

ORIGINAL ARTICLE

# Major depressive disorder and accelerated cellular aging: results from a large psychiatric cohort study

JE Verhoeven<sup>1</sup>, D Révész<sup>1</sup>, ES Epel<sup>2</sup>, J Lin<sup>3</sup>, OM Wolkowitz<sup>2</sup> and BWJH Penninx<sup>1</sup>

Patients with major depressive disorder (MDD) have an increased onset risk of aging-related somatic diseases such as heart disease, 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) was assessed as the telomere sequence copy number (T) compared to a single-copy gene copy number (S) using quantitative polymerase chain reaction. This resulted in a T/S ratio and was converted to base pairs (bp). MDD diagnosis and MDD characteristics were determined by self-report questionnaires and structured psychiatric interviews. Compared with control subjects (mean bp = 5541), sociodemographic-adjusted TL was shorter among remitted MDD patients (mean bp = 5459;  $P = 0.014$ ) and current MDD patients (mean bp = 5461;  $P = 0.012$ ). Adjustment for health and lifestyle variables did not reduce the associations. Within the current MDD patients, separate analyses showed that both higher depression severity ( $P < 0.01$ ) and longer symptom duration in the past 4 years ( $P = 0.01$ ) were associated with shorter TL. Our results demonstrate that depressed patients show accelerated cellular aging according to a 'dose-response' gradient: those with the most severe and chronic MDD showed the shortest TL. We also confirmed the imprint of past exposure to depression, as those with remitted MDD had shorter TL than controls.

*Molecular Psychiatry* (2014) **19**, 895–901; doi:10.1038/mp.2013.151; published online 12 November 2013

**Keywords:** cell aging; depression; mood disorders; telomere shortening

While major depressive disorder (MDD) is commonly known for its affective symptomatology, the disorder is increasingly recognized for the association with impaired somatic health. Depressed individuals evidently show an increased risk of developing various aging-related somatic diseases, such as coronary heart disease,<sup>1</sup> type 2 diabetes,<sup>2</sup> obesity,<sup>3</sup> dementia<sup>4</sup> and cancer.<sup>5</sup> Moreover, MDD enhances subsequent decline in physical<sup>6</sup> and cognitive functioning<sup>4</sup> and increases the overall mortality risk.<sup>7,8</sup> These associations may partly be explained by unhealthy lifestyle behaviors among the depressed, such as smoking, alcohol use and physical inactivity. However, several studies have found independent effects of depression,<sup>2,3,7</sup> which suggests that underlying biological processes are involved as well.

The increase in aging-related somatic conditions has been hypothesized to be a consequence of accelerated biological aging in the depressed population (for example, Wolkowitz *et al.*<sup>9,10</sup>). This process is thought to occur at the cellular level, more specifically, at the level of telomeres. Telomeres are specialized nucleic acid-protein complexes that cap the ends of linear DNA and protect DNA from damage. Due to the 'end-replication problem', the final part of the telomere fails to be replicated during every cell division, causing telomeres to become progressively shorter. When telomeres reach a critically short length, cells become susceptible to senescence or apoptosis.<sup>11</sup> Dysregulation

of immune and metabolic stress systems might contribute to telomere shortening by increasing the oxidative stress, which in turn damages the telomeres.<sup>9,12</sup> In contrast, telomere shortening can be counteracted by telomerase, a ribonucleoprotein enzyme that elongates telomeres by adding nucleotides to the end of chromosomes.<sup>13,14</sup> Shorter telomere length (TL) has been linked to the development of various aging-related diseases such as cardiovascular disease,<sup>15</sup> obesity,<sup>16</sup> diabetes,<sup>17</sup> cancer<sup>18</sup> and cognitive decline,<sup>19</sup> as well as to earlier mortality.<sup>20</sup>

Recently, some studies have associated shortened telomeres with MDD.<sup>21–26</sup> Simon *et al.*<sup>21</sup> were the first to find such an association: they found that 15 MDD and 29 bipolar patients had shorter TL compared with 44 non-depressed subjects. This association was replicated by Lung *et al.*<sup>22</sup> and Hartmann *et al.*<sup>23</sup> but only partially by Wolkowitz *et al.*<sup>25</sup> Also, in somatically diseased patients<sup>24</sup> and in an older sample,<sup>26</sup> MDD was found to be linked to shorter TL. Suggesting a 'dose-response' association, Wolkowitz *et al.*<sup>25</sup> found that TL was inversely correlated with lifetime depression duration, but two other studies could not confirm this link for chronicity or severity.<sup>23,26</sup> All prior studies, however, involved a specific somatic sample,<sup>24</sup> an older sample<sup>24,26</sup> or a small study sample ( $n < 100$ )<sup>21,23,25,26</sup> that did not allow to fully control for influential confounders such as smoking, alcohol use, BMI and physical activity. Consequently,

<sup>1</sup>Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Centre, Amsterdam, The Netherlands; <sup>2</sup>Department of Psychiatry, University of California, San Francisco, School of Medicine, San Francisco, CA, USA and <sup>3</sup>Telome Health Inc., Menlo Park, CA, USA. Correspondence: JE Verhoeven, MSc, Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center, AJ Ernststraat 1187, Room M1.05, PO Box 74077, 1070 BB Amsterdam, 1081 HL Amsterdam, The Netherlands.

E-mail: J.Verhoeven@ggzingeest.nl

Received 30 May 2013; revised 12 September 2013; accepted 16 September 2013; published online 12 November 2013

prior study findings have an unknown generalizability to the population of adults with current or remitted MDD. Considering the conflicting results and limitations discussed above, it remains unclear whether MDD patients indeed show a pattern of accelerated biological aging that might account for the decreased somatic health observed in this patient group.

In this study, we examined whether TL was associated with MDD status in a large adult sample ( $N=2407$ ) including persons with current and remitted MDD and healthy controls. Subjects were recruited from various settings and with different disease stages, thereby largely reflecting the MDD patient population. Our study sample had a broad age range and well-characterized psychiatric diagnoses, and we were able to control for the most important confounding variables. In addition, we examined whether this association depended on specific depression characteristics such as severity, symptom duration, age of onset, childhood trauma, comorbid anxiety and psychoactive medication use.

## MATERIALS AND METHODS

### Study sample

Data are from the baseline assessment of the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study examining the course and consequences of depressive and anxiety disorders. The NESDA sample consists of 2981 persons between 18 and 65 years, including persons with a current or remitted diagnosis of depression and/or anxiety disorders (74%) and healthy controls (26%). To represent various settings and stages of psychopathology, depressed and anxious participants were recruited at three different locations in the Netherlands in different settings: the community, primary care and specialized mental health-care settings. Persons with insufficient command of the Dutch language or a primary clinical diagnosis of other severe psychiatric conditions, such as bipolar disorder, obsessive-compulsive disorder, severe substance use disorder or psychotic disorder, as reported by themselves or their mental health practitioner, were excluded. Participants were recruited between September 2004 and February 2007. The study was approved by the ethical review board of the participating centers, and all participants signed informed consent. Participants in NESDA were assessed during a 4-h clinic visit. The population and methods of the NESDA study have been described in more detail elsewhere.<sup>27</sup>

For the current study, three groups were created: control subjects, persons with remitted MDD and persons with current MDD, leaving out participants that did not meet the criteria of one of three groups ( $N=537$ ). A total of 37 participants were subsequently excluded from analyses because of missing TL data, leaving 2407 individuals. Control subjects ( $N=510$ ) were defined as having no lifetime history of depressive or anxiety disorders as assessed by the *DSM-IV Composite International Diagnostic Interview (CIDI)* version 2.1, and a depression severity score below 14 on the Inventory of Depressive Symptoms.<sup>28</sup> Persons with remitted MDD ( $N=802$ ) had a lifetime history of MDD but no MDD diagnosis in the past 6 months as diagnosed with the CIDI, and current MDD patients ( $N=1095$ ) had CIDI-diagnosed MDD in the past 6 months.

### Measurements

**Telomere length.** Fasting blood was drawn from participants in the morning between 0830 and 0930 hours and DNA samples were stored in a  $-80^{\circ}\text{C}$  freezer afterwards. Leukocyte TL was determined at the laboratory of Telome Health Inc. (Menlo Park, CA, USA), using quantitative polymerase chain reaction, adapted from the published original method by Cawthon.<sup>29</sup> Telomere sequence copy number in each patient's sample (T) was compared with a single-copy gene copy number (S), relative to a reference sample. The resulting T/S ratio is proportional to the mean TL.<sup>29,30</sup> A more detailed report on TL measurement is described in Supplementary 1.

To compare T/S ratios with the telomere restriction fragments reported by other studies using southern blot analysis, we used the following steps to derive a conversion formula. Published work from the Blackburn lab at UCSF used the formula base pairs (bp) =  $3274.2413 \times \text{T/S}$  based on comparison of T/S ratios and telomere restriction fragment analysis of a series of genomic DNA samples from the human fibroblast cell line IMR90.<sup>31</sup> Comparison of the T/S ratios of eight quality control DNA samples (see Supplementary 1) from the Telome Health lab that were included on each

PCR run generated the following formula:  $\text{T/S}_{(\text{UCSF})} = (\text{T/S}_{(\text{Telome Health})} - 0.0545)/1.16$ . Therefore the final formula we used to convert T/S ratios to bp is:  $\text{bp} = 3274.2413 \times ((\text{T/S} - 0.0545)/1.16)$ . 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 ( $\text{CV} = 5.1\%$ ), and the inter-assay CV was also sufficiently low ( $\text{CV} = 4.6\%$ ).

**Depression characteristics.** Severity of symptoms in the past week was assessed by the 30-item Inventory of Depressive Symptoms—Self Report.<sup>28</sup> Overall scores ranged from 0 to 84 and were classified as: 0–13 = normal, 14–25 = mild depression, 26–38 = moderate depression, 39–48 = severe depression and 49–84 = very severe depression.<sup>32</sup> Depression duration in recent years was assessed by the Life Chart interview,<sup>33</sup> which uses a calendar method to assess the number of months in which depressive symptoms were present during the past 4 years. Current (6-month recency) comorbid anxiety disorder (panic disorder, generalized anxiety disorder, agoraphobia, social phobia) and alcohol dependence were assessed with the CIDI. The MDD age of onset was also assessed with the CIDI. To examine the role of childhood trauma, a cumulative childhood trauma index was constructed, using the NEMESIS childhood trauma interview.<sup>34</sup> In this interview, participants were asked whether they were emotionally neglected, psychologically abused, physically abused or sexually abused before age 16 years. The childhood trauma index reports the sum of the categories, which were scored from 0 to 2 (0: never happened; 1: sometimes; 2: happened regularly), resulting in an index score between 0 and 8. Evidence for the construct validity of the CTI has been collected in numerous studies showing that CTI scales are related to the prevalence, incidence and course of psychiatric disorder.<sup>35–37</sup> Currently used psychoactive medication was categorized using the World Health Organization Anatomical Therapeutic Chemical (ATC) classification<sup>38</sup> into antidepressants (tricyclic antidepressants (ATC code N06AA), selective serotonin reuptake inhibitors (ATC code N06AB) and other antidepressants (ATC codes N06AF, N06AG, N06AX)) and benzodiazepines (ATC codes N03Ae, N05BA, N05CD, N05CF).

**Covariates.** Gender, age and years of education were assessed during the interview. BMI was calculated as measured weight divided by squared length and divided into underweight ( $< 18.5$ ), normal ( $18.5\text{--}24.9$ ), overweight ( $25.0\text{--}30.0$ ) and obese ( $> 30.0$ ). Alcohol consumption was categorized as non-drinker (0 drinks), moderate drinker (female  $< 14$  and male  $< 21$  drinks per week) or heavy drinker (female  $\geq 14$  and male  $\geq 21$  drinks per week). BMI and alcohol consumption were added as categorical covariates because they were not linearly associated with TL. Smoking status was categorized into current, former or never smoker. Physical activity was assessed using the International Physical Activity Questionnaire<sup>39</sup> and expressed as overall energy expenditure in Metabolic Equivalent Total (MET)-minutes per week (MET level \* minutes of activity \* events per week), see the Ainsworth *et al.* Compendium.<sup>40</sup> The number of current somatic diseases (that is, heart disease, epilepsy, diabetes, osteoarthritis, stroke, cancer, chronic lung disease, thyroid disease, liver disease, intestinal disorders and ulcers) was assessed during the interview and a disease was regarded as present only if participants received medical treatment.

### Statistical analyses

Baseline characteristics were compared across depression status (controls, remitted and current MDD) using chi-square and analyses of variance. We used analyses of covariance to determine differences in TL in the remitted and current MDD groups compared with the control group, controlling for all covariates. For significant results, Cohen's *d*, defined as the difference in the means of two groups, divided by the pooled standard deviation of these groups, was determined as an effect size estimation. Covariate-adjusted multiple linear regression analyses were used to analyse the association of depression characteristics with TL in the current MDD sample. All analyses were conducted using SPSS version 20 (IBM Corp., Armonk, NY, USA).

## RESULTS

The mean age of the study sample ( $N=2407$ ) was 41.6 years ( $\text{s.d.} = 12.9$ , range 18–65) and 66.8% were female. Characteristics across the three groups are presented in Table 1. The remitted MDD group was slightly older, while the control group included

**Table 1.** Sample characteristics by major depressive disorder (MDD) status

	Control subjects (N = 510)	Remitted MDD (N = 802)	Current MDD (N = 1095)	P-value <sup>a</sup>
<i>Demographics</i>				
Age (mean ± s.d.)	40.5 (14.9)	43.5 (12.5)	40.7 (12.1)	< 0.001
Sex (% female)	60.2	70.3	67.4	0.001
Years of education (mean ± s.d.)	13.1 (3.2)	12.4 (3.2)	11.6 (3.2)	< 0.001
<i>Lifestyle and health</i>				
Body mass index (%)				
Underweight	2.0	1.1	2.8	0.001
Normal	54.5	49.5	48.4	
Overweight	30.0	33.4	28.4	
Obese	13.5	16.0	20.4	
Smoking status (%)				
Never	38.4	23.3	26.7	< 0.001
Former	35.6	26.9	27.8	
Current	26.1	39.8	45.6	
Alcohol status (%)				
Non-drinker	10.0	14.8	21.5	< 0.001
Modest drinker	78.2	72.8	65.0	
Heavy drinker	11.8	12.3	13.5	
Physical activity (in 1000 MET-minutes per week) (mean ± s.d.)	3.8 (3.0)	3.8 (3.0)	3.3 (3.1)	0.039
Number of somatic diseases (%)				
0	67.3	57.5	53.8	< 0.001
1	24.7	28.2	30.4	
2+	8.0	14.3	15.8	
<i>Psychiatric characteristics</i>				
Severity (IDS score) (mean ± s.d.)	5.4 (3.6)	18.0 (10.2)	32.6 (12.2)	< 0.001
Symptom duration (months with symptoms in the past 4 years) (mean ± s.d.)	NA	10.7 (11.0)	20.9 (16.9)	< 0.001
Age of onset (years) (mean ± s.d.)	NA	28.4 (12.1)	27.1 (12.4)	0.025
Comorbid anxiety disorder (%)	NA	36.9	65.7	< 0.001
Comorbid alcohol dependence disorder (%)	5.3	16.6	20.8	< 0.001
Childhood trauma index score (mean ± s.d.)	0.29 (0.68)	0.98 (1.15)	1.22 (1.22)	< 0.001
Antidepressant use (%)				
TCA	0.0	2.9	4.1	< 0.001
SSRI	0.4	16.4	29.6	< 0.001
Other antidepressant	0.0	3.7	11.0	< 0.001
Benzodiazepine use (%)	0.4	4.4	14.6	< 0.001
<i>Telomere length</i>				
T/S ratio (mean ± s.d.)	1.15 (0.31)	1.09 (0.30)	1.11 (0.30)	0.003
Base pairs (mean ± s.d.)	5553 (648)	5433 (617)	5474 (619)	0.003

Abbreviations: IDS, Inventory of Depressive Symptoms; MET-minutes, metabolic equivalent of number of calories spent per minute; NA, not applicable; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. <sup>a</sup>P-value based on one-way analysis of variance for continuous variables and  $\chi^2$  test for categorical variables.

fewer women. The three groups differed on all lifestyle and health variables, with the current and remitted groups being more frequently current smokers and having more somatic diseases than the control group. Furthermore, the current MDD patients were more often underweight or obese, non-drinkers or heavy drinkers, and had less physical activity and more somatic diseases than the control group. The groups also differed on all depression characteristics, with the current MDD group showing the most severe characteristics.

Average TL, which was normally distributed, in the entire sample was 5477 bp (s.d. = 626). TL exhibited a significant negative correlation with age ( $r = -0.326$ ,  $P < 0.001$ ), which corresponded to a shortening rate of 14 bp per year. This shortening rate is comparable to previously reported rates based on cross-sectional data (14 bp/year by Cawthon *et al.*,<sup>20</sup> 20 bp per year by Hartmann *et al.*<sup>23</sup> and 15–17 bp per year by Wigkren *et al.*<sup>26</sup>). Female subjects had longer TL than male subjects ( $F = 14.23$ ;  $P < 0.001$ , corrected for age). TL was, besides age and gender, associated with weight (shorter TL for being underweight, overweight or obese), smoking and drinking status (former and current smokers as well as heavy

drinkers have shorter TL) and number of somatic diseases (more disease, shorter TL), but not with education and physical activity.

Compared with healthy controls (mean bp = 5541), TL was significantly shorter among remitted MDD patients (bp = 5459;  $P = 0.014$ ) and current MDD patients (bp = 5961;  $P = 0.012$ ), adjusted for age, gender and education. Differences remained significant in analyses fully adjusted for health and lifestyle variables: the remitted MDD group had 83 bp shorter TL ( $P = 0.036$ ; Cohen's  $d = 0.12$ ) and the current MDD group had 84 bp shorter TL ( $P = 0.027$ ; Cohen's  $d = 0.12$ ) compared with controls (see Table 2). TL did not differ between current and remitted MDD patients ( $P = 0.974$ ). Within the remitted MDD group, the number of years that patients were in remission was not associated with TL ( $\beta = 0.038$ ;  $P = 0.326$ ). It should be noted, however, that the remitted MDD group still had a relatively high average depression severity score, and a considerable percentage had a comorbid anxiety disorder, as shown in Table 1.

Subsequently, the association between depression characteristics and TL was examined in the group of 1095 current MDD patients. Table 3 shows regression analyses for each characteristic

**Table 2.** Mean telomere length (with s.e.) in base pairs by MDD status in basic and full adjusted analyses

	Controls (N = 510)	Remitted MDD (N = 802)	Remitted MDD versus controls	Current MDD (N = 1095)	Current MDD versus controls
Basic adjustment <sup>a</sup>	5541 (26)	5459 (21)	$P=0.014$ Cohen's $d=0.14$	5461 (18)	$P=0.012$ Cohen's $d=0.13$
Full adjustment <sup>b</sup>	5534 (27)	5463 (21)	$P=0.036$ Cohen's $d=0.12$	5462 (18)	$P=0.027$ Cohen's $d=0.12$

Abbreviation: MDD, major depressive disorder. <sup>a</sup>Adjusted for age, sex, education. <sup>b</sup>Adjusted for age, sex, education, body mass index, smoking, alcohol use, physical activity and somatic diseases.

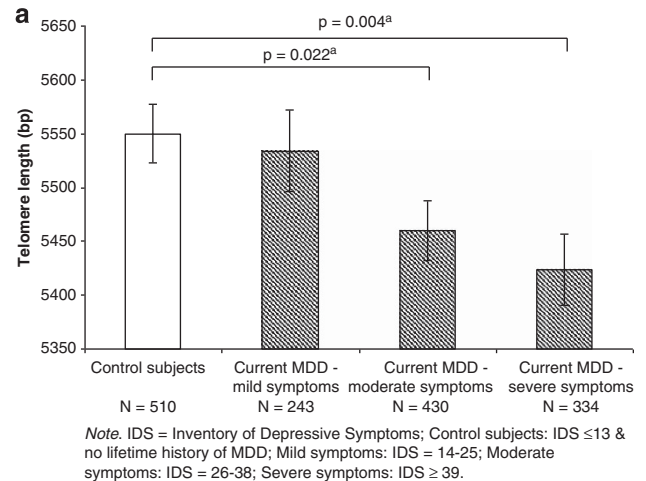
**Table 3.** Associations between telomere length and depression characteristics in the current MDD sample (N = 1095)

	$\beta$	P-value
Severity (IDS score)	-0.087	0.004
Duration (months with symptoms in the past 4 years)	-0.079	0.010
Age of onset (years)	-0.042	0.216
Comorbid anxiety disorder	-0.055	0.057
Comorbid alcohol dependence disorder	-0.042	0.092
Childhood trauma index score	-0.016	0.369
Antidepressant use		
TCA	0.007	0.708
SSRI	-0.021	0.251
Other antidepressant	-0.002	0.921
Benzodiazepine use	-0.003	0.849

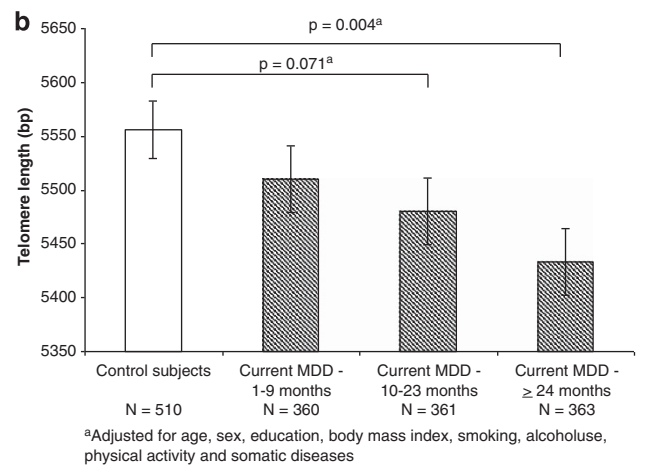
Abbreviations: IDS, Inventory of Depressive Symptoms; MDD, major depressive disorder; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. Analyses are adjusted for age, sex, education, body mass index, smoking, alcohol use, physical activity and somatic diseases.

separately. Higher current depression severity ( $\beta=-0.087$ ;  $P=0.004$ ) and longer symptom duration within the past 4 years ( $\beta=-0.079$ ;  $P=0.010$ ) were associated with shorter TL. The presence of comorbid anxiety ( $\beta=-0.055$ ;  $P=0.057$ ) and comorbid alcohol dependence disorder ( $\beta=-0.042$ ;  $P=0.092$ ) were borderline significantly associated with shorter TL. When these four variables were included in the adjusted linear regression model, depression severity remained significantly associated with TL ( $\beta=-0.074$ ;  $P=0.027$ ), while symptom duration ( $\beta=-0.027$ ;  $P=0.388$ ), comorbid anxiety ( $\beta=-0.036$ ;  $P=0.232$ ) and comorbid alcohol dependence disorder ( $\beta=-0.014$ ;  $P=0.660$ ) were not. No significant associations were found between TL and age of onset ( $P=0.216$ ), childhood trauma ( $P=0.369$ ) or psychoactive medication use.

To graphically illustrate the observed associations between depression severity and symptom duration with TL and explore whether these associations reflect linear trends, we plotted fully adjusted mean TL levels across different depression severity and symptom duration categories. MDD patients were divided into three severity groups: mild symptoms (IDS = 14–25), moderate symptoms (IDS = 26–38) and severe symptoms (IDS  $\geq 39$ ), and were compared with the control group (see Figure 1a). Analyses showed a gradient of risk, with the moderate ( $P=0.022$ ; Cohen's  $d=0.16$ ) and severely ( $P=0.004$ ; Cohen's  $d=0.21$ ) depressed patients having the shortest TL. Figure 1b shows the number of months with depressive symptoms in the past 4 years divided into tertiles creating the following groups: 1–9, 10–23 and  $\geq 24$  months. We found that the latter group had shortened TL compared with the controls ( $P=0.004$ ; Cohen's  $d=0.21$ ).



Note. IDS = Inventory of Depressive Symptoms; Control subjects: IDS  $\leq 13$  & no lifetime history of MDD; Mild symptoms: IDS = 14–25; Moderate symptoms: IDS = 26–38; Severe symptoms: IDS  $\geq 39$ .



<sup>a</sup>Adjusted for age, sex, education, body mass index, smoking, alcohol use, physical activity and somatic diseases

**Figure 1.** Mean telomere length (with s.e.) in base pairs for control subjects and (a) current MDD cases by severity (Inventory of Depressive Symptoms (IDS) scores) and (b) current MDD cases by depression symptom duration (number of months with symptoms in the past 4 years).

## DISCUSSION

In this large cohort study we demonstrated that currently depressed persons had shorter TL than never-depressed controls. Based on an estimated mean telomere shortening rate of 14–20 bp per year as found in this and other studies,<sup>20,23,26</sup> the differences observed indicate 4–6 years of accelerated aging for the current MDD sample as compared to controls. We also showed evidence for the imprint of past exposure to depression since those with remitted MDD also had shorter TL than control subjects. These observed associations remained significant after controlling for lifestyle and somatic health variables, suggesting

that the shortened telomeres were not simply due to unhealthy lifestyle or poorer somatic health among depressed persons. Finally, the association between MDD and TL showed a 'dose-response' gradient, since the most severely and chronically depressed patients had the shortest telomeres. Although the associations between MDD status and TL were of rather small effect size, our found effect sizes (Cohen's  $d=0.12$ – $0.21$ ) are not very different from those described in recent meta-analyses for MDD associations with pathophysiological mechanisms such as increased inflammatory markers (Cohen's  $d=0.15$ – $0.35$ )<sup>41</sup>, decreased brain-derived neurotrophic factor (Cohen's  $d=0.15$ – $0.23$ )<sup>42</sup> and increased cortisol (Cohen's  $d=0.15$ – $0.25$ )<sup>43</sup>. This might be largely due to the heterogeneity of MDD. Overall, this study provides convincing evidence for the suggestion that an emotional stressful condition, such as MDD, may truly impact on the physical 'wear and tear' of a person's body resulting in accelerated biological aging.

Our findings are in line with previous findings in smaller study samples or specific somatic patient groups.<sup>21–24,26</sup> The large and generalizable sample in this study provides confirmatory evidence of accelerated cellular aging in MDD patients, because of the heterogeneous MDD patients, recruited from different clinical settings. Despite earlier conflicting studies,<sup>23,25,26</sup> our study convincingly showed a 'dose-response' association within the current MDD sample, with the shortest TLs observed among MDD patients with the most severe and chronic symptoms, which is further suggestive of an underlying causal association. The most severely depressed group as well as the group with more than 24 months with depressive symptoms over the last 4 years showed 7–10 years of accelerated aging compared with healthy controls, again based on the estimated 14–20 bp per year shortening rate. It should be noted that our duration variable did not reflect the lifetime duration of MDD. Although only borderline significant, both a comorbid anxiety disorder and alcohol dependence disorder did seem to increase the chance of having shorter telomeres, as current MDD patients with a comorbid disorder had shorter TL than patients without comorbidity. This is also consistent with a 'dose-response' relationship, as comorbidity can be seen as a psychiatrically more severe condition. Age of depression onset and childhood trauma were not related to TL within the current MDD group, indicating that when a person is depressed, early-life situations did not further differentiate in cellular aging. This does not suggest that early adverse life events are not an important factor in cellular aging, as our analyses were restricted to MDD patients only, and it is well possible that the impact of adverse life events on cellular aging (as reviewed by Price *et al.*<sup>44</sup>) is mediated by depressive disorders, this remains to be explored. We also did not find an association between current psychoactive medication use and TL, similar to the results of other studies,<sup>23,26</sup> but more specific relationships between duration, type and dose of medication remain to be explored. Interestingly, we found no difference in TL between current and remitted MDD patients, and also no relationship between the duration of remission and TL. This suggests that MDD is a disorder with a chronic course, possibly leaving a biological scar after each episode. Alternatively, shorter TL may be the consequence of the considerable subthreshold depressive symptomatology or comorbid anxiety disorders among the remitted MDD patients in the sample.

MDD is thus associated with shortened TL, which resembles accelerated biological aging. The disorder has previously also been associated with dysregulations of the hypothalamus–pituitary–adrenal (HPA) axis,<sup>43,45</sup> the immune system,<sup>46,47</sup> the autonomic nervous system (ANS)<sup>48,49</sup> and increased oxidative stress.<sup>50</sup> Shortened telomeres, in turn, are suggested to be a consequence or a concomitant of these dysregulated biological stress systems. In line with this, several *in vitro* and *in vivo* studies found increased cortisol,<sup>51</sup> oxidative stress<sup>52</sup> and pro-inflammatory cytokines<sup>53</sup> to be associated with shorter TL. Dysregulations of these stress

systems could contribute to telomere shortening in MDD patients.<sup>9,12</sup> However, the exact biological mechanisms that mediate the relation between depression and telomere shortening, as well as the direction of the link, remain to be further explored.

The major strengths of the present study are the large sample size, including well-characterized current and remitted MDD individuals as well as healthy controls, the wide age range and the assessment of important covariates such as health and lifestyle variables. These strengths allowed us to thoroughly examine the relation between TL and MDD, thereby overcoming the limitations of previous studies. However, some limitations of the present study should also be noted. Our study design had a cross-sectional nature, which might undermine the complexity of TL-regulating mechanisms and does not allow us to draw conclusions regarding causality. As longitudinal studies show complex TL dynamics with both shortening as well as lengthening of telomeres over time,<sup>24,54–56</sup> future studies should explore the relationship longitudinally. It should also be noted that our variable of 'years of aging' is an estimate rather than a directly measured variable. Next, as in nearly all studies we used leukocytes for TL measurement, which is a validated, non-invasive and an often used indicator for cellular aging. It would, however, be worthwhile to examine cellular aging processes in other tissues such as the brain. Two studies on depression and TL in the occipital cortex<sup>57</sup> and cerebellar gray matter<sup>58</sup> failed to find an association. This discrepancy with peripheral studies might be explained by the fact that leukocyte TL is not a direct reflection of TL in most brain tissues as mature neurons are non-mitotic. However, an animal model<sup>59</sup> suggests that some brain parts such as cells in the dentate gyrus of the hippocampus that do undergo mitosis are susceptible to changes in telomerase activity, which is a promising topic for future research. Independently of parallels with brain tissues, the findings of accelerated cell aging in the periphery significantly contribute to understanding the increased risk of medical morbidity and mortality in MDD, as it has become a central assumption that MDD is not merely a disease restricted to the brain but is also associated with dysregulated peripheral stress systems. Last, telomerase activity has not been measured in the current study, but information regarding telomere repair and maintenance would be of great value in future research. It is extremely difficult to separate out the effects of stress from depression in research because stress arousal is inherent in MDD. However, telomerase might help to disambiguate the effects of stress from MDD, as its activity was found to be decreased in a chronically stressed sample<sup>60</sup> while it was increased in the presence of a MDD diagnosis.<sup>53,61</sup>

This large-scale study provides convincing evidence that depression is associated with several years of biological aging, especially among those with the most severe and chronic symptoms. An important question remains whether this aging process can be reversed, and whether this would impact on depression. In other research areas, lifestyle interventions have shown to favorably impact on cellular aging.<sup>62–65</sup> It needs to be tested whether these may be fruitful interventions in MDD patients, resulting not only in a reversal of depression symptomatology but also in restoring biological aging and consequent somatic health.

## CONFLICT OF INTEREST

EE is a co-founder of Telome Health, Inc, a telomere measurement company. JL is an Associate Research Biochemist in the Department of Biochemistry and Biophysics at UCSF. The other authors declare no conflict of interest.

## ACKNOWLEDGMENTS

The infrastructure for the NESDA study ([www.nesda.nl](http://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 supported by participating universities and mental health-care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Institute for Quality of Health Care (IQ Healthcare), Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos)). BP, JV, DR and telomere length assaying were supported through a NWO-VICI grant (number 91811602).

## REFERENCES

- 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.
- Mezuk B, Eaton WW, Albrecht S, Golden SH. Depression and type 2 diabetes over the lifespan: a meta-analysis. *Diabetes Care* 2008; **31**: 2383–2390.
- Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW *et al*. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry* 2010; **67**: 220–229.
- Gao Y, Huang C, Zhao K, Ma L, Qiu X, Zhang L *et al*. Depression as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. *Int J Geriatr Psychiatry* 2012; **28**: 441–449.
- Chida Y, Hamer M, Wardle J, Steptoe A. Do stress-related psychosocial factors contribute to cancer incidence and survival? *Nat Clin Pract Oncol* 2008; **5**: 466–475.
- Penninx BW, Guralnik JM, Ferrucci L, Simonsick EM, Deeg DJ, Wallace RB. Depressive symptoms and physical decline in community-dwelling older persons. *JAMA* 1998; **279**: 1720–1726.
- Schulz R, Beach SR, Ives DG, Martire LM, Ariyo AA, Kop WJ. Association between depression and mortality in older adults: the Cardiovascular Health Study. *Arch Intern Med* 2000; **160**: 1761–1768.
- Cuijpers P, Smit F. Excess mortality in depression: a meta-analysis of community studies. *J Affect Disord* 2002; **72**: 227–236.
- Wolkowitz OM, Epel ES, Reus VI, Mellon SH. Depression gets old fast: do stress and depression accelerate cell aging? *Depress Anxiety* 2010; **27**: 327–338.
- Wolkowitz OM, Reus VI, Mellon SH. Of sound mind and body: depression, disease, and accelerated aging. *Dialogues Clin Neurosci* 2011; **13**: 25–39.
- Blackburn EH. Switching and signaling at the telomere. *Cell* 2001; **106**: 661–673.
- Epel ES. Psychological and metabolic stress: a recipe for accelerated cellular aging? *Hormones (Athens)* 2009; **8**: 7–22.
- Olovnikov AM. Telomeres, telomerase, and aging: origin of the theory. *Exp Gerontol* 1996; **31**: 443–448.
- Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med* 2006; **12**: 1133–1138.
- Fitzpatrick AL, Kronmal RA, Gardner JP, Psaty BM, Jenny NS, Tracy RP *et al*. Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol* 2007; **165**: 14–21.
- Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF *et al*. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005; **366**: 662–664.
- Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care* 2006; **29**: 283–289.
- Willeit P, Willeit J, Mayr A, Weger S, Oberhollenzer F, Brandstätter A *et al*. Telomere length and risk of incident cancer and cancer mortality. *JAMA* 2010; **304**: 69–75.
- Martin-Ruiz C, Dickinson HO, Keys B, Rowan E, Kenny RA, von Zglinicki T. Telomere length predicts poststroke mortality, dementia, and cognitive decline. *Ann Neurol* 2006; **60**: 174–180.
- Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003; **361**: 393–395.
- 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.
- Lung FW, Chen NC, Shu BC. Genetic pathway of major depressive disorder in shortening telomeric length. *Psychiatr Genet* 2007; **17**: 195–199.
- Hartmann N, Boehner M, Groenen F, Kalb R. Telomere length of patients with major depression is shortened but independent from therapy and severity of the disease. *Depress Anxiety* 2010; **27**: 1111–1116.
- Hoen PW, de Jonge P, Na BY, Farzaneh-Far R, Epel E, Lin J *et al*. Depression and leukocyte telomere length in patients with coronary heart disease: data from the Heart and Soul Study. *Psychosom Med* 2011; **73**: 541–547.
- Wolkowitz OM, Mellon SH, Epel ES, Lin J, Dhabhar FS, Su Y *et al*. Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress—preliminary findings. *PLoS One* 2011; **6**: e17837.
- Wikgren M, Maripuu M, Karlsson T, Nordfjäll K, Bergdahl J, Hultdin J *et al*. Short telomeres in depression and the general population are associated with a hypocortisolemic state. *Biol Psychiatry* 2012; **71**: 294–300.
- 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 Psychiatry Res* 2008; **17**: 121–140.
- Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The Inventory of Depressive Symptomatology (IDS): psychometric properties. *Psychol Med* 1996; **26**: 477–486.
- Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002; **30**: e47.
- 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.
- 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.
- Rush AJ, Trivedi MH, Ibrahim HM, Carmody TJ, Arnow B, Klein DN *et al*. The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. *Biol Psychiatry* 2003; **54**: 573–583.
- Lyketsos CG, Nestadt G, Cwi J, Heithoff K. The life-chart interview: a standardized method to describe the course of psychopathology. *Int J Meth Psychiatr Res* 1994; **4**: 143–155.
- de Graaf R, Bijl RV, 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.
- Hovens JG, Wiersma JE, Giltay EJ, van Oppen P, Spinhoven P, Penninx BW *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.
- Wiersma JE, Hovens JG, van OP, Giltay EJ, van Schaik DJ, Beekman AT *et al*. The importance of childhood trauma and childhood life events for chronicity of depression in adults. *J Clin Psychiatry* 2009; **70**: 983–989.
- Spinhoven P, Elzinga BM, Hovens JG, Roelofs K, Zitman FG, van Oppen P *et al*. The specificity of childhood adversities and negative life events across the life span to anxiety and depressive disorders. *J Affect Disord* 2010; **126**: 103–112.
- World Health Organization Collaboration Centre for Drug Statistics Methodology. *Anatomical Therapeutic Chemical (ATC) Classification System*. World Health Organization Collaboration Centre for Drug Statistics Methodology: Oslo, Norway, 2007.
- Craig CL, Marshall AL, Sjoström 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.
- Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR Jr, Tudor-Locke C *et al*. 2011 Compendium of physical activities: a second update of codes and MET values. *Med Sci Sports Exerc* 2011; **43**: 1575–1581.
- 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.
- Molendijk M, Bus B, Oude Voshaar R, Spinhoven P, Penninx BW, Elzinga B. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state—trait issues, clinical features, and pharmacological treatment. *Mol Psychiatry* 2010; **16**: 1088–1095.
- Vreeburg SA, Hoogendijk WJ, van PJ, Derijk RH, Verhagen JC, 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.
- Price LH, Kao HT, Burgers DE, Carpenter LL, Tyrka AR. Telomeres and early-life stress: an overview. *Biol Psychiatry* 2013; **73**: 15–23.
- Penninx BW, Beekman AT, Bandinelli S, Corsi AM, Bremner M, Hoogendijk WJ *et al*. Late-life depressive symptoms are associated with both hyperactivity and hypoactivity of the hypothalamo-pituitary-adrenal axis. *Am J Geriatr Psychiatry* 2007; **15**: 522–529.
- Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK *et al*. A meta-analysis of cytokines in major depression. *Biol Psychiatry* 2010; **67**: 446–457.
- Vogelzangs N, Duijvis HE, Beekman ATF, Kluff C, Neuteboom J, Hoogendijk W *et al*. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. *Transl Psychiatry* 2012; **2**: e79.
- Rotenberg J. Cardiac vagal control in depression: a critical analysis. *Biol Psychol* 2007; **74**: 200–211.
- Licht CM, de Geus EJ, Zitman FG, Hoogendijk WJ, van DR, Penninx BW. 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.
- Michel TM, Pulschen D, Thome J. The role of oxidative stress in depressive disorders. *Curr Pharm Des* 2012; **18**: 5890–5899.

- 51 Choi J, Fauce SR, Effros RB. Reduced telomerase activity in human T lymphocytes exposed to cortisol. *Brain Behav Immun* 2008; **22**: 600–605.
- 52 von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002; **27**: 339–344.
- 53 Damjanovic AK, Yang Y, Glaser R, Kiecolt-Glaser JK, Nguyen H, Laskowski B *et al*. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. *J Immunol* 2007; **179**: 4249–4254.
- 54 Aviv A, Chen W, Gardner JP, Kimura M, Brimacombe M, Cao X *et al*. Leukocyte telomere dynamics: longitudinal findings among young adults in the Bogalusa Heart Study. *Am J Epidemiol* 2009; **169**: 323–329.
- 55 Nordfjall K, Svenson U, Norrback KF, Adolfsson R, Lenner P, Roos G. The individual blood cell telomere attrition rate is telomere length dependent. *PLoS Genet* 2009; **5**: e1000375.
- 56 Shalev I, Moffitt T, 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.
- 57 Teyssier JR, Ragot S, Donzel A, Chauvet-Gelinier JC. [Telomeres in the brain cortex of depressive patients]. *Encephale* 2010; **36**: 491–494.
- 58 Zhang D, Cheng L, Craig DW, Redman M, Liu C. Cerebellar telomere length and psychiatric disorders. *Behav Genet* 2010; **40**: 250–254.
- 59 Zhou QG, Hu Y, Wu DL, Zhu LJ, Chen C, Jin X *et al*. Hippocampal telomerase is involved in the modulation of depressive behaviors. *J Neurosci* 2011; **31**: 12258–12269.
- 60 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 USA* 2004; **101**: 17312–17315.
- 61 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.
- 62 Puterman E, Lin J, Blackburn E, O'Donovan A, Adler N, Epel E. The power of exercise: buffering the effect of chronic stress on telomere length. *PLoS One* 2010; **5**: e10837.
- 63 Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE *et al*. Physical activity, sedentary behavior, and leukocyte telomere length in women. *Am J Epidemiol* 2012; **175**: 414–422.
- 64 Lin J, Epel E, Blackburn E. Telomeres and lifestyle factors: roles in cellular aging. *Mutat Res* 2012; **730**: 85–89.
- 65 Osthus IB, Sgura A, Berardinelli F, Alsnes IV, Brønstad E, Rehn T *et al*. Telomere length and long-term endurance exercise: does exercise training affect biological age? A pilot study. *PLoS One* 2012; **7**: e52769.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)