Chapter 8

Depressive and anxiety disorders show robust, but non-dynamic, 6-year longitudinal association with short leukocyte telomere length

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Published in *The American Journal of Psychiatry 2016;* doi: 10.1176/appi.ajp.2015.15070907

ABSTRACT

Objective: Several cross-sectional studies have related depressive and anxiety disorders to shorter leukocyte telomere length (LTL) as an indicator of cellular aging. However, these studies have left many unresolved questions about underlying causality and ordering of associations. The objective of this large longitudinal study is to examine the relationship between depressive and anxiety disorders and LTL over a 6-year time period.
Methods: Data are from the Netherlands Study of Depression and Anxiety including 2292 subjects with remitted and current diagnoses of depressive or anxiety disorders and 644 healthy controls. LTL was assessed using qPCR and measured at baseline and after 6 years; depressive and anxiety disorder diagnoses and characteristics (course, duration and severity) were determined at baseline and after 2, 4 and 6 years.
Results: Results showed that persons with remitted (B=-52.6; p=.021) and current (B= -60.8; p=.007) depressive or anxiety disorder had consistently shorter LTL compared to controls across baseline and 6-year follow-up, also when controlling for lifestyle and somatic health variables. Changes in the course of depressive or anxiety disorder characteristics over 6 years, however, was not associated with different LTL attrition rates.
Conclusions: This study confirmed robust associations of depressive and anxiety disorders with shorter telomeres, but interestingly, it did not demonstrate that depressive and anxiety disorders and LTL change together over time, suggesting the absence of a direct within-person relationship. Short LTL is suggested to be either a long-term consequence or an underlying vulnerability factor for depressive or anxiety disorders.

Keywords: major depressive disorder; depression; anxiety disorder; telomere length; cellular aging; longitudinal

INTRODUCTION

Depressive and anxiety disorders are associated with shorter leukocyte telomere length (LTL), an indicator of cellular aging (1). This might help explain why depressed or anxious persons have increased onset risks of several aging-related somatic conditions, such as cardiovascular disease, diabetes type II and obesity (2,3). One of the proposed pathways is that various biological abnormalities in depressive and anxiety disorders, such as a dysregulated immune system or increased oxidative stress (4,5), may lead to cellular damage including shortened telomere length. Another less-explored explanation is that short telomere length might be a risk factor for persons to develop a depressive or anxiety disorder. Alternatively, an underlying third factor, such as genotype or lifestyle, may make persons vulnerable for both short telomeres and depressive or anxiety disorders (6). As human life expectancy increases, research into aforementioned pathways becomes increasingly important. A better understanding of biological mechanisms may yield novel interventions that prevent aging to be inevitably accompanied by aging-related somatic diseases and disability.

LTL has emerged as an indicator of biological or cellular, rather than chronological, age, since 1) it decreases progressively with every cell division (unless counteracted by sufficient activity of the ribonucleo-protein enzyme, telomerase), and thus with age (7); 2) reflects damage accumulated over the years by lifestyle, psychological stress and cytotoxic environments (8); and 3) it correlates and predicts the incidence of numerous serious medical illnesses (9). Several cross-sectional studies, with a few exceptions (10-12), found shorter LTL in depressed patients compared to controls (13-16), including investigations in the current sample (17). This was further confirmed by recent meta-analyses that found significant effect sizes for the association between depression and shorter LTL (1,18). Also, although less often studied, shorter LTL was found in anxiety disorder patients compared to controls (19,20). A major shortcoming of most studies published until now is their cross-sectional nature, limiting the ability to draw any inferences regarding cause and effect. Only two studies employed longitudinal designs: Hoen et al. (13) found that depression status in 608 heart disease patients did not predict 5-year change in LTL, while Shalev et al. (21) found that 193 men with a depression or anxiety disorder between age 26 and 38 showed greater LTL decrease in that time frame compared to 226 healthy men, but this association was not present in women. In summary, it remains unclear to what extent possible telomere shortening is reversible when persons recover, whether chronicity of a disorder leads to accelerated shortening of LTL over time, whether there are common (genetic) risk factors for both, or, alternatively, whether short LTL is a vulnerability factor that may antecede the development of depressive and anxiety disorders. Longitudinal data are needed to explore these questions.

This study examines the 6-year longitudinal relationship between LTL and depressive and anxiety disorders using a large sample (N=2936). Objectives were to examine whether presence of a diagnosis and symptom severity were consistently associated with LTL over time, and whether changes in depressive and anxiety disorder characteristics (course, duration and severity) corresponded with changes in LTL.

METHODS AND MATERIALS

Study sample

Data were from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study examining the course and consequences of depressive and anxiety disorders. Study sample and methods have been described in detail elsewhere (22). In short, the NESDA baseline sample consisted of 2981 persons between 18 and 65 years, including persons with a current or remitted depressive and/or anxiety disorder (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. In order to maintain representativity, there were only two exclusion criteria: 1) insufficient command of the Dutch language, and 2) a primary clinical diagnosis of bipolar disorder, obsessive-compulsive disorder, PTSD, severe substance use disorder or a psychotic disorder. The study was approved by the Ethical Review Board of participating centres and all participants signed informed consent. Participants were assessed during a 4-hour clinic visit. Every 2 years after the baseline assessment, face-to-face follow-up assessments were conducted. Follow-up assessments had a response of 87.1% (N=2596) at 2-year follow-up , 80.6% (N=2402) at 4-year follow-up and 75.7% (N=2256) at 6-year follow-up.

Measurements

Depressive and/or anxiety disorder status. At each assessment, persons were classified as control subjects, having a remitted depressive or anxiety disorder, or having a current diagnosis. Control subjects were defined as having no lifetime history of depressive or anxiety disorders at all as assessed by the DSM-IV Composite International Diagnostic Interview (CIDI) version 2.1. Persons in the remitted group had a lifetime history of depression or anxiety disorder but no diagnosis in the past 6 months as diagnosed with the CIDI, and current patients had CIDI-diagnosed depressive disorders (major depressive disorder, dysthymia) and/or an anxiety disorder (social phobia, panic disorder with/without agoraphobia, GAD or agoraphobia) in the past 6 months. At baseline, 45 participants were excluded because of missing LTL data, leaving 2936 individuals (644 controls, 620 remitted and 1672 current patients). At 6-year follow-up blood samples

were available for 2003 participants, of whom 120 had unreliable LTL measurement, leaving 1883 persons (440 controls, 915 remitted and 528 current patients, see Table 1). Persons who did not participate at the 6-year follow-up were younger, less educated had longer LTL at baseline, higher depression and anxiety severity scores and had more often a lifetime depression or anxiety diagnosis (all p<.01).

Psychiatric characteristics. Severity of depressive symptoms in the past week was assessed in all subjects with the 30-item Inventory of Depressive Symptoms - Self Report (IDS-SR) (23). Anxiety severity was assessed with the 21-item Beck's Anxiety Inventory (BAI) (24). For both severity measures, a change score was calculated by subtracting the score at baseline from the score at 6-year follow-up. Duration was determined as the percentage of time with depressive or anxiety symptoms during each of the 2-year intervals between the follow-up assessments, as assessed by the calendar method of the Life Chart interview (LCI) (25). Time with symptoms between assessments were averaged to calculate the average percentage of time with symptoms over the 6-year period. As another measure of chronicity, the number of assessments (baseline, 2-year, 4-year, 6-year follow-up) with a CIDI-diagnosed depressive and/or anxiety disorder were summed, ranging from 0 to 4. Finally, to investigate the associations of course of depressive or anxiety disorders with LTL attrition, we created six groups based on diagnosis status at baseline and at 2-year, 4-year and 6-year follow-up (persons were allowed to have one missing): 1) control group; 2) new onset; 3) persistent remitted; 4) relapse; 5) remission; 6) chronic. See Table 2 for details.

Leukocyte telomere length. LTL was assessed at baseline and 6-year follow-up. Fasting blood was drawn from participants in the morning and stored in a -20°C freezer. Baseline and 6-year LTL were determined at the laboratories of Telomere Diagnostics, Inc. (Menlo Park, CA) and University of California, San Francisco in 2012 and 2014, respectively, using quantitative polymerase chain reaction (qPCR) adapted from the published original method by Cawthon (26). 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 LTL (39, 40). The detailed method is described elsewhere (17). As previously described, T/S ratios were converted into base pairs (bp) with the following formula: bp=3274+2413x((T/S-0.0545)/1.16).

The reliability of the assays was adequate: the included quality control DNA samples on each PCR run illustrated that the inter-assay coefficient of variation (CV) was sufficiently low (baseline: CV=4.6%; 6-year: CV=3.0%), as well as for the telomere (baseline: CV=2.0%; 6-year: CV=5.4%) and the single-gene assays (baseline: CV=1.6%; 6-year: CV=4.8%) separately. Variances caused by these CVs are negligible on a group level.

The 6-year follow-up T/S ratios were adjusted relative to the baseline samples for systematic differences caused by different reference samples, by rerunning and comparing samples from baseline sample plates (N=226, up to 8 samples from each of the baseline plate), together with 6-year follow-up samples. On average, the T/S ratios of the 6-year follow-up runs were at 76% of the T/S ratios of baseline, consequently, the follow-up T/S ratios they were divided by 0.76. DNA samples were de-identified and the labs that performed the assays were blind to all other measurements, thus cases and controls were randomly distributed over the plates. Overall, 1860 persons had complete LTL at both time points. For those persons, a LTL change score was calculated by subtracting baseline values from 6-year values. Also, percent change was calculated and this variable was categorized into 1) shorteners: persons who showed >5% telomere shortening, 2) lengtheners: >5% telomere lengthening, and a stable group: those that did not substantially change their LTL (<5% change) over six years. Additionally, a categorization variable with a cutoff of 10% was presented.

Covariates. Sex and age were administered during the baseline interview, years of education was assessed at baseline and 6-year assessments.. Measured body mass index (BMI, weight/height2) was 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, mild-moderate (female<14, male<21 drinks/week) or heavy drinker (female≥14, male≥21 drinks/week). Smoking status was categorized into current, former or never smoker. Physical activity was assessed using the IPAQ-questionnaire (27) and expressed as overall energy expenditure in Metabolic Equivalent Total (MET) minutes per week. The number of self-reported somatic diseases for which participants received medical treatment (i.e. diabetes, osteoarthritis, stroke, cancer, heart, chronic lung, intestinal or thyroid diseases) was counted. Finally, current regularly used antidepressant medication was included as a binary covariate (yes/no): tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), and other antidepressants were defined using WHO classifications (28). Further, we calculated a derived daily dose (DDD) for all participants, which consists of the daily dose used by participants divided by the defined daily dose (the mean advised dose) assigned by the WHO for their specific medication. While no baseline associations between antidepressants and LTL were found in this sample (17), and although correcting for antidepressant use might be over-adjusting since the most severe cases are more likely to use them, we checked whether associations between LTL and psychiatric characteristics were influenced by antidepressant use.

Data analyses

Sample characteristics for those who had complete LTL and psychiatric status data on both time points were compared with paired t-tests for continuous variables and with Wilcoxon signed rank statistics for categorical variables. Associations between LTL and covariates from both time points (baseline and 6-year follow-up) were analyzed using generalized estimated equations (GEE) analyses. GEE analyses were performed with an exchangeable correlation structure, which takes within-person correlations due to multiple observations per participant into account (29). Time was coded as 1 (baseline) and 2 (6-year follow-up).

GEE analyses were conducted with diagnosis status (control, remitted or current) or symptom severity (IDS-SR and BAI) at both time points as predictors and LTL at both times as outcome variable, to test whether diagnosis status and symptom severity were consistently related to LTL. All participants with available LTL and diagnosis status on at least one time point were included, because GEE analyses tolerates missing observations. Analyses included covariates at both time points in different models: 1) sociodemographics (age, sex, education), 2) addition of health and lifestyle variables (smoking, alcohol use, BMI, chronic disease and activity), 3) addition of current medication use (TCA, SSRI, other antidepressants). We tested whether sex was an effect modifier by adding a group-by-sex interaction to the model. Similar GEE analyses were performed to test if baseline diagnosis status predicted LTL attrition.

Six-year changes in depression/anxiety status and and 6-year change in LTL were first examined using GEE analyses with the six-year course variable (see Table 2) as predictor and LTL across both time points as outcome. A group-by-time interaction was added to test if the six course groups predicted differential 6-year TL attrition over time. Second, linear regression models were used with changes in 1) symptom severity (IDS-SR and BAI), 2) symptom duration over the 6-year time period and 3) the number of assessments with diagnoses as predictors, and LTL change score as the outcome, corrected for LTL at baseline.

RESULTS

The study sample's age was 41.8 years (SD=13.1) at baseline and 66.7% was female (see Table 1). Overall, LTL decreased significantly over the 6-year follow-up period (p<.001) which corresponded to an average shortening rate of 13.3 bp per year. Baseline LTL predicted LTL at 6-year follow-up (β=.478; p<.001), and explained 22.9% of the variance. There was a strong inverse correlation between baseline LTL and LTL change (β=-.718; p<.001), confirming earlier findings that persons with long LTL at baseline have a higher chance of shortening and vice versa (30,31). At 6-year follow-up, 27% of the persons showed telomere shortening (27% of controls versus 26% of current patients), 26% showed telomere lengthening (52 % versus 46%), and 47% did not substantially change (21% versus 28%) their LTL. Additionally, 13.2% showed >10% shortening and 11.7% showed >10% lengthening. GEE analyses, using LTL and covariates from both baseline and 6-year follow-up, showed that LTL was negatively associated with age (B=-13.3; p<.001), male sex (B=-95.5; p<.001), smoking (B=-80.1; p<.001), being overweight (B=-48.4; p=.008) or obese (B=-49.6; p=.027) and heavy alcohol use (B=-59.5; p=.013), but not with education, physical activity, the number of chronic somatic diseases or antidepressant medication and their associated DDDs.

Table 1. Sample characteristics at baseline and at 6-year follow-up

	Baseline (N=2936)		6-year follow-up (N=1883)	
Demographics				
	Mean	SD	Mean	SD
Age	41.8	13.1	48.6	12.9
Years of education	12.2	3.3	12.9	3.3
	% female	N	% female	N
Sex	66.4	1950	65.4	1231
Lifestyle & health				
	Mean	SD	Mean	SD
Physical Activity (in 1000 MET-minutes/week)	3.7	3.0	4.0	3.4
Number of somatic diseases	0.6	0.9	0.6	0.9
	%	N	%	N
Body Mass Index				
Underweight	2.2	64	1.6	30
Normal	50.7	1489	45.0	847
Overweight	30.4	893	33.6	633
Obese	16.7	490	19.8	373

Smoking status (%)				
Never	28.1	825	29.5	555
Former	33.2	975	42.3	797
Current	38.7	1136	28.2	531
Alcohol Status (%)				
Non-drinker	17.0	499	17.5	330
Mild-moderate drinker	70.3	2064	72.6	1367
Heavy drinker	12.7	373	9.9	186
Psychiatric characteristics				
	Mean	SD	Mean	SD
Depressive symptoms (IDS)	21.4	14.1	15.2	11.9
Anxiety symptoms (BAI)	12.1	10.6	8.3	8.4
Percent time with depressive symptoms	19.1	26.9	12.8	22.2
Percent time with anxiety symptoms	25.5	32.8	16.3	24.9
	%	N	%	N
Diagnosis status				
Controls	21.9	644	23.4	440
Remitted diagnosis	21.1	620	48.6	915
Current diagnosis	56.9	1672	28.0	528
Within current diagnosis				
Depressive disorder	23.3	389	30.1	159
Anxiety disorder	32.0	535	36.0	190
Comorbid	44.7	748	33.9	179
Antidepressant use				
Tricyclic antidepressant	2.7	79	3.0	56
Selective serotonin reuptake inhibitor	17.1	502	11.9	224
Other antidepressants	5.6	164	5.5	104
Leukocyte telomere length				
	Mean	SD	Mean	SD
Base pairs	5467	617	5387	433

Abbreviations: BAI = Beck's Anxiety Inventory; IDS = Inventory of Depressive Symptoms; MET-minutes = metabolic equivalent of number of calories spent per minute; SD = standard deviation;

Table 2. Six groups classified based on the course of depressive and anxiety disorder diagnoses over four assessments

Group	N	Baseline	2-year	4-year	6-year
Control group	368	No lifetime diagnosis	No diagnosis	No diagnosis	No diagnosis
New onset	76	No lifetime diagnosis	At least one current diagnosis at one follow-up assessment		
Persistent remitted	199	Remitted diagnosis	Remitted diagnosis	Remitted diagnosis	Remitted diagnosis
Relapse	150	Remitted diagnosis	At least one current diagnosis at one follow-up assessment		
Remission	465	Current diagnosis	Remitted/Current diagnosis	Remitted/Current diagnosis	Remitted diagnosis
Chronic	501	Current diagnosis	Remitted/Current diagnosis	Remitted/Current diagnosis	Current diagnosis

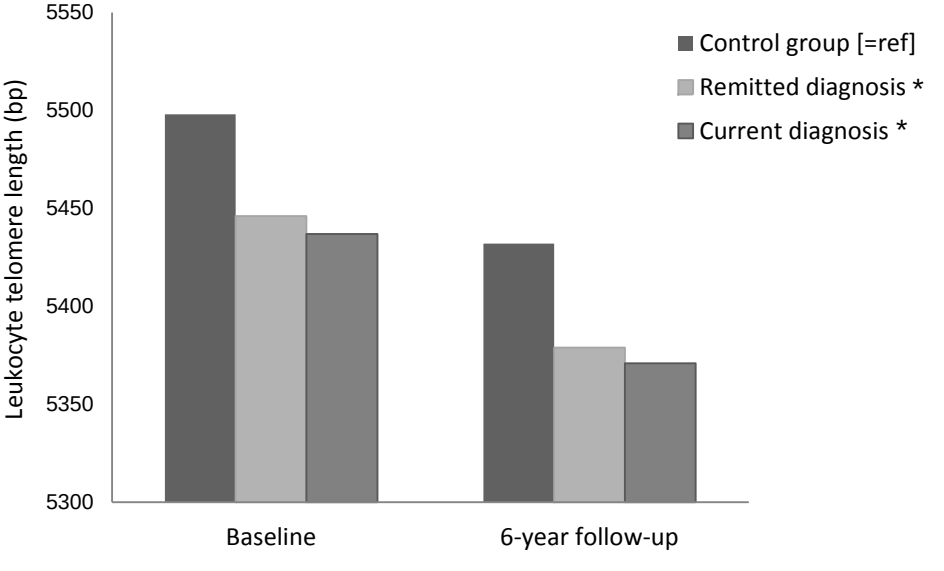
Note. The total N is less than 1860 because of missing data at the 2-year or 4-year assessments

Are diagnosis status and symptom severity consistently associated with LTL over two time points?

GEE analyses including LTL and diagnosis status from both baseline and 6-year follow-up showed that LTL was shorter in persons with a remitted and a current depressive and/or anxiety disorder, compared to controls (see Figure 1, Table 3), replicating our earlier cross-sectional findings (17,19). LTL of persons with a current or remitted diagnosis did not differ from each other (p=.645). These associations remained significant after adjustment for health and lifestyle variables (remitted: B=-46.7; p=.046; current disorder: B=-54.3; p=.017), and current medication use variables. Associations were not different for men and women (diagnosis-by-sex interactions>.05)

Associations were rather similar for depressive and anxiety disorders (depression: remitted (B=-56.1; p=.015), current (B=-51.8; p=.033); anxiety: remitted (B=-34.8; p=.144), current (B=-71.8; p=.002). This, in combination with the high comorbidity between disorders (63% (32)), led us to conduct further analyses with depressive and anxiety disorders combined.

Figure 1. Leukocyte telomere length at two time points by depressive and anxiety disorder diagnosis status (N=2936)



Note. * p<.05; p-values based on comparison of LTL at both time points with control group [reference] in analyses adjusted for age at baseline, sex, education and time

Table 3. Relationship of leukocyte telomere length with depressive and anxiety disorder diagnosis status and symptom severity over two time points (N=2936)

	B	SE	p-value[1]
Control group [reference]			
Remitted diagnosis	-52.6	22.8	.021
Current diagnosis	-60.8	22.4	.007
Time	-66.2	12.4	<.001
IDS-SR	-1.7	0.6	.007
Time	-71.9	12.1	<.001
BAI	-2.3	0.9	.009
Time	-69.4	11.9	<.001

Abbreviations: BAI = Beck's Anxiety Inventory; IDS-SR = Inventory of Depressive Symptoms Self-Report; diagnosis refers to a depressive and/or anxiety disorder
[1]GEE analyses were adjusted for age at baseline, sex and education

GEE analyses examining the association between LTL and symptoms severity across baseline and 6-year follow-up showed that LTL was negatively associated with IDS-SR and BAI scores (Table 3), also in adjusted models. Results of unadjusted analyses of LTL with diagnosis status and symptom severity (data not shown) closely resembled sociodemographic-adjusted analyses.

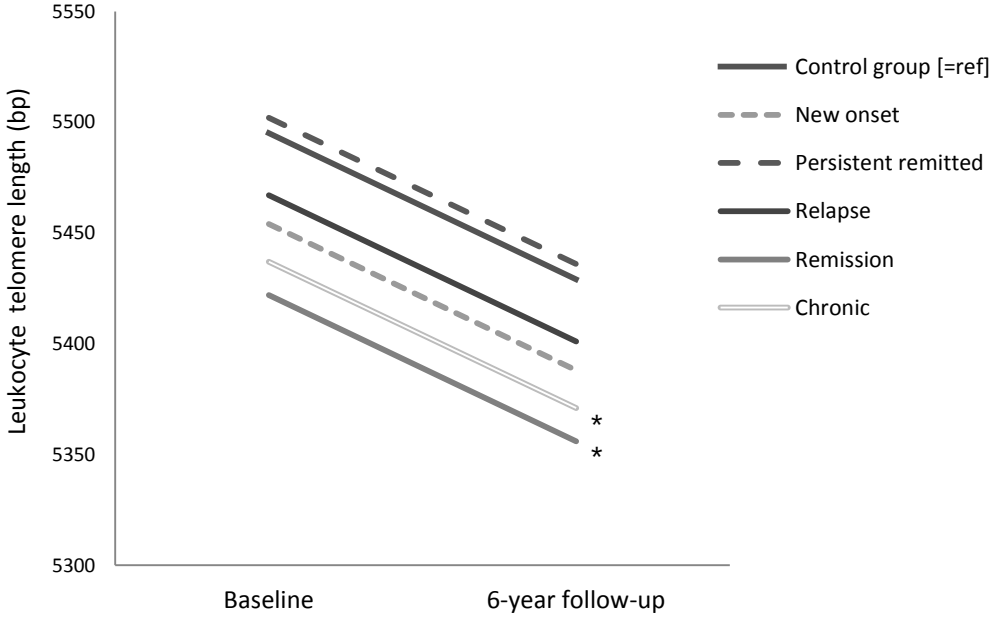
Does baseline diagnosis status predict LTL attrition over 6 years?
Persons with a current depressive or anxiety disorder at baseline did not show different LTL attrition rates compared to controls, as shown by non-significant time interaction terms (remitted diagnosis-by-time: p=.796; current diagnosis-by-time: p=.410) in a sociodemographic adjusted GEE model.

Is the 6-year course of depressive and anxiety disorders related to 6-year LTL change?
GEE analyses tested whether six groups based on diagnosis status at baseline, at 2-year, 4-year and 6-year follow up (see Table 2) predicted average LTL across baseline and 6-year follow-up. Figure 2 shows that the remitted group (B=-72.9; p=.011) and the chronic patients (B=-58.1; p=.041) had on average shorter LTL than controls, adjusted for sociodemographics. Adjustment for health, lifestyle and current antidepressant medication use showed similar LTL differences for the remitted group (p=.015) and the chronic group (p=.048). To investigate the associations of incidence, remission or

chronicity of depressive or anxiety disorders with LTL attrition rate, a group-by-time interaction was added to the sociodemographic-adjusted model. The absence of a significant interaction (p=.926) showed that there was no difference in slope between the six groups, i.e. LTL attrition rates were not dependent on the incidence or remission of depression and anxiety disorders. Thus, the pre-existing TL differences at baseline did not become more pronounced or diminished over 6 years.

In addition, changes in depression and anxiety characteristics were analyzed against changes in TL, adjusted for sociodemographics (Table 4). This showed that 6-year change in depression (IDS-SR score) or anxiety (BAI score) severity, percent of time with symptoms over 6 years, and the number of time points with a diagnosis over 6 years were not significantly associated with 6-year change in LTL.

Figure 2. Leukocyte telomere length at baseline and 6-year follow-up based on the course of depressive and anxiety disorders



Note. * p<.05; p-values based on comparison with control group [reference] in analyses adjusted for age at baseline, sex, education and time

Table 4. Associations between 6-year change in depressive and anxiety disorder characteristics and 6-year change in leukocyte telomere length (N=1860)

	β	p-value
Change in IDS-SR score[1]	.021	.271
Change in BAI score[2]	.005	.795
Percent of time with depressive symptoms	-.017	.311
Percent of time with anxiety symptoms	-.007	.649
Number of waves with diagnosis	-.017	.288

Abbreviations: BAI = Beck's Anxiety Inventory; IDS-SR = Inventory of Depressive Symptoms Self-Report
All analyses are adjusted for age, sex, education and leukocyte telomere length at baseline
[1] analyses are additionally adjusted for baseline IDS-SR
[2] analyses are additionally adjusted for baseline BAI

DISCUSSION

This large study with 6-year longitudinal data demonstrated that persons with a lifetime depressive or anxiety disorder had consistently shorter leukocyte telomere length (LTL) than non-psychiatric controls at two time points, irrespective of the current diagnosis status. In line with a dose-response association, we found negative associations between LTL and the severity of depressive and anxiety symptoms, indicating that the most severe patients had the shortest telomeres. In different analytical approaches, diagnosis status or changes in diagnosis status, however, did not correspond with changes in LTL. First, persons with depression or anxiety disorder at baseline did not show accelerated 6-year LTL attrition compared to controls. Second, six groups based on the course of depressive and anxiety disorders over four assessments (i.e. controls, persistently remitted persons, persons with new onset, persons with relapse/remission, chronic persons), showed no difference in telomere attrition rate. Third, changes in symptom severity, duration of symptoms and number of study waves with a diagnosis did not correlate with change in LTL. Overall, these findings do not suggest a dynamic relationship: existing differences in LTL did not become larger or reduced over time depending on e.g. whether a person developed more severe, chronic symptoms or recovered.

Our results point towards a between-person rather than a within-person relation of depressive and anxiety disorders and LTL, since a person's LTL did not change along with changes in that person's psychopathology. In other words, persons with a lifetime diagnosis were found to have shorter LTL, which represented a difference in cellular age of 4 years (remitted diagnosis) and 4.5 years (current diagnosis); and this was thus irrespective of whether they relapsed or recovered from a disorder. This either suggests

that 1) shorter LTL is indeed a consequence of depressive and anxiety disorder-related physiological disturbances, leaving a long-term (cellular) scar; or 2) persons with short LTL, possibly due to genetic heritability (6,33), are more prone to developing a depressive or anxiety disorder, and TL attrition rate is not necessarily accelerated when those persons meet a disorder diagnosis. Our results resemble those of Hoen et al. (13) in depressed patients with heart disease. Shalev and colleagues (21), however, did find greater LTL decrease between age 26 and 38 in 193 men with one or more depressive or anxiety disorders (depression and generalized anxiety disorder) in a 12-year time frame compared to 226 men without a diagnosis, but this association was not reported for women. Substantial study differences might account for the conflicting outcomes regarding LTL change: Shalev et al. had a younger (26 vs. 42 years at baseline) and smaller (N=758 vs. 1860) study sample and a longer follow-up period (e.g. 12 years vs. 6 years). Moreover, unlike Shalev et al., our study assessed LTL at baseline and 6-year follow-up in different batches which might have caused noise between the two time points. Further, Shalev et al. did not report, however, whether the number of phases with an internalizing disorder increasingly impacted LTL change and whether the effect was still significant after adjustment for lifestyle variables. It is important to note that we found that LTL at baseline was negatively associated with LTL change, indicating that those with short LTL at baseline had a higher chance of lengthening, and vice versa (30). However, despite this compensatory effect which is possibly due to an internal telomere homeostasis system (34), we still found that persons with a current and remitted depressive or anxiety disorder had consistently shorter LTL over the two time points. Something else to consider is that LTL shortening might not be limited to diagnostic categories and it may be possible that LTL reflects underlying pathophysiological processes that surpass traditional diagnoses (8). Evidence for this comes from studies that confirm shorter LTL among other psychiatric cases, such as those with schizophrenia (35) or PTSD (36).

The major strengths of this study are its large sample size, including well-characterized current and remitted depressive and anxiety disorders, as well as healthy controls. Moreover, the sample had a wide age range, and important covariates such as health and lifestyle variables were assessed at multiple time points. Also, LTL has been measured reliably with qPCR and inter-assay coefficients of variation were sufficiently low. These strengths allowed us to thoroughly examine the longitudinal relation between LTL and depressive and anxiety disorders. However, some limitations of this study should also be noted. First, It should be noted that results of this observational study did not take into account whether persons recovered spontaneously or as a result of psychological or pharmacological treatment, whereas this may actually have an impact on LTL or its regulatory mechanisms (31); possible short-term changes due to treatment may thus not have been captured. Hence, although results of this study are not suggestive of

reversibility of LTL shortening after recovery from a depressive or anxiety disorder, multiple *in vitro* (37,38) and *in vivo* (39) studies have systematically shown that such reversibility is indeed possible, possibly even as a result of psychotropic treatment (38,40,41). Further, LTL from baseline and 6-year follow-up was measured two year apart, which could have caused noise between the time points. To adjust for possible systematic differences, samples from both time points were rerun together and LTL at follow-up was converted accordingly. Next, as in most studies, we used leukocytes for TL measurement, which is a validated and often used indicator for cellular aging. A limitation of using average leukocyte TL is that it consists of different cell types. A recent study found different TL change rates for T-cells, B-cells and monocytes, which makes it difficult to distinguish whether TL differences are due to actual shortening/lengthening or rather to a redistribution of cell types (42). However, LTL has been found to have a rather high consistency and rather similar shortening rates across various tissues (i.e. buccal cells, skeletal muscle, skin and subcutaneous fat) (43), but not necessarily all tissues. This suggests that the results of studies conducted in leukocytes are to some extent generalizable to other cell types. Another limitation is that telomerase activity has not been measured, which would be interesting in future research, since telomerase activity may play an important role in the maintenance of LTL, as suggested by several intervention studies (31), and may even mediate beneficial effects of psychotropic medication (44).

In conclusion, this 6-year longitudinal study showed that LTL was consistently shorter for those with a lifetime depressive or anxiety disorder diagnosis, especially among those with the most severe symptoms (dose-response). Importantly, we found that LTL differences over six years did not become more pronounced or diminished as a consequence of incidence, remission or chronicity of depressive or anxiety disorders. While it should be noted that possible short-term treatment effects might not have been captured in this observational study, absolute LTL changes were not related to changes in diagnosis status, symptom severity or duration. This indicates a static, non-dynamic relationship between depressive or anxiety disorders and short LTL and suggests the absence of a direct within-person relationship, since changes in psychiatric characteristics did not correspond with changes in LTL. Future research should elucidate whether short leukocyte telomere length is a long-term consequence or a pre-existing risk factor for the development of depressive or anxiety disorders.

Acknowledgements

The infrastructure for the NESDA study (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).

Grant support

BP, JV and DR and telomere length assaying were supported through a NWO-VICI grant (number 91811602).

Conflicts of interest

The authors declare no relevant conflict of interest.

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