Chapter 2

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

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ABSTRACT

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.

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

INTRODUCTION

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 (CHD) (1), type 2 diabetes (2), obesity (3), dementia (4) and cancer (5). Moreover, MDD enhances subsequent decline in physical (6) and cognitive functioning (4) and increases overall mortality risk (7,8). These associations may partly be explained by unhealthy lifestyle behaviors among the depressed, such as smoking, alcohol use and physical inactivity. However, several studies found independent effects of depression (2,3,7), 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 (e.g., Wolkowitz et al. (9,10)). 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 (11). Dysregulation of immune and metabolic stress systems might contribute to telomere shortening by increasing oxidative stress, which in turn damages telomeres (9,12). In contrast, telomere shortening can be counteracted by telomerase, a ribonucleoprotein enzyme that elongates telomeres by adding nucleotides to the end of chromosomes (13,14). Shorter telomere length (TL) has been linked to the development of various aging-related diseases such as cardiovascular disease (15), obesity (16), diabetes (17), cancer (18) and cognitive decline (19), as well as to earlier mortality (20).

Recently, some studies have associated shortened telomeres with MDD (21-26). Simon and colleagues (21) were the first to find such an association: they found that 15 MDD and 29 bipolar patients had shorter TL compared to 44 non-depressed subjects. This association was replicated by Lung et al. (22) and Hartmann et al. (23), but only partially by Wolkowitz et al. (25). Also, in somatically diseased patients (24) and in an older sample (26), MDD was found to be linked to shorter TL. Suggesting a "dose-response" association, Wolkowitz et al. (25) found that TL was inversely correlated with lifetime depression duration, but two other studies could not confirm this link for chronicity or severity (23,26). All prior studies, however, involved either a specific somatic sample (24), an older sample (24,26), or a small study sample (n<100) (21,23,25,26) that did not allow to fully control for influential confounders such as smoking, alcohol use, body mass index (BMI) and physical activity. Consequently, 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 participating centres, and all participants signed informed consent. Participants in NESDA were assessed during a 4-hour clinic visit. The population and methods of the NESDA study have been described in more detail elsewhere (27).

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 (28). 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 8:30 and 9:30 am and DNA samples were stored in a -20°C freezer afterwards. Leukocyte TL was determined at the laboratory of Telome Health Inc. (Menlo Park, USA), using quantitative polymerase chain reaction (qPCR), adapted from the published original method by Cawthon et al. (29). 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 TL (29,30). A more detailed report on TL measurement is described in Supplement 1.

To compare T/S ratios to telomere restriction fragments (TRF) 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 a formula of base pairs (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 (31). Comparison of the T/S ratios of 8 quality control DNA samples (see Supplement 1) from the Telome Health lab that were included on each PCR run, generated the following formula: $T/S_{(UCSF)}=(T/S_{(TelomeHealth)}-0.0545)/1.16$. Therefore the final formula we used to convert T/S ratios to bp is: bp=3274+2413x((T/S-0.0545)/1.16). The reliability of the assay was adequate: 8 included quality control DNA samples on each PCR run illustrated a small intra-assay coefficient of variation (CV=5.1%), and the inter-assay CV was also sufficiently low (CV=4.6%).

Depression characteristics. Severity of symptoms in the past week was assessed by the 30-item Inventory of Depressive Symptoms - Self Report (IDS-SR) (28). Overall scores range 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 (32). Depression duration in recent years was assessed by the Life Chart interview (LCI) (33), 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 (CTI) was constructed, using the NEMESIS childhood trauma interview (34). In this interview, participants were asked whether they were emotionally neglected,

psychologically abused, physically abused or sexually abused before age 16 years. The CTI 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 prevalence, incidence and course of psychiatric disorder (35-37). Currently used psychoactive medication was categorized using the World Health Organization Anatomical Therapeutic Chemical (ATC) classification (38) into antidepressants (tricyclic antidepressants [N06AA], selective serotonin reuptake inhibitors [N06AB], and other antidepressants [N06AF, N06AG, N06AX]) and benzodiazepines [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 (18.5–24.9), overweight (25.0–30.0) and obese (>30.0). Alcohol consumption was categorized as non-drinker (0 drinks), moderate drinker (female<14 and male<21 drinks/week) or heavy drinker (female≥14 and male≥21 drinks/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 (IPAQ) (39), 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 (40). The number of current somatic diseases (i.e. 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 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 ANOVAs. We used ANCOVAs to determine differences in TL in the remitted and current MDD groups compared to the control group, controlling for all covariates. For significant results, Cohen's d, defined as the difference in the means of 2 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 (SD=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 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 (SD=626). TL exhibited a significant negative correlation with age (r=-.326, p<.001), which corresponded to a shortening rate of 14 bp/year. This shortening rate is comparable to previously reported rates based on cross-sectional data (14 bp/year by Cawthon et al. (20), 20 bp/year by Hartmann et al. (23) and 15-17 bp/year by Wigkren et al. (26)). Female subjects had longer TL than male subjects (F=14.23; p<.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 to healthy controls (mean bp=5541), TL was significantly shorter among remitted MDD patients (bp=5459; p=.014) and current MDD patients (bp=5961; p=.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=.036; Cohen's d=0.12) and the current MDD group had 84 bp shorter TL (p=.027; Cohen's d=0.12) compared to controls (see Table 2). TL did not differ between current and remitted MDD patients (p=.974). Within the remitted MDD group, the number of years that patients were in remission was not associated with TL (β=.038; p=.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.

Table 1. Sample characteristics by Major Depressive Disorder (MDD) status

	Control subjects N = 510	Remitted MDD N = 802	Current MDD N = 1095	p-value [a]
Demographics				
Age (mean ± s.d.)	40.5 (14.9)	43.5 (12.5)	40.7 (12.1)	<.001
Sex (% female)	60.2	70.3	67.4	.001
Years of education (mean ± s.d.)	13.1 (3.2)	12.4 (3.2)	11.6 (3.2)	<.001
Lifestyle & health				
Body Mass Index (%)				
Underweight	2.0	1.1	2.8	.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	<.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	<.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)	.039

Number of somatic diseases (%)				
0	67.3	57.5	53.8	<.001
1	24.7	28.2	30.4	
2+	8.0	14.3	15.8	
Psychiatric characteristics				
Severity (Inventory of Depressive Symptoms) (mean ± s.d.)	5.4 (3.6)	18.0 (10.2)	32.6 (12.2)	<.001
Symptom duration (months with symptoms in past 4 years) (mean ± s.d.)	NA	10.7 (11.0)	20.9 (16.9)	<.001
Age of onset (year) (mean ± s.d.)	NA	28.4 (12.1)	27.1 (12.4)	.025
Comorbid anxiety disorder (%)	NA	36.9	65.7	<.001
Comorbid alcohol dependence disorder (%)	5.3	16.6	20.8	<.001
Childhood trauma (mean ± s.d.)	0.29 (0.68)	0.98 (1.15)	1.22 (1.22)	<.001
Antidepressant use (%)				
Tricyclic antidepressant	0.0	2.9	4.1	<.001
Selective serotonin reuptake inhibitor	0.4	16.4	29.6	<.001
Other antidepressant	0.0	3.7	11.0	<.001
Benzodiazepine use (%)	0.4	4.4	14.6	<.001
Telomere Length				
T/S ratio (mean ± s.d.)	1.15 (0.31)	1.09 (0.30)	1.11 (0.30)	.003
Base pairs (mean ± s.d.)	5553 (648)	5433 (617)	5474 (619)	.003

Abbreviations: MET-minutes = metabolic equivalent of number of calories spent per minute; NA = Not Applicable; s.d. = standard deviation

[a] p-value based on one-way ANOVA for continuous variables and Chi square test for categorical variables.

Table 2. Mean telomere length (with SE) 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 [a]	5541 (26)	5459 (21)	p = .014 Cohen's d = 0.14	5461 (18)	p = .012 Cohen's d = 0.13
Full adjustment [b]	5534 (27)	5463 (21)	p = .036 Cohen's d = 0.12	5462 (18)	p = .027 Cohen's d = 0.12

Abbreviations: MDD = Major Depressive Disorder; SE = Standard Error

[a] Adjusted for age, sex, education.

[b] Adjusted for age, sex, education, body mass index, smoking, alcohol use, physical activity and somatic diseases.

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 separately. Higher current depression severity (β=-.087; p=.004) and longer symptom duration within the past 4 years (β=-.079; p=.010) were associated with shorter TL. The presence of comorbid anxiety (β=-.055; p=.057) and comorbid alcohol dependence disorder (β=-.042; p=.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 (β=-.074; p=.027), while symptom duration (β=-.027; p=.388), comorbid anxiety (β=-.036; p=.232) and comorbid alcohol dependence disorder (β=-.014; p=.660) were not. No significant associations were found between TL and age-of-onset (p=.216), childhood trauma (p=.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≥39) and were compared to the control group (see Figure 1A). Analyses showed a gradient of risk with the moderate (p=.022; Cohen's d =0.16) and severely (p=.004; Cohen's d=0.21) depressed patients having the shortest TL. In figure 1B we showed the number of months with depressive symptoms in the past 4 years divided into tertiles creating the following groups: 1-9 months, 10-23 months and ≥24 months. We found that the latter group had shortened TL compared to the controls (p=.004; Cohen's d=0.21).

Table 3. Associations of telomere length with depression characteristics in current MDD sample (N=1095)

	β	p-value
Severity (Inventory of Depressive Symptoms score)	-.087	.004
Duration (months with symptoms in past 4 years)	-.079	.010
Age of onset (years)	-.042	.216
Comorbid anxiety disorder	-.055	.057
Comorbid alcohol dependence disorder	-.042	.092
Childhood trauma index score	-.016	.369
Antidepressant use		
Tricyclic antidepressant	.007	.708
Selective serotonin reuptake inhibitor	-.021	.251
Other antidepressant	-.002	.921
Benzodiazepine use	-.003	.849

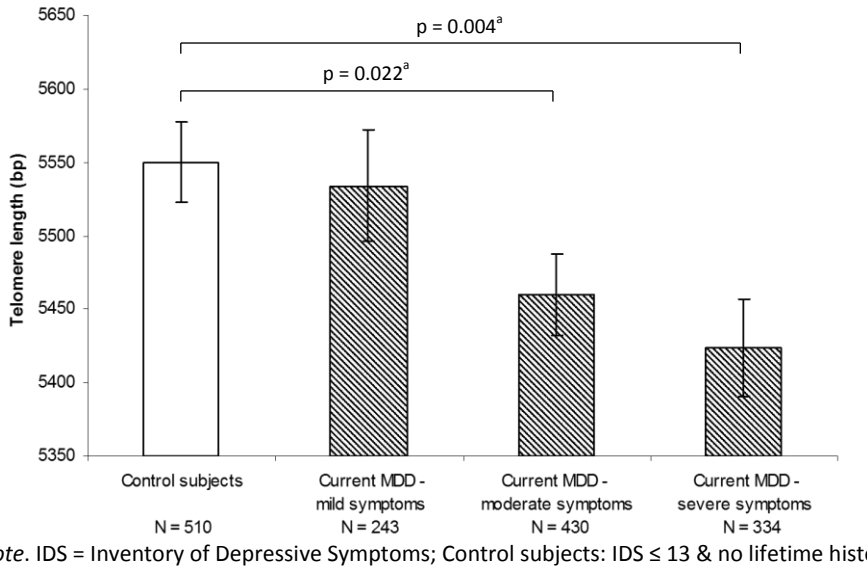
Abbreviation: MDD = Major Depressive Disorder.
Analyses are adjusted for age, sex, education, body mass index, smoking, alcohol use, physical activity and somatic diseases.

DISCUSISON

In this large cohort study we demonstrated that currently depressed persons had shorter telomere length (TL) than never-depressed controls. Based on an estimated mean telomere shortening rate of 14-20 bp/year as found in this and other studies (20,23,26), the differences observed indicate 4 to 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 telomere length 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) (41), decreased brain-derived neurotrophic factor (Cohen's d = 0.15-0.23) (42) and increased cortisol (Cohen's d = 0.15-0.25) (43). This might be largely due to the heterogeneity of MDD. Overall, this study provides convincing evidence for the suggestion that an emotional stressful condition,

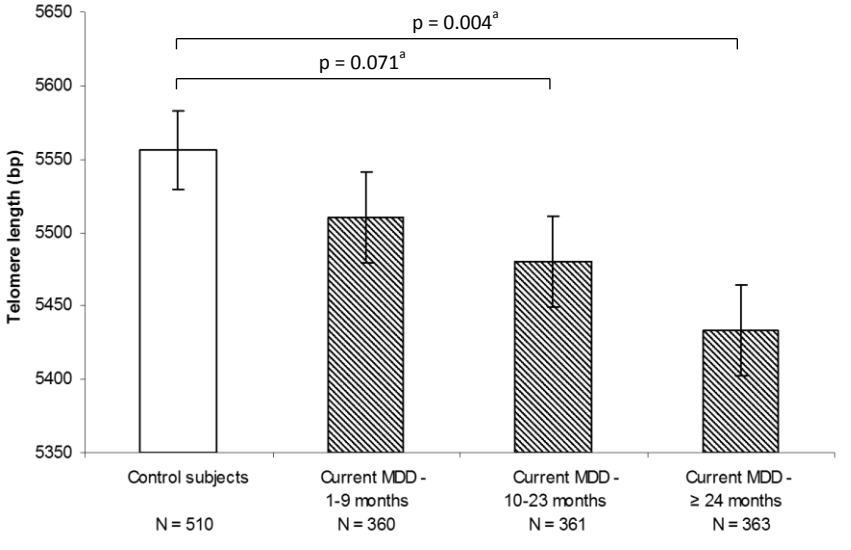
Figure 1. Mean telomere length (with SE) in base pairs for control subjects and (A) current MDD cases by severity (IDS scores) and (B) current MDD cases by depression symptom duration (number of months with symptoms in past 4 years)

A



Note. IDS = Inventory of Depressive Symptoms; Control subjects: IDS ≤ 13 & no lifetime history of MDD; Mild symptoms: IDS = 14-25; Moderate symptoms: IDS = 26-38; Severe symptoms: IDS ≥ 39.

B



[a] Adjusted for age, sex, education, body mass index, smoking, alcohol use, physical activity and somatic diseases

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 in specific somatic patient groups (21-24,26). 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 (23,25,26), our study convincingly showed a "dose-response" association within the current MDD sample, with the shortest TLs among MDD patients with the most severe and chronic symptoms, which is further suggestive of a 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 to 10 years of accelerated aging compared to healthy controls, again based on the estimated 14-20 bp/year shortening rate. It should be noted that our duration variable did not reflect 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, since 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, since 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. (44)) are 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 (23,26), 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 (43,45), the immune system (46,47), the autonomic nervous system (ANS) (48,49), and increased oxidative stress (50). 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 (51), oxidative stress (52), and pro-inflammatory cytokines (53) to be associated with shorter TL. Dysregulations of these stress systems could contribute to telomere shortening in MDD patients (9,12). 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 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. Since longitudinal studies show complex TL dynamics with both shortening as well as lengthening of telomeres over time (24,54-56), 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 (57) and cerrebellar gray matter (58) 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 (59) 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 effects of stress from MDD, as its activity was found decreased in a chronically stressed sample (60) while it was increased in the presence of a MDD diagnosis (53,61).

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 favourably impact on cellular aging (62-65). It needs to be tested whether these may be fruitful interventions in MDD patients, resulting not only in a reversal of depression symptomatology but in restoring biological aging and consequent somatic health.

SUPPLEMENT 1

Supplemental report on Telomere Length (TL) measurement

Leukocyte TL was determined at the laboratory of Telome Health Inc. (Menlo Park, CA, USA), using quantitative polymerase chain reaction (qPCR), adapted from the published original method by Cawthon et al. (29). 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 TL (29,30).

Primers for the telomere sequence copy gene (T runs) were tel1b [5'-CGGTTT $(GTTTGG)_5$GTT-3'], used at a final concentration of 100 nM, and tel2b [5'-GGCTTG $(CCTTAC)_5$CCT-3'], used at a final concentration of 900 nM. The primers for the single-copy gene (human beta-globin) qPCR (S runs) were hbg1 [5' GCTTCTGACACAACTGTGTTCACT AGC-3'], used at a final concentration of 300 nM, and hbg2 [5'-CACCAACTTCATCCACGTT CACC-3'], used at a final concentration of 700 nM. The final reaction mix contained 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 200 µM each dNTP; 1% DMSO; 0.5x SRBR Green I; 16.5 ng E. coli DNA per reaction; 0.4 Units of Platinum Taq DNA polymerase per 11 µL reaction.

Genomic DNA from pooled 100 male donors (Mosaic human gDNA, M, from Aldevron, cat# 5080-25) was used as the standard reference. This reference DNA was included in each PCR run so that the quantity of targeted templates in each research sample could be determined relative to the reference DNA sample by the standard curve method. All PCRs were carried out on a Roche Lightcycler 480 realtime PCR machine with 384-well capacity (Roche Diagnostics Corporation, Indianapolis, IN).

The thermal cycling profiles are:

- T run: denature at 96°C for 1 second, anneal/extend at 54°C for 60 seconds, with fluorescence data collection, 30 cycles.
- S run: denature at 95°C for 15 seconds, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, 8 cycles; followed by denature at 96°C for 1 second, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, hold at 83°C for 5 seconds with data collection, 35 cycles.

Each sample was run in triplicate wells in the 384-well assay plate and the T/S ratio is calculated using the average T and S concentration from triplicates. Each sample was assayed three times, resulting in three T/S values. Data analysis was performed with a software developed at Telome Heatlh. QC criteria included linearity test and PCR amplification of the standard curve, outliner removal of individual well as well as individual T/S ratios.

Genomic DNA from 8 cancer cell lines were included in each run to assess the analytic performance of each PCR run. The average assay coefficient of variation is 5.1% for the control DNAs in the entire study. Participant samples with CV greater than 12.5% from the 3 T/S values were re-assayed and the average of CV of the study samples is 4.6% (SD=3.1%).

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Conflict of interest

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

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