Telomere length and the course of major depressive disorder
ABSTRACT

Background → The course of MDD has been found to be worse in older patients compared to younger patients, and cannot be entirely explained by a range of clinical, social, and health factors. Possibly, biological age is an underlying factor for this unfavorable course. Biological age, as indexed by telomere length (TL), has been associated with MDD previously. The aim of this study is to assess whether TL is associated with the two-year course of MDD. Methods → Data were from the Netherlands Study of Depression and Anxiety (NESDA) and the Netherlands Study of Depression in Older Persons (NESDO). Participants were 1,023 persons aged 18 to 88 (mean age 47.3 (SD 16.3) years) with a current MDD diagnosis and a depression severity score of at least 14 on the Inventory of Depressive Symptomatology at baseline. Leukocyte TL was determined at baseline using fasting blood samples by performing quantitative polymerase chain reaction (qPCR) and was expressed in kilo base pairs (kbp). Four depression outcomes were assessed during and after two years: still having a depression diagnosis, having a chronic course, time to remission, and depression severity change. Results → TL was found to be unrelated to having a depression diagnosis at two year follow-up, having a chronic symptom course, and time to remission. Shorter TL was associated with a smaller decrease in depression severity, but this association disappeared when adjusting for other prognostic covariates and chronological age. Conclusions → Shorter TL is not associated with the two-year course of MDD, and therefore biological age does not seem to explain the more unfavorable course for MDD in older age. It remains to be examined which other mechanisms are responsible for the poorer MDD outcome observed in the older patient population.
INTRODUCTION

Major depressive disorder (MDD) is a highly prevalent disorder with serious consequences for one’s mental as well as somatic health. Nearly one in six adults is expected to go through at least one depressive episode sometime during their life course.\(^1,2\) The prognosis of such an episode may be highly variable, depending on a range of coinciding circumstances. According to a recent meta-analysis, 35-60% of depressed adults experience stable recovery with no recurrence, 70-85% recovers at least once, and 10-17% has a chronic course.\(^3\)

In a 20-year update on late-life depression, Haigh and colleagues\(^4\) conclude that older persons experience a worse trajectory with higher relapse and recurrence rates. Depression severity, the number of previous episodes, and medical comorbidity were put forward as likely explaining factors. In a recent study using a large sample of depressed persons (N = 1,042) aged 18 to 88 years, we indeed established that the (two-year) course of MDD worsened with age: all four course indicators under study were least favorable for those aged 70+ years. The oldest patients were twice as likely to still have a depression diagnosis after two years and to experience a chronic symptom course, showed longer time to remission, and had only half as much improvement in depression severity compared to those aged 18-29 years. For most indicators, the course seemed to worsen linearly with age.\(^5\) We were able to test a wide range of clinical, social, and health-related explanatory factors, however, these factors explained only a fraction of the deteriorating prognosis of depression. Thus, additional research is needed to elucidate which factors or mechanisms are responsible for this unfavorable age trend.

In the past years, attention has not only been paid to the consequences of chronological aging, but has also shifted towards biological (or cellular) aging. Biological aging focuses on the aging of the body and its cells, regardless of chronological age. One marker of biological aging is telomere length (TL). Telomeres are nucleic-acid protein complexes situated at the ends of chromosomes, protecting DNA from damage. During cell division, the very ends of chromosomes are not fully replicated, resulting in telomere shortening during each cell division, which makes TL an interesting marker of biological aging. Once telomeres become critically short they eventually lose their protective function, which in turn could result in cell senescence and apoptosis.\(^6,7\) Short TL has been associated with a range of unfavorable health outcomes, such as cardiovascular diseases,\(^8\) dementia,\(^9\) indicators of arterial stiffness,\(^10\) cancer,\(^11\) and mortality.\(^9,11\)

Although TL shortens naturally with increasing age, other factors (such as chronic diseases, lifestyle factors, and stress)\(^12,13\) can accelerate this process. MDD has also been shown to be associated with TL-shortening; multiple meta-analyses have linked MDD cross-sectionally to shorter TL,\(^14-16\) although studies performed in older populations (aged ~60 years and older) found no convincing evidence for such an association.\(^17-19\) MDD, and stress in general, are thought to affect TL through dysregulations of physiological stress systems, such as the immune system.\(^20\) MDD and anxiety disorders have been associated with increased levels of inflammatory markers interleukin-6 (IL-6) and C-reactive protein\(^21\) and with multiple metabolic dysregulations (larger waist
circumference, high triglycerides and low HDL cholesterol). In addition, stress has been shown to affect the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. These disruptions have shown to be associated with increased oxidative stress and telomere shortening.

Longitudinally, associations between MDD and subsequent change in TL have been inconclusive. Some studies found between-person but not within-person associations with TL over time, whereas another study did find persistence of MDD to be associated with differential TL shortening, but only for men and in a young sample (participants were followed from age 11 to age 38). Although the evidence for changes in TL based on different MDD courses is lacking, the opposite relationship may still be present. Short TL, and thus more advanced biological age, may be indicative of physical exhaustion and excessive stress system dysregulations, which may signify a more unfavorable MDD course. Whether short TL subsequently affects the course of MDD, has not been studied before.

The aim of the current study is therefore to examine whether TL is associated with the two-year naturalistic course and prognosis of depression (i.e. persistence of depression, chronic symptom course, time to remission and depression severity change), in a sample of 1,023 persons aged 18 to 88 years. If associations between TL and the course of MDD are found, it will be examined whether these associations last when chronological age and other prognostic covariates for the course of MDD are taken into account as well. A unique aspect of this study is the fact that biological and chronological aging can be compared simultaneously, in a large cohort, covering almost the entire adult life span. We hypothesize that short TL is associated with a more unfavourable course of MDD and may partly explain the earlier observed unfavorable impact of chronological age on this course.

METHODS

Study sample
Data were derived from two longitudinal multi-center cohort studies: the Netherlands Study of Depression and Anxiety (NESDA), and the Netherlands Study of Depression in Older Persons (NESDO). As NESDA and NESDO use similar measurements and infrastructures and both investigate the natural course, determinants and consequences of depression, data from these studies were combined. Detailed descriptions of both studies can be found elsewhere.

This study used baseline and 2-year follow-up data from both NESDA and NESDO. NESDA’s baseline sample initially consisted of 2,981 persons aged 18-65 years. Participants had a current (6-month recency) diagnosis of a depressive and/or anxiety disorder (n = 1,701), a remitted depressive and/or anxiety disorder (n = 628), or no history of depressive and anxiety disorders (n = 652), and were recruited from the general population, primary health care and outpatient mental health care facilities. The follow-up rate after two years was 87.1% (N = 2,596). At baseline, the NESDO sample consisted of 510 adults aged 60-93 years with a current (6-month recency) diagnosis of...
a depressive disorder (n = 378), or no history of depressive and anxiety disorders (n = 132). After two years, 78.6% (N = 401) still participated in NESDO. Participants were recruited from primary health care and both out- and inpatient mental health care facilities. Exclusion criteria for NESDA and NESDO were: 1) insufficient fluency in Dutch; 2) a primary clinical diagnosis of a severe psychiatric disorder other than depression and anxiety; and for NESDO additionally 3) (clinician-suspected) dementia, or having a Mini-Mental State Examination (MMSE) score below 18 (out of 30). Clinical diagnoses were used to determine the presence of depressive and/or anxiety disorders, using the DSM-IV based Composite International Diagnostic Interview (CIDI, lifetime version), conducted by trained research staff.

Selection criteria for the current study were: not being an inpatient (inpatients are not part of NESDA), and having a current 6-month MDD diagnosis (and possibly co-morbid dysthymia) at baseline with a score of at least 14 on the self-report Inventory of Depressive Symptomatology (IDS-SR) to indicate substantial depression severity. Also, TL assessment at baseline and two-year follow-up assessment of depression diagnoses needed to be available. Eventually, 1,023 participants were included in this study (817 from NESDA, 206 from NESDO).

Baseline and two-year follow-up assessments were face-to-face interviews, completed at participating centers between 2004 and 2007 (baseline) and 2007 and 2010 (2-year follow-up) for NESDA, and between 2007 and 2010 (baseline) and 2009 and 2012 (2-year follow-up) for NESDO. All participants provided written informed consent, and all Ethical Review Boards of the participating centers provided approval.

Measures

Telomere length

Leukocyte TL was determined by Telomere Diagnostics, Inc. (TDx, Menlo Park, CA, USA) using fasting blood samples collected between 8:30 and 9:30 AM, which were subsequently stored at -80°C. Quantitative polymerase chain reaction (qPCR) was used to compare the telomere sequence copy number (T) in each patient’s sample to a single-copy gene copy number (S), relative to a reference sample. The resulting T/S ratio was proportional to mean TL. A detailed description of the method is provided elsewhere. To be able to compare the T/S ratio to outcomes of TL from studies using Southern blot analysis (telomere restriction fragments, TRF), the T/S ratio was converted into base pairs (bp). Lin and colleagues used the following formula: bp=3274+2413*T/S based on comparison of T/S ratios and TRF analysis of a series of genomic DNA samples from the human fibroblast cell line IMR90. Comparison of the T/S ratios of 8 quality control DNA samples from the TDx lab that were included on each PCR run, generated the following formula: T/S_{(Lin et al.)}=(T/S_{(TDx)}-0.0545)/1.16. Therefore, the final formula used to convert T/S ratios to bp is: bp=3274+2413x((T/S-0.0545)/1.16). The 8 included quality control DNA samples on each PCR run illustrated a small intra-assay coefficient of variation (CV=5.1%), and sufficiently low inter-assay CV (CV=4.6%), indicating that the reliability of the assay was adequate. In the current study TL served as a predictor variable,
therefore we examined TL in kilo bp (bp/1000) for interpretation purposes.

**Two-year depression course**

The CIDI was used to determine the presence of a depressive disorder (MDD and/or dysthymia with a 6-month recency). When a depressive disorder was present, subsequently the Life Chart Interview (LCI), which showed to be valid and reliable, was conducted. In the LCI, using a calendar method participants reported presence (yes/no) of depressive symptoms and the extent of their severity (no or minimal severity, mild, moderate to severe, very severe) for each month of a two-year follow-up period. As a tool to help recall depressive symptoms, life events within the two-year period were reported. Symptoms were considered to be present when of at least mild severity in a specific month. Using the CIDI, LCI and the IDS-SR we created four outcome variables displaying the two-year course of MDD in order to cover different aspects of the prognosis of MDD.

First, we used the CIDI to establish the presence (yes/no) of 6-month depressive disorders (MDD and/or dysthymia) after two years. Second, we used the LCI to determine whether participants experienced a chronic course of depressive symptoms (yes/no), which was defined as experiencing depressive symptoms for at least 80% of the time during the two-year follow-up period. Third, time to remission was also calculated using the LCI and represented the time since baseline assessment to the first time point at which no depressive symptoms or only symptoms of mild severity were reported for three consecutive months. Fourth, we used the 30-item IDS-SR (total score range from 0 to 84) to assess the change in depression severity within the two-year follow-up period. Depression severity change was defined as the depression severity score after two years minus baseline depression severity.

**Covariates**

A number of covariates was included in the study as these may affect the course of depression as well and could play a role in associations between TL and the course of depression. Age (range 18-88 years), sex and years of education were asked during the baseline interview using standard questions.

Baseline clinical covariates were depression severity, assessed using the IDS-SR, having a co-morbid 6-month anxiety disorder, having a first or recurrent MDD episode, and the number of MDD episodes experienced (all determined using the CIDI), anxiety severity, assessed using the Beck Anxiety Inventory (BAI), and antidepressant use. Antidepressant use was categorized using the World Health Organization Anatomical Therapeutic Chemical (ATC) classification, into tricyclic antidepressant (TCA) use [ATC-code N06AA], selective serotonin inhibitors (SSRI) use [ATC-code N06AB], or use of other antidepressants [ATC-codes N06AF, N06AG, N06AX].

Prognostic lifestyle and somatic health covariates were also included in order to help explain potential associations between TL and two-year depression outcomes. We included smoking, alcohol use, and physical activity as lifestyle covariates, and Body Mass Index (BMI), functional limitations, pain, and the number of chronic diseases as
health covariates. Smoking was measured in cigarette years, the average number of cigarettes smoked per day multiplied by the number of years of smoking (per 100). Alcohol use was the number of alcoholic drinks per week. Physical activity was assessed by the International Physical Activity Questionnaire (IPAQ) and expressed in 1000 Metabolic Equivalent Total (MET)-minutes per week (\((\text{MET-level} \times \text{minutes of activity} \times \text{events per week})/1000\)). BMI (weight/(length²)) was used continuously. Functional limitations were assessed using the World Health Organization Disability Assessment Schedule II, which examines several aspects of daily life (e.g. household activities and participation in society). Pain was assessed using the Chronic Pain Grade, and participants were categorized into five grades based on a combination of pain intensity and pain disability experienced: grade 0 (no pain symptoms); grade 1 (low pain intensity–low disability); grade 2 (high intensity–low disability); grade 3 (high disability–moderately limiting); grade 4 (high disability–severely limiting). These grades were used as a continuous variable. Furthermore, the number of chronic diseases under treatment was established. Included diseases were lung disease, heart disease, diabetes mellitus, stroke, osteoarthritis, cancer, ulcers, intestinal disease, liver disease, epilepsy, and thyroid disease.

**Statistical analysis**

For three tertiles of TL, percentages and means were displayed for baseline characteristics and two-year MDD outcomes. In addition, continuous TL was associated with these outcomes using regression analyses.

Associations between TL (in kilo base pairs) and presence of current depressive disorders after two years were examined using logistic regression. We used four adjustment models: 1) adjusted for sex, 2) additionally adjusted for clinical, lifestyle, and health covariates that showed significant associations with continuous TL at baseline, 3) additionally adjusted for chronological age. We then analyzed the association between TL and having a chronic course (yes/no) in the same way. We analyzed the association between TL and time to remission using Cox proportional hazards analysis. Time to remission was the dependent variable and calculated as the number of months between baseline and remission. If no remission was reached, time to remission was censored at 24 months. Finally, using multiple linear regression analysis, the association between TL and change in depression severity was assessed. In addition to covariates, this analysis was also adjusted for baseline depression severity.

Missing values (ranging from 0.3% for pain to 4.8% for physical activity) were imputed using multiple imputation (5 imputation sets) in which TL and all socio-demographic, clinical, and lifestyle and health covariates were used as predictors. Pooled estimates from the imputed models were used for multivariate analyses. An alpha of 5% was used to determine statistical significance, and all analyses were performed in SPSS version 22.
RESULTS

Baseline characteristics
Shorter TL was associated with older age, male sex, higher baseline depression and anxiety severity, antidepressant use, more cigarette years, a higher number of alcoholic drinks per week, higher BMI, higher levels of pain and a higher number of chronic diseases (Table 1). As a result, subsequent analyses were adjusted for these covariates. TL was unrelated to years of education, having a first or recurrent episode, having a concurrent anxiety diagnosis, physical activity, and functional limitations. Correlation between TL and age at baseline was weak to moderate ($r = -0.40$).

Is TL related to the two-year course of MDD?
Table 2 shows that in models only adjusted for sex (Model 1), TL was only significantly associated with depression severity change. Shorter TL was associated with a smaller decrease, thus a smaller improvement, in depression severity. After adjusting for clinical, lifestyle and health covariates (Model 2), this association disappeared. When adding chronological age to the model, chronological age, but not TL, was significantly associated with all four course outcomes of MDD (Model 3). Post-hoc analyses further demonstrated that TL was also not quadratically associated with the course of depression (data not shown).

As can be seen from Table 3, 46.8% of participants still had a depression diagnosis after two years (42.2% had MDD and 23.2% had dysthymia), 28.6% had a chronic course, and depression severity reduced on average by 9.57 (SD 11.52) points. Looking at TL tertiles, Table 3 confirms that two-year outcomes are not associated with TL, although outcomes for those with short TL seem somewhat less favorable.
Table 1. Baseline characteristics across telomere length (TL) tertiles, and linear associations with continuous TL (N = 1,023).

<table>
<thead>
<tr>
<th></th>
<th>Short TL (3.95-5.06 kbp, n = 341)</th>
<th>Middle TL (5.06-5.52 kbp, n = 341)</th>
<th>Long TL (5.52-7.30 kbp, n = 341)</th>
<th>TL continuous (bp) B</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere length in bp, M (SD)</td>
<td>4814.64 (201.33)</td>
<td>5269.12 (128.38)</td>
<td>6046.09 (460.03)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age, M (SD)</td>
<td>55.5 (15.30)</td>
<td>46.7 (15.0)</td>
<td>39.5 (14.44)</td>
<td>-146.28*</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sex, female, %</td>
<td>55.4</td>
<td>69.5</td>
<td>73.0</td>
<td>165.31</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Education, years, M (SD)</td>
<td>11.29 (3.40)</td>
<td>11.52 (3.27)</td>
<td>11.33 (3.19)</td>
<td>5.33</td>
<td>.34</td>
</tr>
<tr>
<td>First episode, yes, %</td>
<td>46.9</td>
<td>47.2</td>
<td>47.5</td>
<td>-12.20</td>
<td>.74</td>
</tr>
<tr>
<td>Number of episodes</td>
<td></td>
<td></td>
<td></td>
<td>-29.88</td>
<td>.14</td>
</tr>
<tr>
<td>1 episode, %</td>
<td>39.2</td>
<td>44.2</td>
<td>47.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 episodes, %</td>
<td>17.3</td>
<td>10.9</td>
<td>11.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 or more episodes, %</td>
<td>43.5</td>
<td>44.8</td>
<td>40.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression severity, M (SD)</td>
<td>34.11 (10.99)</td>
<td>34.35 (11.21)</td>
<td>32.06 (10.28)</td>
<td>-4.91</td>
<td>.004</td>
</tr>
<tr>
<td>Anxiety diagnosis, yes, %</td>
<td>55.1</td>
<td>63.3</td>
<td>59.8</td>
<td>47.14</td>
<td>.21</td>
</tr>
<tr>
<td>Anxiety severity, M (SD)</td>
<td>18.89 (11.17)</td>
<td>18.86 (10.52)</td>
<td>17.16 (10.39)</td>
<td>-3.82</td>
<td>.03</td>
</tr>
<tr>
<td>Antidepressant use, yes, %</td>
<td>50.9</td>
<td>53.0</td>
<td>43.9</td>
<td>-82.53</td>
<td>.03</td>
</tr>
<tr>
<td>Cigarette years per 100 years, M (SD)</td>
<td>3.24 (3.88)</td>
<td>2.46 (3.66)</td>
<td>2.03 (2.91)</td>
<td>-25.85</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Number of alcoholic drinks per week, M (SD)</td>
<td>7.28 (10.88)</td>
<td>5.49 (9.64)</td>
<td>5.97 (10.11)</td>
<td>-3.99</td>
<td>.03</td>
</tr>
<tr>
<td>Physical activity per 1000 MET-minutes, M (SD)</td>
<td>3.06 (2.93)</td>
<td>3.42 (3.21)</td>
<td>3.38 (3.00)</td>
<td>8.96</td>
<td>.15</td>
</tr>
<tr>
<td>Functional limitations, M (SD)</td>
<td>28.25 (12.58)</td>
<td>27.98 (11.75)</td>
<td>27.58 (11.46)</td>
<td>-1.09</td>
<td>.48</td>
</tr>
<tr>
<td>Body Mass Index, M (SD)</td>
<td>26.63 (5.13)</td>
<td>26.21 (5.22)</td>
<td>25.49 (5.54)</td>
<td>-11.23</td>
<td>.001</td>
</tr>
<tr>
<td>Pain, M (SD)</td>
<td>2.06 (1.19)</td>
<td>2.07 (1.13)</td>
<td>1.87 (1.17)</td>
<td>-42.63</td>
<td>.01</td>
</tr>
<tr>
<td>Number of chronic diseases, M (SD)</td>
<td>1.21 (1.26)</td>
<td>0.90 (1.04)</td>
<td>0.70 (0.94)</td>
<td>-90.42</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*aAge per 10 years.*
Table 2. Associations between continuous TL (in kbp) at baseline and the two-year course of depression (N = 1,023).

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biological age (TL)</td>
<td>Biological age (TL)</td>
<td>Biological age (TL)</td>
</tr>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Presence of any depressive disorder</td>
<td>0.98 0.80-1.21 .87</td>
<td>1.15 0.92-1.44 .24</td>
<td>1.26 0.99-1.60 .07</td>
</tr>
<tr>
<td>Presence of major depressive disorder</td>
<td>0.96 0.78-1.19 .73</td>
<td>1.11 0.88-1.39 .38</td>
<td>1.18 0.93-1.50 .18</td>
</tr>
<tr>
<td>Presence of dysthymia</td>
<td>0.85 0.66-1.10 .22</td>
<td>0.97 0.74-1.27 .82</td>
<td>1.15 0.86-1.54 .35</td>
</tr>
<tr>
<td>Chronic course</td>
<td>0.92 0.70-1.21 .55</td>
<td>0.89 0.68-1.16 .39</td>
<td>1.12 0.83-1.52 .45</td>
</tr>
<tr>
<td>Chronic course</td>
<td>1.14 0.96-1.36 .13</td>
<td>1.06 0.92-1.23 .41</td>
<td>0.97 0.83-1.14 .72</td>
</tr>
<tr>
<td>Time to remission</td>
<td>-1.37 0.59 .02</td>
<td>-0.83 0.59 .16</td>
<td>-0.01 0.63 .99</td>
</tr>
</tbody>
</table>

Model 1: adjusted for sex. Model 2: additionally adjusted for depression severity, anxiety severity, antidepressant use, cigarette years, number of alcoholic drinks per week, Body Mass Index, pain, and the number of chronic diseases. Model 3: additionally adjusted for chronological age (per 10 years). TL = telomere length, bp = base pairs.

Table 3. Means and percentages for two-year depression outcomes across telomere length (TL) tertiles, and linear associations with continuous TL.

<table>
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<th>Middle TL (5.06-5.52 kbp, n = 341)</th>
<th>Long TL (5.52-7.30 kbp, n = 341)</th>
<th>Total group (N = 1,023)</th>
<th>TL continuous B p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent depressive disorder, yes, %</td>
<td>47.5</td>
<td>46.6</td>
<td>46.3</td>
<td>46.8</td>
<td>-9.15</td>
</tr>
<tr>
<td>Major depressive disorder, yes, %</td>
<td>42.2</td>
<td>43.4</td>
<td>41.1</td>
<td>42.2</td>
<td>-12.60</td>
</tr>
<tr>
<td>Dysthymia, yes, %</td>
<td>25.8</td>
<td>21.4</td>
<td>22.3</td>
<td>23.2</td>
<td>-64.02</td>
</tr>
<tr>
<td>Chronic course, yes, %</td>
<td>31.0</td>
<td>30.0</td>
<td>24.8</td>
<td>28.6</td>
<td>-65.86</td>
</tr>
<tr>
<td>Remission, incidence rate/100 person-yrs</td>
<td>62.22</td>
<td>57.57</td>
<td>75.79</td>
<td>64.55</td>
<td>-</td>
</tr>
<tr>
<td>Depression severity change</td>
<td>-9.10 (11.99)</td>
<td>-10.10 (11.92)</td>
<td>-9.50 (10.60)</td>
<td>-9.57 (11.52)</td>
<td>-1.96</td>
</tr>
</tbody>
</table>
DISCUSSION
This study examined whether TL was associated with several aspects of the two-year course of MDD, but overall, failed to find such association. TL was found to be associated with a smaller decline in depression severity, but this association did not last when taking other prognostic factors and chronological age into account as well. For the other aspects (having a depression diagnosis, having a chronic course, and time to remission), we found no significant associations with TL. When looking at TL and chronological age simultaneously, for all two-year outcomes only chronological age was a significant predictor. Thus, although we expected that shorter TL could have been an explanatory factor for the worse depression course in older age, we found no convincing evidence to support this notion.

Longitudinally, evidence for associations between depression and anxiety and changes in TL has been inconclusive. Two longitudinal studies showed that those with psychopathology had shorter TL at baseline, but TL shortened at similar rates across groups with different course trajectories.\(^{25,43}\) One study did demonstrate changes in TL over time, although only in men,\(^ {26}\) but the current study adds to the evidence for a non-dynamic association between MDD and TL.

Most importantly, our findings demonstrate that, in line with clinical, social, and health factors, biological age as indexed by TL cannot explain the more unfavorable MDD course experienced by those with a higher chronological age. This implies that TL is of limited clinical use, but it also means more research is needed to elucidate which factors or mechanisms are responsible for the poorer MDD course in older persons.

Possibly, TL by itself does not fully represent biological age, and incorporating multiple markers of biological aging may provide more detailed insights. For example, future studies could also include mitochondrial DNA copy number (mtDNAcn), and DNA methylation age (DNAm), although previously depressive symptoms were found to be unrelated to mtDNAcn over a 10-year time period.\(^ {25}\) In addition, a better reflection of physiological stress system dysregulations, such as inflammation and HPA-axis functioning) could help explain whether overactivation or exhaustion of these biological systems could contribute to the observed age-association for the course of MDD.

Unique aspects of this study are its large sample size and wide age range, enabling us to study TL across the entire adult life span. In addition, to our knowledge this is the first study to examine whether TL was related to longitudinal changes in depression, rather than the other way around. Limitations of this study include the use of only one marker to determine biological age. Whereas leukocyte TL is a widely used measure, it has been shown that TL from different leukocytes (i.e. T-cells, B-cells, and monocytes) has different attrition rates.\(^ {44}\) In the current study, we used TL from whole blood so some variation may exist due to the different cells included. In addition, TL is not limited to leukocytes and could be observed in other somatic tissues as well. Nevertheless, Daniali et al.\(^ {45}\) found leukocyte TL to be correlated with TL from skeletal muscle, skin, and subcutaneous fat, which suggests that our results are not compromised as a result of using leukocyte TL. Another limitation of this study is attrition, as those who did not participate at two-year follow-up could be those with the
most severe consequences of shorter TL. Moreover, those suffering the most from short TL may not have participated in NESDA or NESDO in the first place.

Taken together, this study could not confirm an association between accelerated biological aging and the course of MDD, leaving us with the challenge to unravel which other factors are responsible for the worsening course of MDD with increasing chronological age.
REFERENCES


