar oud (chronologische leeftijd) 42 jaar oud voorspeld wordt door het algoritme (voorspelde biologische leeftijd), overtreft de biologische leeftijd van die persoon (en dus ouder lijkende biologische toestand) zijn of haar chronologische leeftijd met +2 jaar. Dit leeftijdsverschil wordt "biologische veroudering" of "het biologische verouderingseffect" genoemd.

Toepassing van voorspellingsmodellen voor biologische leeftijd bij depressie

Het doel van het bouwen van een voorspellingsmodel voor de biologische leeftijd is om het vervolgens toe te passen op een patiëntenpopulatie met depressie en/of angststoornissen om de hypothese te testen of hun biologische leeftijd de chronologische leeftijd overtreft. Het verschil tussen iemands chronologische en biologische leeftijd kan dan ook gerelateerd worden aan andere ziektekenmerken. Het begrijpen van het verband tussen biologische veroudering en depressie zal helpen bij het identificeren en voorspellen van de kans of iemand kwetsbaar is voor leeftijd gerelateerde gezondheidsproblemen. Het is belangrijk om te vermelden dat voorspellingsmodellen voor de biologische leeftijd niet alleen relevant zijn voor het onderzoeken van depressie. Met andere woorden, voorspellingsmodellen voor de biologische leeftijd kunnen worden toegepast op de algemene bevolking of andere klinische populaties om individuele afwijkingen van "normale" veroudering te onderzoeken. Dit proefschrift onderzoekt met name de biologische verouderingspatronen die worden waargenomen bij personen met depressie.

STUDIEPOPULATIES IN DIT PROEFSCHRIFT

In het onderzoek voor dit proefschrift zijn voornamelijk empirische gegevens gebruikt uit drie hoofdbronnen: 1) The Netherlands Study of Depression and Anxiety (NESDA), 2) The Great Smoky Mountains Study (GSMS), en 3) De depressie werkgroep van het Enhancing Neuroimaging Genetics through Meta-analysis (ENIGMA) consortium.

Het NESDA-cohort is een longitudinaal onderzoek dat het ontstaan, beloop en de gevolgen van depressie en angststoornissen onderzoekt. Naast klinische en medische vragenlijsten worden er biologische en genetische factoren onderzocht. Tussen 2004 en 2007 werden 2.981 deelnemers (78% voldeed aan de criteria voor depressie en/of angststoornissen) geïncludeerd die werden geworven uit de algemene bevolking, de eerstelijnszorg en de

gespecialiseerde geestelijke gezondheidszorg. Een subgroep van 1.130 deelnemers (18-64 jaar) onderging een volledige profilering van het methyloom. Een (gedeeltelijk overlappende) deelgroep van 301 deelnemers (18-55 jaar oud) nam deel aan het neuroimaging-onderzoek.

Het tweede cohort, GSMS, is een unieke longitudinale populatie-gebaseerde cohortstudie van 1.420 kinderen waarvan de werving plaatsvond in 11 overwegend landelijke gebieden van North Carolina, de Verenigde Staten van Amerika. In 1993 waren de deelnemers bij de intake tussen de 9 en 13 jaar oud. Van hen werd gedetailleerde informatie over verschillende gezondheidsfactoren en bloedmonsters verzameld tijdens jaarlijkse metingen tot 2015. De deelnemers zijn nu begin 30 en in dit proefschrift zijn gegevens van 539 deelnemers gebruikt (9-35 jaar oud) met een totaal van 1.029 metingen.

De derde bron van gegevens, het ENIGMA-consortium, is een wereldwijde neurowetenschappelijke alliantie van ruim 1.400 wetenschappers uit 43 landen die zich richten op het onderzoeken van fundamentele vragen in de neurowetenschappen en genetica. Dit consortium bestaat uit verschillende werkgroepen, waarbij de ENIGMA depressie werkgroep de belangrijkste databron is die voor dit proefschrift is gebruikt. Gegevens van meer dan 19 cohorten, waaronder meer dan 6.900 deelnemers (18-75 jaar oud) waarvan 38,3% depressieve patiënten, werden opgenomen in een gepoolde mega-analyse.

DOELEN VAN DIT PROEFSCHRIFT

In dit proefschrift onderzoeken we verschillende berekeningen van de biologische leeftijd in personen met een depressie om meer te weten te komen over de complexe wisselwerking tussen mentale gezondheid en biologische veroudering. Het eerste doel is om richting te geven aan onderzoekers die interesse hebben in het bestuderen van stress, psychopathologie en biologische veroudering. **Hoofdstuk 2** geeft daarom een literatuuroverzicht van de relaties tussen biologische veroudering en mentale gezondheid, evenals een beschrijving van dit snelgroeiende onderzoeksgebied met de bijbehorende beperkingen en uitdagingen. Ook geeft **Hoofdstuk 2** aan aantal aanbevelingen voor dit onderzoeksgebied.

Het tweede doel van dit proefschrift is het uitvoeren van experimenteel onderzoek om biologische veroudering te meten. In **Hoofdstuk 3** en **Hoofdstuk 4** wordt biologische veroudering gemeten door middel van DNA methylatie patronen, ofwel epigenetica. In **Hoofdstuk 3** wordt in een cross-sectionele studie onderzocht of depressie geassocieerd is met een oudere

epigenetische leeftijd bepaald aan de hand van het DNA uit het bloed en hersenweefsel. In **Hoofdstuk 4** wordt een longitudinaal onderzoek beschreven naar de epigenetische leeftijd vanaf de kindertijd en adolescentie tot aan de jongvolwassenheid. Hierin wordt onderzocht of veranderingen in verschillende gezondheidsrisico's leiden tot veranderingen in epigenetische veroudering, een verband dat zou moet worden waargenomen als het gezondheidsrisico een causaal effect heeft op de epigenetische veroudering.

Het derde doel van dit proefschrift is om te onderzoeken of vroegtijdige biologische veroudering ook kan worden waargenomen in de hersenen, gemeten met MRI-scans. De volgende twee hoofdstukken gaan daarom over de biologische leeftijd gebaseerd op hersenstructuur (hersenleeftijd). **Hoofdstuk 5** beschrijft de ontwikkeling van een machine learning model voor het berekenen van de biologische hersenleeftijd en onderzoekt of mensen met een depressie meer hersenveroudering laten zien. In **Hoofdstuk 6** wordt op die bevindingen voortgebouwd. Hierin wordt beschreven hoe het ontwikkelde model toegepast wordt op controles en patiënten met depressie en/of angststoornissen en associëren we de hersenleeftijd met meer gedetailleerde klinische, psychologische en biologische factoren. Het vierde en laatste doel van dit proefschrift is om meerdere biologische leeftijden te onderzoeken en deze te combineren en te integreren in één studie. In **Hoofdstuk 7** worden daarom de onderlinge correlaties onderzocht tussen vijf biologische klokken op basis van telomeren en vier omics-niveaus en hun unieke en gedeelde associaties met een breed scala aan lichamelijke en mentale gezondheidsrisico's.

SAMENVATTING VAN DE BELANGRIJKSTE BEVINDINGEN

Het doel van dit onderzoek was om verschillende kwantificaties van de biologische leeftijd bij depressieve stoornissen te bestuderen, om een beter begrip te verkrijgen van de complexe relatie tussen mentale gezondheid en biologische veroudering. Daartoe werden een literatuuronderzoek en vier empirische studies uitgevoerd om te onderzoeken of patiënten met depressie, gemeten aan de hand van leeftijd gerelateerde patronen afgeleid van perifere en hersenbiologie, een hogere biologische leeftijd hebben. Deze paragrafen vatten de belangrijkste bevindingen samen. In dit proefschrift worden de termen "biologische klok" of "biologische leeftijd voorspeller" gebruikt om te verwijzen naar de methode die voorspellingen over de "biologische leeftijd" genereert die sterk correleren met de chronologische leeftijd. De termen "biologische veroudering" en "'biologische verouderingsindicator"

worden door elkaar gebruikt en geven het verschil weer tussen de voorspelde biologische leeftijd en de chronologische leeftijd of zijn gelijk aan de residuen van een lineair regressiemodel van de voorspelde biologische leeftijd op chronologische leeftijd.

Hoofdstuk 2, het literatuuroverzicht, beschrijft dat veroudering niet plaatsvindt op één biologisch niveau of in een enkel celtype, maar multi-systemisch is en zich kan manifesteren in meerdere organen en weefsels op verschillende biologische niveaus. Om de vooruitgang op het gebied van onderzoek naar biologische veroudering te versnellen, zijn zes huidige uitdagingen en aanbevelingen geschetst. Ten eerste is voorzichtigheid geboden bij het interpreteren van cross-sectionele correlaties als causaal. Het wordt aanbevolen om longitudinale gegevens te verzamelen, experimenten te overwegen en de term voorspelling te verkiezen boven de term verklaring. Ten tweede zijn individuele biologische leeftijdsindicatoren meestal niet gecorreleerd en kunnen deze mogelijk beter geïntegreerd worden. Het wordt daarom aanbevolen om indices van biologische leeftijdsindicatoren samen te stellen die relevant zijn voor de mentale gezondheid. Ten derde kan biologische veroudering weefsel- en celtype-specifiek zijn, en daarom wordt aanbevolen om celtypen te zuiveren met behulp van gevestigde moleculaire markers. Ten vierde kunnen de varianties binnen de groep groter zijn dan de varianties tussen de groepen. Het wordt aanbevolen om gegevens te visualiseren, bekende invloeden zorgvuldig te beoordelen, verder te gaan dan groepsanalyses en gebruik te maken van modellen met meerdere meetmomenten binnen een proefpersoon. Ten vijfde zijn grote steekproeven nodig om kleine effectgroottes te detecteren. Het wordt aanbevolen om daarbij samen te werken en de dataverzameling en protocollen voor analyses te harmoniseren. Dit kan data pooling wereldwijd vergemakkelijken. Ten slotte worden cellulaire metingen van biologische veroudering vaak verkregen uit niet-uniforme laboratoriumtesten en opslagomstandigheden. Het wordt aanbevolen voor toekomstige studies om metingen en opslagomstandigheden te harmoniseren. Een voorlopige conclusie is dat het huidige veld baat zou kunnen hebben bij naleving van een minimumnorm voor rapportage om de harmonisatie van datasets tussen laboratoria en cohort studies te vergemakkelijken. Toekomstige studies die deze kansen benutten zullen hopelijk ons begrip van de psychobiologische factoren die het verouderingsproces beïnvloeden vergroten.

In **Hoofdstuk 3** is onderzocht of depressie geassocieerd is met meer biologische veroudering gemeten aan de hand van DNA-methylatiepatronen verkregen uit bloed. Patiënten met depressie waren gemiddeld +0,64 jaar ouder in vergelijking met controles. Dit effect was meer uitgesproken (+1,06 jaar) in een subgroep van patiënten die zowel depressie als jeugdtrauma's hebben meegemaakt. Verkennende analyses toonden aan dat meer epigenetische veroudering verder geassocieerd is met een hogere ernst van de depressie, het mannelijk geslacht, een hogere BMI en lage lichamelijke activiteit bij alle proefpersonen en een hoger cumulatief trauma uit de kindertijd, maar niet met de ernst van de depressie alleen in de patiëntengroep. Er waren geen significante associaties tussen epigenetische veroudering en roken/alcoholgebruik of de duur van of leeftijd waarop de depressie begon, noch met het gebruik van antidepressiva (d.w.z. tricyclische antidepressiva, selectieve serotonineheropnameremmers, andere antidepressiva). Om de robuustheid van de bevindingen te testen, werd depressie ook geassocieerd met biologische veroudering, zoals gemeten door DNA-methylatiepatronen verkregen uit postmortaal hersenweefsel, en er werd vastgesteld dat patiënten met depressie een gemiddeld verschil van +1,11 jaar vertoonden in vergelijking met controles. Ten slotte werden de biologische processen onderzocht die ten grondslag liggen aan epigenetische veroudering in beide weefsels. Hiervoor werden Gene Ontology (GO)-analyses uitgevoerd op de 1.094 overlappende CpG-sites die geassocieerd zijn met epigenetische veroudering, resulterend in 330 genen die aanwezig waren in ten minste één GO-categorie. De top "enriched" GO-termen omvatten neuronale processen zoals neurogenese, neuron differentiatie en regulering van de dood van neuronen, wat aangeeft dat verschillende depressie relevante biologische processen waren verrijkt.

Het belangrijkste doel van **Hoofdstuk 4** was om te bepalen of eerder gerapporteerde cross-sectionele epigenetische veroudering in de loop van de tijd ook versnelt (d.w.z. daadwerkelijk versnelde veroudering). Aan de hand van longitudinale data werd gevonden dat 45% van de totale variantie in epigenetische veroudering kan worden toegeschreven aan verschillen tussen proefpersonen, terwijl de resterende 55% kan worden toegeschreven aan veranderingen in de tijd. Deze bevindingen suggereren dat epigenetische veroudering zowel stabiel als veranderlijk is. Bij het ontleden van de covariantie tussen een grote verscheidenheid aan gezondheidsrisico's en epigenetische veroudering werden echter hogere bijdragen gevonden op het niveau van de deelnemer in plaats van op het niveau van tijd. In andere woorden, hoewel er verschillende zwakke maar significante bijdragen op het tijdsniveau werden waargenomen, ging een verandering in een gezondheidsrisico bij een bepaald meetmoment (bijvoorbeeld een toename in BMI) vaak niet gepaard met een parallelle verandering in epigenetische veroudering. De belangrijkste bevindingen van dit hoofdstuk waarschuwen daarom voor de causale interpretatie van correlaten van epigenetische veroudering en benadrukken dat de veelgebruikte term "versnelde" epigenetische veroudering in cross-sectionele studies wellicht onjuist is.

De studie in **Hoofdstuk 5** is erop gericht om te onderzoeken of depressie geassocieerd is met meer biologische veroudering, gemeten met een structurele MRI van de hersenen. Daartoe werden voorspellingsmodellen voor de hersenleeftijd ontwikkeld in 2.188 gezonde controles (afzonderlijk voor mannen en vrouwen) uit 19 verschillende cohorten wereldwijd. De modellen werden verder gevalideerd in 23 onafhankelijke cohorten en scanners. De gevonden model coëfficiënten werden toegepast op 2.126 onafhankelijke controles en 2.675 patiënten met depressie om de verschillen in hersenveroudering tussen de diagnostische groepen te berekenen. Gemiddeld vertoonden depressieve patiënten +1,08 jaar hersenveroudering in vergelijking met de controlegroep, maar post-hoc-vergelijkingen tussen depressie subgroepen lieten geen significante verschillen zien (d.w.z. status van eerste vs. recidiverende episode, vroege vs. volwassen vs. late aanvang van depressie, antidepressiva medicatievrij vs. antidepressiva-gebruikers, patiënten in remissie vs. momenteel depressieve patiënten).

Hoofdstuk 6 bouwt voort op het werk beschreven in Hoofdstuk 5 door te onderzoeken welke symptoomclusters (stemming/cognitie, immunometabolisch, somatisch) van depressie geassocieerd zijn met hersenveroudering en of de hersenen van patiënten met angststoornissen er ouder uit lijken te zien. Na correctie voor antidepressivagebruik vertoonden zowel patiënten met depressie (+2,78 jaar) als angststoornissen (+2,91 jaar) significant meer hersenveroudering dan controles. Deze studie wees verder op unieke bijdragen van de ernst van somatische symptomen van depressie aan hersenveroudering en een mogelijk beschermend effect van antidepressiva (-2,53 jaar) bij patiënten met depressie en/of angststoornissen. Er waren geen significante associaties met leefstijl (d.w.z. alcohol, roken, lichaamsbeweging) of biologische stresssystemen (d.w.z. ontstekingsmarkers, autonoom zenuwstelsel, hypothalamus-hypofyse-bijnier-as).

Ten slotte combineert de studie beschreven in **Hoofdstuk 7** één “traditionele” biologische leeftijdsindicator (d.w.z. telomeerlengte) met vier moderne op omics gebaseerde biologische klokken (d.w.z. epigenetica, transcriptomics, proteomics, metabolomics) om te onderzoeken of de verschillende biologische klokken dezelfde of verschillende aspecten van biologische veroudering duiden. De vijf biologische klokken werden geïntegreerd en geresidualiseerd voor leeftijd en correlaties daartussen werden berekend. Hieruit bleek dat de onderlinge correlaties klein waren, wat duidt op een geringe overlap. Om te onderzoeken of de verschillende biologische verouderingsindicatoren gelinkt waren met vergelijkbare determinanten werden ze geassocieerd met een breed scala aan lichamelijke en mentale gezondheidsvariabelen. Consistente associaties werden gevonden tussen het mannelijk geslacht, hogere BMI, metabool syndroom, roken en depressie en meer biologische veroudering over meerdere biologische niveaus. In vergelijking met de individuele biologische verouderingsindicatoren werd een samengestelde index van alle vijf biologische verouderingsindicatoren berekend die de sterkste associaties vertoonde met gezondheidsdeterminanten. Alles bij elkaar genomen suggereren de sterkere effecten (tot uiting komend in grotere effectgroottes) van de samengestelde index en de lage correlaties tussen de verschillende biologische verouderingsindicatoren dat biologische veroudering het beste wordt weerspiegeld door informatie van meerdere biologische niveaus te combineren.

CONCLUSIES

Het doel van dit proefschrift is om verschillende kwantificaties van de biologische leeftijd bij depressie te onderzoeken om de complexe wisselwerking tussen mentale gezondheid en biologische veroudering beter te begrijpen. De belangrijkste bevinding uit dit proefschrift is dat er convergerend bewijs is van meerdere biologische systemen dat depressie geassocieerd is met een ouder lijkende biologische staat van de hersenen en het lichaam, zoals gemeten door epigenetica, transcriptomics, proteomics en op hersenscans gebaseerde biologische klokken. Dit verklaart mogelijk waarom depressieve personen eerder in hun leven een verhoogd risico hebben om ouderdomsziekten te ontwikkelen dan niet-depressieve leeftijdsgenoten. Verschillende factoren dragen bij aan de waargenomen biologische veroudering, maar BMI was het meest consistent geassocieerd met meer veroudering over zes biologische niveaus en studies. Dit proefschrift heeft geen *versnelde* biologische veroudering bij depressie aangetoond en heeft zelfs gedeeltelijk bevestigd dat, althans op epigenetisch niveau, de meeste bestudeerde factoren waarschijnlijk geen causale versnellende effecten hebben op biologische veroudering. Hoewel nog moet worden opgehelderd of de verschillende biologische verouderingsindicatoren die in dit proefschrift worden besproken ook potentiële interventiedoelen zijn, benadrukt het nogmaals sterk dat depressie gevolgen heeft voor de gezondheid die verder gaan dan psychologische verstoringen. Een veelbelovende aanwijzing die vervolgonderzoek vereist, is de bevinding dat het gebruik van antidepressiva misschien wel beschermende effecten kan hebben op hersenveroudering. Toekomstige robuust opgezette longitudinale studies met meerdere beoordelingen zijn nodig om de complexe wisselwerking tussen psychologische, biologische en sociale factoren en veroudering verder te karakteriseren.

CURRICULUM VITAE

Laura Kim Mae Han was born on December 2nd 1989, in Amstelveen, the Netherlands. She graduated from high school in 2008, and spent a year working and travelling. She started her bachelor's degree in Psychobiology in 2009, followed by the Research Master Brain and Cognitive Sciences in 2012, both at the University of Amsterdam. During her master's degree, Laura investigated telomere length and brain activation at GGZ inGeest under supervision of Dr. Lianne Schmaal. During the second year, she visited the University of California, San Francisco (UCSF) for a six-month research internship in the group of Dr. Tony Yang, where she published her first paper on telomere length and hippocampal volume in adolescents with depression. She then worked as a student consultant for TNO before graduating from the Institute for Interdisciplinary Studies in 2015 (cum laude). In 2016, she started her PhD trajectory focused on biological aging and depression at GGZ inGeest and the Amsterdam University Medical Center. During this time, Laura has visited the Virginia Commonwealth University (VCU) in Richmond, Virginia, to learn how to work with DNA methylation data at the Center for Biomarker Research and Precision Medicine (CBRPM) led by Prof. Dr. Edwin van den Oord. In the final year of her PhD, Laura received a competitive Endeavour Research Leadership award by the Department of Education of the Government of Australia to visit The University of Melbourne for six months to examine age-related structural brain patterns in depression, using data from the ENIGMA consortium. In the fall of 2021, Laura will start her position as a postdoctoral researcher in the Mood and Anxiety Profiling and Prediction (MAPP) lab with Dr. Lianne Schmaal at the University of Melbourne and Orygen Youth Mental Health.

PUBLICATION LIST

First author publications (published)

- Jansen, R.*, **Han, L. K. M.***, Verhoeven, J. E., Aberg, K. A., van den Oord, E., Milaneschi, Y., & Penninx, B. W. J. H. (2021). An integrative study of five biological clocks in somatic and mental health. *eLife,* 10, e59479. * These authors contributed equally.
- **Han, L. K. M.**, Dinga, R., Hahn, T., Ching, C. R. K., Eyler, L. T., Aftanas, L., Aghajani, M., Aleman, A., Baune, B. T., Berger, K., Brak, I., Filho, G. B., Carballedo, A., Connolly, C. G., Couvy-Duchesne, B., Cullen, K. R., Dannlowski, U., Davey, C. G., Dima, D., … Schmaal, L. (2020). Brain aging in major depressive disorder: results from the ENIGMA major depressive disorder working group. *Molecular Psychiatry,* 1-16.
- **Han, L. K. M.**, Verhoeven, J. E., Tyrka, A. R., Penninx, B. W. J. H., Wolkowitz, O. M., Månsson, K. N. T., Lindqvist, D., Boks, M. P., Révész, D., Mellon, S. H., & Picard, M. (2019). Accelerating research on biological aging and mental health: Current challenges and future directions. *Psychoneuroendocrinology*, 106, 293–311.
- **Han, L. K. M.**, Aghajani, M., Clark, S. L., Chan, R. F., Hattab, M. W., Shabalin, A. A., Zhao, M., Kumar, G., Xie, L. Y., Jansen, R., Milaneschi, Y., Dean, B., Aberg, K. A., van den Oord, E. J. C. G., & Penninx, B. W. J. H. (2018). Epigenetic Aging in Major Depressive Disorder. *The American Journal of Psychiatry*, 175(8), 774–782.
- Henje Blom, E.*, **Han, L. K. M.***, Connolly, C. G., Ho, T. C., Lin, J., LeWinn, K. Z., Simmons, A. N., Sacchet, M. D., Mobayed, N., Luna, M. E., Paulus, M., Epel, E. S., Blackburn, E. H., Wolkowitz, O. M., & Yang, T. T. (2015). Peripheral telomere length and hippocampal volume in adolescents with major depressive disorder. *Translational Psychiatry*, 5(August), e676. *These authors contributed equally.

First author publications (in progress)

- **Han, L. K. M.**, Schnack, H. G., Brouwer, R. M., Veltman, D. J., van der Wee, N. J. A., van Tol, M.-J., Aghajani, M., & Penninx, B. W. (2020). Contributing Factors to Advanced Brain Aging in Depression and Anxiety Disorders. *medRxiv.* Submitted for publication.
- **Han, L. K. M.**, Aghajani, M., Copeland, W., Penninx, B. W. J. H., Aberg, K. A. & van den Oord, E. J. C. G. (2021). Do psychiatric problems, lifestyle variables and adversities accelerate epigenetic aging? Submitted for publication.
- **Han, L.K.M.**, Marquand, A.F. & Dinga, R. Brain age differences and how to test them. In preparation.
- **Han, L.K.M.**, Toenders, Y.J., Shen, X., Whalley, H.C., Leenings, R., Hahn, T., … & Schmaal, L. Brain age: associations with environmental and genetic factors. In preparation.
- Boateng, C.*, **Han, L.K.M.***, Klapwijk, E., Colins, O., van der Wee, N.A., Vermeiren, R. & Aghajani, M. Disorder-specific and transdiagnostic impact of major youth psychiatric disorders on brain morphology. In preparation. *These authors are contributing equally.
- **Han, L.K.M.**, Verhoeven, J.E., Schmaal, L., Veltman, D.J. & Penninx, B.W.J.H. The effects of antidepressant and running therapy on brain age and morphology in depression and anxiety. In preparation.
- **Han, L.K.M.**, Verhoeven, J.E., Schmaal, L., Veltman, D.J. & Penninx, B.W.J.H. The effects of antidepressant and running therapy on emotional face processing and working memory in depression and anxiety. In preparation.

Co-author publications (published)

- Aberg, K. A., Dean, B., Shabalin, A. A., Chan, R. F., **Han, L. K. M**., Zhao, M., van Grootheest, G., Xie, L. Y., Milaneschi, Y., Clark, S. L., Turecki, G., Penninx, B. W. J. H., & van den Oord, E. J. C. G. (2018). Methylome-wide association findings for major depressive disorder overlap in blood and brain and replicate in independent brain samples. *Molecular Psychiatry.*
- Hattab, M. W., Shabalin, A. A., Clark, S. L., Zhao, M., Kumar, G., Chan, R. F., Xie, L. Y., Jansen, R., **Han, L. K. M.**, Magnusson, P. K. E., van Grootheest, G., Hultman, C. M., Penninx, B. W. J. H., Aberg, K. A., & van den Oord, E. J. C. G. (2017). Correcting for cell-type effects in DNA methylation studies: reference-based method outperforms latent variable approaches in empirical studies. *Genome Biology,* 18(24).
- Henje Blom, E., Connolly, C. G., Ho, T. C., LeWinn, K. Z., Mobayed, N., **Han, L.**, Paulus, M. P., Wu, J., Simmons, A. N., & Yang, T. T. (2015). Altered insular activation and increased insular functional connectivity during sad and happy face processing in adolescent major depressive disorder. *Journal of Affective Disorders*, 178, 215–223.
- Ho, T. C., Zhang, S., Sacchet, M. D., Weng, H., Connolly, C. G., Henje Blom, E., **Han, L. K. M.**, Mobayed, N. O., & Yang, T. T. (2016). Fusiform Gyrus Dysfunction is Associated with Perceptual Processing Efficiency to Emotional Faces in Adolescent Depression: A Model-Based Approach. *Frontiers in Psychology,* 7, 40.
- Lever-van Milligen, B. A., Verhoeven, J. E., Schmaal, L., van Velzen, L. S., Révész, D., Black, C. N., **Han, L. K. M.**, Horsfall, M., Batelaan, N. M., van Balkom, A. J. L. M., van Schaik, D. J. F., van Oppen, P., & Penninx, B. W. J. H. (2019). The impact of depression and anxiety treatment on biological aging and metabolic stress: study protocol of the MOod treatment with antidepressants or running (MOTAR) study. *BMC Psychiatry*, 19(1), 425.
- Sacchet, M. D., Ho, T. C., Connolly, C. G., Tymofiyeva, O., Lewinn, K. Z., **Han, L. K.**, Blom, E. H., Tapert, S. F., Max, J. E., Frank, G. K., Paulus, M. P., Simmons, A. N., Gotlib, I. H., & Yang, T. T. (2016). Large-Scale Hypoconnectivity Between Resting-State Functional Networks in Unmedicated Adolescent Major Depressive Disorder. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 41(12), 2951–2960.
- Sämann, P. G., Iglesias, J. E., Gutman, B. A., Grotegerd, D., Leenings, R., Flint, C., Dannlowski, U., Clarke-Rubright, E. K., Morey, R. A., van Erp, T. G. M., Whelan, C. D., **Han, L. K. M.**, van Velzen, L. S., Cao, B., Augustinack, J. C., Thompson, P., Jahanshad, N., & Schmaal, L. (2020). FreeSurfer based segmentation of hippocampal subfields: a review of methods and applications, with a novel quality control procedure for ENIGMA studies and other collaborative efforts. *Human Brain Mapping.*
- Schmaal, L., Pozzi, E., C Ho, T., van Velzen, L. S., Veer, I. M., Opel, N., Van Someren, E. J. W., **Han, L. K. M.**, Aftanas, L., Aleman, A., Baune, B. T., Berger, K., Blanken, T. F., Capitão, L., Couvy-Duchesne, B., R Cullen, K., Dannlowski, U., Davey, C., Erwin-Grabner, T., … Veltman, D. J. (2020). ENIGMA MDD: seven years of global neuroimaging studies of major depression through worldwide data sharing. *Translational Psychiatry,* 10(1), 172.
- Thompson, P. M., Jahanshad, N., Ching, C. R. K., Salminen, L. E., Thomopoulos, S. I., Bright, J., Baune, B. T., Bertolín, S., Bralten, J., Bruin, W. B., Bülow, R., Chen, J., Chye, Y., Dannlowski, U., de Kovel, C. G. F., Donohoe, G., Eyler, L. T., Faraone, S. V., Favre, P., … ENIGMA Consortium. (2020). ENIGMA and global neuroscience: A decade of large-scale studies of

the brain in health and disease across more than 40 countries. *Translational Psychiatry,* 10(1), 100.
- Tymofiyeva, O., Henje Blom, E., Ho, T. C., Connolly, C. G., Lindqvist, D., Wolkowitz, O. M., Lin, J., LeWinn, K. Z., Sacchet, M. D., **Han, L. K. M.**, Yuan, J. P., Bhandari, S. P., Xu, D., & Yang, T. T. (2018). High levels of mitochondrial DNA are associated with adolescent brain structural hypoconnectivity and increased anxiety but not depression. *Journal of Affective Disorders*, 232, 283–290.

Co-author publications (in progress)

- Brouwer, R. M., Klein, M., Grasby, K. L., Schnack, H. G., Jahanshad, N., Teeuw, J., … & Kremen, W. S. (2020). Dynamics of Brain Structure and its Genetic Architecture over the Lifespan. BioRxiv. Submitted for publication.
- Clausen, A.N., Fercho, K.A., Monsour, M., Disner, S., Salminen, L., Haswell, C.C., … & Morey, R.A. (2021). Assessment of brain age in posttraumatic stress disorder: findings from the ENIGMA PTSD and brain age working groups. Submitted for publication.
- Eyler, L.T. et al. Assessment of brain age in Bipolar Disorder: findings from the ENIGMA BD and brain age working groups. In preparation.
- Rutherford, S., Ruhé, E., **Han, L.K.M.**, Beckmann, C.F., Penninx, B.W.J.H., Marquand, A.F. Predicting resilience and recurrence in major depressive disorder using normative models. In preparation.
- Schnack, H.G., Brouwer, R.M., **Han, L. K. M.**, Penninx, B.W.J.H., Boomsma, D., Hulshoff Pol, H. Influence of feature type on brain-age model performance and how to measure it. In preparation.

DISSERTATION SERIES

Department of Psychiatry, Amsterdam University Medical Centers

- N.M. (Neeltje) Batelaan (2010). Panic and Public Health: Diagnosis, Prognosis and Consequences. Vrije Universiteit Amsterdam. ISBN: 978-90-8659-411-5.
- G.E. (Gideon) Anholt (2010). Obsessive-Compulsive Disorder: Spectrum Theory and Issues in Measurement. Vrije Universiteit Amsterdam.
- N. (Nicole) Vogelzangs (2010). Depression & Metabolic Syndrome. Vrije Universiteit Amsterdam. ISBN: 978-90-8659-447-4.
- C.M.M. (Carmilla) Licht (2010). Autonomic Nervous System Functioning in Major Depression and Anxiety Disorders. Vrije Universiteit Amsterdam. ISBN: 978-90-8659-487-0.
- S.A. (Sophie) Vreeburg (2010). Hypothalamic-Pituitary-Adrenal Axis Activity in Depressive and Anxiety Disorders. Vrije Universiteit Amsterdam. ISBN: 978-90-8659-491-7.
- S.N.T.M. (Sigfried) Schouws (2011). Cognitive Impairment in Older Persons with Bipolar Disorder. Vrije Universiteit Amsterdam. ISBN: 978-90-9025-904-8.
- P.L. (Peter) Remijnse (2011). Cognitive Flexibility in Obsessive-Compulsive Disorder and Major Depression – Functional Neuroimaging Studies on Reversal Learning and Task Switching. Vrije Universiteit Amsterdam. ISBN: 978-90-6464-449-8.
- S.P. (Saskia) Wolfensberger (2011). Functional, Structural, and Molecular Imaging of the Risk for Anxiety and Depression. Vrije Universiteit Amsterdam. ISBN: 978-90-8659-536-5.
- J.E. (Jenneke) Wiersma (2011). Psychological Characteristics and Treatment of Chronic Depression. Vrije Universiteit Amsterdam. ISBN: 978-94-9121-150-8.
- P.D. (Paul David) Meesters (2011). Schizophrenia in Later Life. Studies on Prevalence, Phenomenology and Care Needs (SOUL Study). Vrije Universiteit Amsterdam. ISBN: 978-90-8659-563-1.
- R. (Ritsaert) Lieverse (2011). Chronobiopsychosocial Perspectives of Old Age Major Depression: a Randomized Placebo Controlled Trial with Bright Light. Vrije Universiteit Amsterdam. ISBN: 978-90-8570-858-2.
- A. (Adrie) Seldenrijk (2011). Depression, Anxiety and Subclinical Cardiovascular Disease. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-052-3.

- Y. (Yuri) Milaneschi (2012). Biological Aspects of Late-life Depression. Vrije Universiteit Amsterdam. ISBN: 978-90-8659-608-9.
- L. (Lynn) Boschloo (2012). The Co-occurrence of Depression and Anxiety with Alcohol Use Disorders. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-327-2.
- D. (Didi) Rhebergen (2012). Insight into the heterogeneity of depressive disorders. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-387-6.
- T.M. (Michiel) van den Boogaard (2012). The Negotiated Approach in the Treatment of Depressive Disorders: the impact on patient-treatment compatibility and outcome. Vrije Universiteit Amsterdam. ISBN: 978-90-8891-495-9.
- M. (Marjon) Nadort (2012). The implementation of outpatient schema therapy for borderline personality disorder in regular mental healthcare. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-463-7.
- U. (Ursula) Klumpers (2013). Neuroreceptor imaging of mood disorder related systems. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-575-7.
- E. (Ethy) Dorrepaal (2013). Before and beyond. Stabilizing Group treatment for Complex posttraumatic stress disorder related to child abuse based on psycho-education and cognitive behavioral therapy. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-601-3.
- K. (Kathleen) Thomaes (2013). Child abuse and recovery. Brain structure and function in child abuse related complex posttraumatic stress disorder and effects of treatment. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-600-6.
- K.M.L.(Klaas) Huijbregts (2013). Effectiveness and cost-effectiveness of the implementation of a collaborative care model for depressive patients in primary care. Vrije Universiteit Amsterdam. ISBN: 978-90-9027404-1.
- T.O. (Tessa) van den Beukel (2013). Ethnic differences in survival on dialysis in Europe. The role of demographic, clinical and psychosocial factors. Vrije Universiteit Amsterdam. ISBN: 978-94-6108410-1.
- A. (Agnes) Schrier (2013). Depression and anxiety in migrants in the Netherlands. Population studies on diagnosis and risk factors. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-719-5.
- B. (Barbara) Stringer (2013). Collaborative Care for patients with severe personality disorders. Challenges for the nursing profession. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-809-3.
- C.M. (Caroline) Sonnenberg (2013). Late life depression: sex differences in clinical presentation and medication use. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-866-6.

- Z. (Zsuzsika) Sjoerds (2013). Alcohol dependence across the brain: from vulnerability to compulsive drinking. Vrije Universiteit Amsterdam. ISBN: 978-90-8891-695-3.
- V.J.A. (Victor) Buwalda (2013). Routine Outcome Monitoring in Dutch Psychiatry: Measurement, Instruments, Implementation and Outcome. Vrije Universiteit Amsterdam. ISBN: 978-94-6191-905-2.
- J.G. (Josine) van Mill (2013). Sleep, depression and anxiety: an epidemiological perspective. Vrije Universiteit Amsterdam. ISBN: 978-94-6108-525-2.
- S. (Saskia) Woudstra (2013). Framing depression in a SN[a]Pshot: Imaging risk factors of MDD. Vrije Universiteit Amsterdam. ISBN: 978-90-8891-751-6.
- N.C.M. (Nicole) Korten (2014). Stress, depression and cognition across the lifespan. Vrije Universiteit Amsterdam. ISBN: 978-94-6108-748-5.
- M.K. (Maarten) van Dijk (2014). Applicability and effectiveness of the Dutch Multidisciplinary Guidelines for the treatment of Anxiety Disorders in everyday clinical practice. Vrije Universiteit Amsterdam. ISBN: 978-94-92096-00-5.
- I.M.J. (Ilse) van Beljouw (2015). Need for Help for Depressive Symptoms from Older Persons Perspectives: The Implementation of an Outreaching Intervention Programme. Vrije Universiteit Amsterdam. ISBN: 978-94-6259-496-8.
- A.M.J. (Annemarie) Braamse (2015). Psychological aspects of hematopoietic stem cell transplantation in patients with hematological malignancies. Vrije Universiteit Amsterdam. ISBN: 978-94-6259-594-1.
- A. (Annelies) van Loon (2015). The role of ethnicity in access to care and treatment of outpatients with depression and/or anxiety disorders in specialised care in Amsterdam the Netherlands. Vrije Universiteit Amsterdam. ISBN: 978-94-90791-34-6.
- C. (Chris) Vriend (2015). (Dis)inhibition: imaging neuropsychiatry in Parkinson’s disease. Vrije Universiteit Amsterdam. ISBN: 978-94-6295-115-0.
- A.M. (Andrea) Ruissen (2015). Patient competence in obsessive compulsive disorder. An empirical ethical study. Vrije Universiteit Amsterdam. ISBN: 978-90-6464-856-4.
- H.M.M. (Henny) Sinnema (2015). Tailored interventions to implement guideline recommendations for patients with anxiety and depression in general practice. Vrije Universiteit Amsterdam. ISBN: 978-94-6169-653-3.
- T.Y.G. (Nienke) van der Voort (2015). Collaborative Care for patients with bipolar disorder. Vrije Universiteit Amsterdam. ISBN: 978-94-6259-646-7.

- W. (Wim) Houtjes (2015). Needs of elderly people with late-life depression; challenges for care improvement. Vrije Universiteit Amsterdam. ISBN: 978-94-6108-985-4.
- M. (Marieke) Michielsen (2015). ADHD in older adults. Prevalence and psychosocial functioning. Vrije Universiteit Amsterdam. ISBN: 978-90-5383-132-8.
- S.M. (Sanne) Hendriks (2016). Anxiety disorders. Symptom dimensions, course and disability. Vrije Universiteit Amsterdam. ISBN: 978-94-6259-963-5.
- E.J. (Evert) Semeijn (2016). ADHD in older adults; diagnosis, physical health and mental functioning. Vrije Universiteit Amsterdam. ISBN: 978-94-6233-190-7.
- N. (Noera) Kieviet (2016). Neonatal symptoms after exposure to antidepressants in utero. Vrije Universiteit Amsterdam. ISBN: 978-94-6169-794-3.
- W.L. (Bert) Loosman (2016). Depressive and anxiety symptoms in Dutch chronic kidney disease patients. Vrije Universiteit Amsterdam. ISBN: 987-94-6169-793-6.
- E. (Ellen) Generaal (2016). Chronic pain: the role of biological and psychosocial factors. Vrije Universiteit Amsterdam. ISBN: 978-94-028-0032-6.
- D. (Dóra) Révész (2016). The interplay between biological stress and cellular aging: An epidemiological perspective. Vrije Universiteit Amsterdam. ISBN: 978-94-028-0109-5.
- F.E. (Froukje) de Vries (2016). The obsessive-compulsive and tic-related brain. Vrije Universiteit Amsterdam. ISBN: 978-94-629-5481-6.
- J.E. (Josine) Verhoeven (2016). Depression, anxiety and cellular aging: does feeling blue make you grey? Vrije Universiteit Amsterdam. ISBN: 978-94-028-0069-2.
- A.M. (Marijke) van Haeften-van Dijk (2016). Social participation and quality of life in dementia: Implementation and effects of interventions using social participation as strategy to improve quality of life of people with dementia and their carers. Vrije Universiteit Amsterdam. ISBN: 978-94-6233-341-3. P.M. (Pierre) Bet (2016). Pharmacoepidemiology of depression and anxiety. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-388-4.
- M.L. (Mardien) Oudega (2016). Late life depression, brain characteristics and response to ECT. Vrije Universiteit Amsterdam. ISBN: 978-94-6295-396-3.

- H.A.D. (Henny) Visser (2016). Obsessive-Compulsive Disorder; Unresolved Issues, Poor Insight and Psychological Treatment. Vrije Universiteit Amsterdam. ISBN: 978-94-028-0259-7.
- E.C. (Eva) Verbeek (2017). Fine mapping candidate genes for major depressive disorder: Connecting the dots. Vrije Universiteit Amsterdam. ISBN: 978-94-028-0439-3.
- S. (Stella) de Wit (2017). In de loop: Neuroimaging Cognitive Control in Obsessive-Compulsive Disorder. Vrije Universiteit Amsterdam. ISBN: 978-90-5383-225 7.
- W.J. (Wouter) Peyrot (2017). The complex link between genetic effects and environment in depression. Vrije Universiteit Amsterdam. ISBN: 978-94-6182-735-7.
- R.E. (Rosa) Boeschoten (2017). Depression in Multiple Sclerosis: Prevalence Profile and Treatment. Vrije Universiteit Amsterdam. ISBN: 978-94-028-0474-4.
- G.L.G. (Gerlinde) Haverkamp (2017). Depressive symptoms in an ethnically DIVERSe cohort of chronic dialysis patients: The role of patient characteristics, cultural and inflammatory factors. Vrije Universiteit Amsterdam. ISBN: 978-94-6233-528-8.
- T.J. (Tjalling) Holwerda (2017). Burden of loneliness and depression in late life. Vrije Universiteit Amsterdam. ISBN: 978-94-6233-598–1.
- J. (Judith) Verduijn (2017). Staging of Major Depressive Disorder. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-597-0.
- C.N. (Catherine) Black (2017). Oxidative stress in depression and anxiety disorders. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-672-4.
- J.B. (Joost) Sanders (2017). Slowing and Depressive Symptoms in Aging People. Vrije Universiteit Amsterdam. ISBN: 978-94-6233-650-6.
- W. (Willemijn) Scholten (2017). Waxing and waning of anxiety disorders: relapse and relapse prevention. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-606-9.
- P. (Petra) Boersma (2017). Person-centred communication with people with dementia living in nursing homes; a study into implementation success and influencing factors. Vrije Universiteit Amsterdam. ISBN: 978-94-6233-725-1.
- T.I. (Annet) Bron (2017). Lifestyle in adult ADHD from a Picasso point of view. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-685-4.
- S.W.N. (Suzan) Vogel (2017). ADHD IN ADULTS: seasons, stress, sleep and societal impact. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-673-1.

- R.(Roxanne) Schaakxs (2018). Major depressive disorder across the life span: the role of chronological and biological age. Vrije Universiteit Amsterdam. ISBN: 978-94-6299-819-3.
- J.J. (Bart) Hattink (2018). Needs-based enabling- and care technology for people with dementia and their carers. Vrije Universiteit Amsterdam. ISBN: 978-94-6295-880-7.
- F.T. (Flora) Gossink (2018). Late Onset Behavioral Changes differentiating between bvFTD and psychiatric disorders in clinical practice. Vrije Universiteit Amsterdam. ISBN: 978-94-6295-899-9.
- R. (Roxanne) Gaspersz (2018). Heterogeneity of Major Depressive Disorder. The role of anxious distress. Vrije Universiteit Amsterdam. ISBN: 978-94-028-1076-9.
- M.M. (Marleen) Wildschut (2018). Survivors of early childhood trauma and emotional neglect: who are they and what's their diagnosis? Vrije Universiteit Amsterdam. ISBN: 978-94-6332-401-4.
- J.A.C. (Jolanda) Meeuwissen (2018). The case for stepped care. Exploring the applicability and cost-utility of stepped-care strategies in the management of depression. Vrije Universiteit Amsterdam. ISBN: 978-90-5383-359-9.
- D.S. (Dora) Wynchank (2018). The rhythm of adult ADHD. On the relationship between ADHD, sleep and aging. Vrije Universiteit Amsterdam. ISBN: 978-94-6375-034-9.
- M.J.(Margot) Metz (2018). Shared Decision Making in mental health care: the added value for patients and clinicians. Vrije Universiteit Amsterdam. ISBN: 978-94-6332-403-8.
- I.(Ilse) Wielaard (2018). Childhood abuse and late life depression. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-072-3.
- L.S.(Laura) van Velzen (2019). The stressed and depressed brain. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-062-4.
- S. (Sonja) Rutten (2019). Shedding light on depressive, anxiety and sleep disorders in Parkinson's disease. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-176-8.
- N.P.G. (Nadine) Paans (2019). When you carry the weight of the world not only on your shoulders. Factors associating depression and obesity. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-141-6.
- D.J. (Deborah) Gibson-Smith (2019). The Weight of Depression. Epidemiological studies into obesity, dietary intake and mental health. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-144-7.
- C.S.E.W. (Claudia) Schuurhuizen (2019). Optimizing psychosocial support and symptom management for patients with advanced cancer. Vrije Universiteit Amsterdam. ISBN: 978-94-6323-468-9.

- M.X. (Mandy) Hu (2019). Cardiac autonomic activity in depression and anxiety: heartfelt afflictions of the mind. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-206-2.
- J..K.(Jan) Mokkenstorm (2019). On the road to zero suicides: Implementation studies. Vrije Universiteit Amsterdam. ISBN: 978-94-6361-224-1.
- S.Y. (Sascha) Struijs (2019). Psychological vulnerability in depressive and anxiety disorders. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-244-4.
- H.W. (Hans) Jeuring (2019). Time trends and long-term outcome of late-life depression: an epidemiological perspective. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-228-4.
- R. (Ruth) Klaming Miller (2019). Vulnerability of memory function and the hippocampus: Risk and protective factors from neuropsychological and neuroimaging perspectives. Vrije Universiteit Amsterdam. ISBN: 978-94-6182-955-5.
- P.S.W. (Premika) Boedhoe (2019) The structure of the obsessive-compulsive brain – a worldwide effort. Vrije Universiteit Amsterdam. ISBN: 978-94-6380-329-8.
- C.S. (Carisha) Thesing (2020). Fatty acids in depressive and anxiety disorders: fishing for answers. Vrije Universiteit Amsterdam. ISBN: 978-94-6375-846-8.
- R.D. (Richard) Dinga (2020). Evaluation of machine learning models in psychiatry. Vrije Universiteit Amsterdam.
- M. (Mayke) Mol (2020). Uptake of internet-based therapy for depression: the role of the therapist. Vrije Universiteit Amsterdam. ISBN: 978-94-6416-150-2.
- R.C. (Renske) Bosman (2020). Improving the long-term prognosis of anxiety disorders: Clinical course, chronicity and antidepressant use. Vrije Universiteit Amsterdam. ISBN: 978-94-6375-736-2.
- R.W. (Robbert) Schouten (2020). Anxiety, depression and adverse clinical outcomes in dialysis patients. Should we do more? Vrije Universiteit Amsterdam. ISBN: 978-94-6416-179-3.
- T.T. (Trees) Juurlink (2021). Occupational functioning in personality disorders: a quantitative, qualitative and semi-experimental approach. Vrije Universiteit Amsterdam. ISBN: 978-94-6421-117-1.
- I.P.H. (Ires) Ghielen (2021). Surfing the waves of Parkinson's disease. Understanding and treating anxiety in the context of motor symptoms. Vrije Universiteit Amsterdam. ISBN: 978-94-6416-493-0
- L.K.M. (Laura) Han (2021). Biological aging in major depressive disorder. Vrije Universiteit Amsterdam. ISBN: 978-94-93184-91-6