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ORIGINAL ARTICLE

Internalizing disorders and leukocyte telomere erosion: a prospective study of depression, generalized anxiety disorder and post-traumatic stress disorder

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There is evidence that persistent psychiatric disorders lead to age-related disease and premature mortality. Telomere length has emerged as a promising biomarker in studies that test the hypothesis that internalizing psychiatric disorders are associated with accumulating cellular damage. We tested the association between the persistence of internalizing disorders (depression, generalized anxiety disorder and post-traumatic stress disorder) and leukocyte telomere length (LTL) in the prospective longitudinal Dunedin Study ($n = 1037$). Analyses showed that the persistence of internalizing disorders across repeated assessments from ages 11 to 38 years predicted shorter LTL at age 38 years in a dose–response manner, specifically in men ($\beta = -0.137$, 95% confidence interval (CI): −0.232, −0.042, $P = 0.005$). This association was not accounted for by alternative explanatory factors, including childhood maltreatment, tobacco smoking, substance dependence, psychiatric medication use, poor physical health or low socioeconomic status. Additional analyses using DNA from blood collected at two time points (ages 26 and 38 years) showed that LTL erosion was accelerated among men who were diagnosed with internalizing disorder in the interim ($\beta = -0.111$, 95% CI: −0.184, −0.037, $P = 0.003$). No significant associations were found among women in any analysis, highlighting potential sex differences in internalizing-related telomere biology. These findings point to a potential mechanism linking internalizing disorders to accelerated biological aging in the first half of the life course, particularly in men. Because internalizing disorders are treatable, the findings suggest the hypothesis that treating psychiatric disorders in the first half of the life course may reduce the population burden of age-related disease and extend health expectancy.

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INTRODUCTION

Human life expectancy is increasing,[1] and policy makers and citizens alike are concerned that these extra years of life should be healthy, productive and enjoyable, not extra years of prolonged disease and disability. The science of age-related diseases has recently turned to a life-course developmental view, based on evidence that the underlying pathogenesis of age-related diseases involves gradually accumulating physiological damage to organ systems, beginning in the first half of the life course.[2–4] This developmental view raises the possibility that primary prevention could reverse disease-causing processes while people are still healthy.[5]

The hope of preventing age-related diseases is fostering efforts to identify candidate risk targets that can be treated in the first half of the life course[6]. Internalizing psychiatric disorder is a promising novel candidate to investigate for several reasons. First, compared to the general population, patients with internalizing disorders such as depression, generalized anxiety disorder (GAD) and post-traumatic stress disorder (PTSD) have higher mortality rates, but die of the same age-related diseases as the population, such as heart disease, cerebrovascular disease and cancer.[7–10] Second, internalizing disorders are together sufficiently common to be a public-health prevention target,[11] and the timing is right because internalizing disorders peak during the first half of the life course, whereas age-related diseases onset in the second half.[11] Third, internalizing disorders are themselves treatable. If the hypothesis that internalizing disorders accelerate progression toward age-related disease were true, this would imply that the population burden of age-related disease could be reduced by screening for and treating internalizing psychiatric conditions early in life. Further, we studied internalizing disorders instead of life stressors for several reasons: (a) there is an implicit assumption that if stress is consequential enough, it will generate symptoms of mental disorder, (b) persistent disorder is measured more reliably

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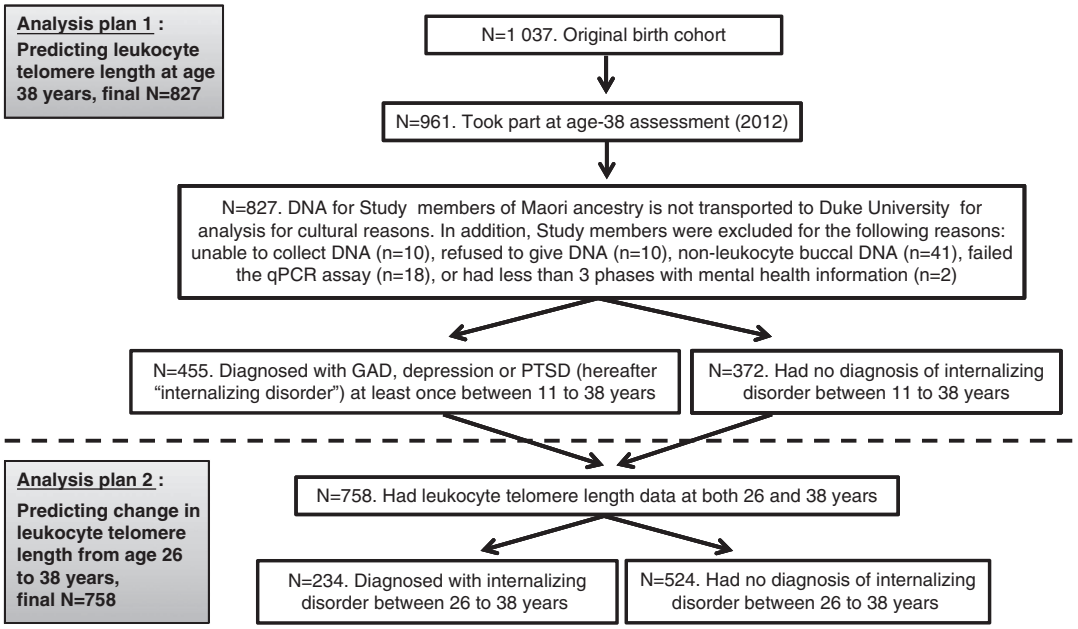

Figure 1. Analysis plan flow chart to test the hypothesis of association between internalizing disorder and leukocyte telomere length (LTL).

than stress events, and (c) disorders can be treated and thus have translational relevance, whereas stress is generally not a treatment target.

We report a test of the association between internalizing disorders and leukocyte telomere length (LTL). The length of telomeres has been proposed as a biomarker for studying accumulating cellular damage throughout the life course.[12] Telomeres, the protective caps at the end of linear chromosomes, erode in somatic tissues with each division of a cell. Ultimately, telomere shortening in cells leads to mitochondrial and metabolic dysfunction and cell cycle arrest.[13] Experimental and longitudinal studies have implicated shorter telomere length in disease susceptibility and greater risk of age-related disease and early mortality.[14–16]

Previous studies have provided mixed support for the association between internalizing disorders and shorter telomere length.[17–23] In the only study to date of internalizing disorder with repeated telomere length measurements, depression was associated with shorter LTL among elderly patients with coronary heart disease, but did not predict 5-year change in LTL.[24] Thus, it remains unclear whether internalizing disorder is associated with shortening of telomeres, that is, actual erosion.

Our study aimed to elaborate current knowledge by using a prospective longitudinal design to test the link between lifetime assessment of internalizing disorders and LTL in the Dunedin birth cohort. Given that persistent depression, GAD and PTSD have high comorbidity,[11,25] shared etiological mechanisms[26] and overlap in symptomatology, we examined all three internalizing disorders. As longer illness duration has previously correlated with shorter LTL,[19] we used data from our 4-decade prospective study to test the association between persistence of internalizing disorder diagnoses and LTL. Further, given reports of sex differences in the (1) prevalence of internalizing disorders,[27,28] (2) telomere length,[29] and (3) mortality risk associated with mental disorders,[9,30] we tested for sex differences in all analyses.

We carried out two sets of analyses. The first set (Figure 1, analysis plan 1) tested the hypothesis that persistence of internalizing disorders across repeated assessments from ages 11 to 38 years would predict shorter LTL at age 38 years. This analysis considered alternative explanatory variables (including childhood maltreatment, cigarette consumption, substance dependence, psychiatric medication use, poor physical health and low socioeconomic status) thought to be associated with both internalizing disorders and LTL. The second set of analyses (Figure 1, analysis plan 2) took advantage of our two LTL measurements to test the hypothesis that LTL erosion between ages 26 and 38 years would be associated with internalizing disorder diagnosed in the interim. This hypothesis was also tested for depression, GAD and PTSD separately.

MATERIALS AND METHODS

Participants

Participants are members of the Dunedin Multidisciplinary Health and Development Study, a longitudinal investigation of health and behavior in a complete birth cohort. Study members ($n = 1037$; 91% of eligible births; 52% male) were all individuals born between April 1972 and March 1973 in Dunedin, New Zealand, who were eligible for the longitudinal study based on residence in the Otago province at age 3 years and who participated in the first follow-up assessment at age 3 years. The cohort represents the full range of socioeconomic status in the general population of New Zealand's South Island and is primarily White. Assessments were carried out at birth and at ages 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32 and, most recently, 38 years, when 95% of the 1007 living Study members underwent assessment in 2010–2012. At each assessment wave, each Study member is brought to the Dunedin Research Unit for a full day of interviews and examinations.

Assessments of internalizing disorders

The Dunedin Study longitudinally ascertains psychiatric disorders using a strategy akin to experience sampling; every 2–6 years, we interview participants about past-year symptoms. Past-year reports maximize reliability and validity because recall of symptoms over longer periods has been shown to be inaccurate. Of course, it is possible that past-year reports separated by 1–5 years miss episodes of mental disorder occurring only in gaps between assessments. We tested this by using life-history calendar interviews to ascertain indicators of mental disorder occurring in the gaps, including inpatient treatment, outpatient treatment or spells taking prescribed psychiatric medication (these indicators are salient and recalled more reliably than individual symptoms).[31] Life-history calendar data indicated that virtually all participants having disorder consequential enough to be associated with treatment have been detected in our net of past-year diagnoses made at ages 18, 21, 26, 32 and 38 years. Specifically, we identified only 11 people who reported treatment but had not been captured in our net of diagnoses from ages 18–38 years.

Table 1. Description of alternative explanatory variables that may explain the association between internalizing disorder and leukocyte telomere length

Alternative explanatory variable	*Description*	*Assessment age(s)*
Childhood maltreatment	Childhood maltreatment is a risk factor for internalizing disorders in adulthood,[73] and has also been associated with shorter telomere length (reviewed in Price *et al.*[56] and Shalev[57]). As previously described,[74] adverse childhood experiences during the first decade of life (ages 3–11 years) were ascertained using behavioral observations, parental reports and retrospective reports by Study members once they reached adulthood. Measures of childhood maltreatment in the Dunedin study included: (1) maternal rejection assessed at age 3 years by observational ratings of mothers' interaction with the study children, (2) harsh discipline assessed at ages 7 and 9 years by parental report of disciplinary behaviors, (3) exposure to disruptive caregiver changes assessed through age 11 and defined by two or more changes of the child's primary caregiver, (4) exposure to physical abuse, and (5) exposure to sexual abuse assessed retrospectively at age 26 years. The number of indicators was summed to a scale of 0 to 2+. 65% of children experienced no maltreatment, 27% experienced one indicator of maltreatment, and 8% experienced two or more indicators of maltreatment.	3–11
Lifetime cigarette consumption (pack-years)	Higher cigarette consumption is associated with both internalizing disorders[75] and shorter telomere length.[76] Here, we defined lifetime cigarette consumption as: pack-years = the number of cigarettes smoked per day, divided by 20 and multiplied by the number of years smoked at that rate through age 38 years (M = 5.77, s.d. = 8.36).	Lifetime up to 38
Substance dependence disorders lifetime	Substance dependence and internalizing disorders tend to co-occur in the same individuals,[77] and there is some evidence that substance-use disorders may be associated with shorter telomere length.[78,79] Past-year substance dependence in the Dunedin Study was assessed at ages 18, 21, 26, 32 and 38 years. Diagnoses included: cannabis, alcohol and hard-drug dependence. Diagnoses at each age followed the then current version of the *DSM* (M = 0.76 diagnoses, s.d. = 1.21).	18–38
Psychiatric medication use	There is concern about potential adverse effects of psychiatric medications on health and physiological processes.[80] Use of psychiatric medications in the Dunedin Study was assessed using successive life-history calendars[31] covering the period from ages 20 to 38 years. 28.8% reported taking medication for a psychiatric problem.	20–38
Physical health problem index	Poor physical health has been associated with both internalizing disorders[81] and shorter telomere length.[61] Here, physical health at age 38 years was measured by nine clinical indicators of poor adult health, including metabolic abnormalities (waist circumference, high-density lipoprotein level, triglyceride level, blood pressure and glycated hemoglobin), cardiorespiratory fitness, pulmonary function, periodontal disease and systemic inflammation (high-sensitivity C-reactive protein). Pregnant women were excluded from the reported analyses. Descriptions for each clinical indicator and clinical cutoffs are provided in Supplementary Table S1. We summed the number of clinical indicators on which Study members exceeded clinical cutoffs. 24.7% had 0 clinical indicators, 23.8% had 1, 20.8% had 2, 14.3% had 3, 7.7% had 4, 4.9% had 5 and 3.9% had 6 clinical indicators or more.	38
Adult SES	Low SES has been associated with psychiatric morbidity,[82] and weakly associated with telomere length.[83] Adult SES at age 38 years was determined based on the Study members' occupation following the New Zealand socioeconomic index and coded to a six-point scale[84] (M = 3.79, s.d. = 1.41).	38

Abbreviations: DSM, Diagnostic and Statistical Manual of Mental Disorders; SES, socioeconomic status.

At ages 11, 13 and 15 years, the Diagnostic Interview Schedule for Children[32] was used to assess past-year depression and overanxious disorder of childhood (which in adulthood is subsumed by GAD), according to Diagnostic and Statistical Manual of Mental Disorders III (*DSM-III*). At ages 18, 21, 26, 32 and 38 years, the Diagnostic Interview Schedule[33] was used to assess past-year major depression and GAD, according to the then current versions of *DSM-III-R* and *DSM-IV*. PTSD was assessed for the first time at age 26 years, when lifetime reports were obtained, and subsequently at ages 32 and 38 years past 6 years PTSD was assessed. Interviewers were health professionals. All disorders were diagnosed regardless of the presence of other disorders. We included GAD and not phobias because GAD entails distress comparable to depression and PTSD, whereas most phobias include avoidance and thus are not accompanied by ongoing distress. The Dunedin cohort 12-month prevalence rates of internalizing disorders match rates from the United States of America and New Zealand national surveys.[34]

For this study, given high comorbidity between internalizing disorders, we summed the number of assessments during which each Study member met diagnostic criteria for any internalizing disorder at each phase/age: 372 Study members (45.0%) had no history of internalizing disorder from 11 to 38 years; 210 (25.4%) met diagnostic criteria for an internalizing disorder at one assessment phase/age; 124 (15.0%) met criteria at two assessment phases/ages; 68 (8.2%) at three; 32 (3.9%) at four; and 21 (2.5%) at five or more assessment phases/ages.

Measurement of mean relative LTL

Leukocyte DNA was extracted from blood using standard procedures.[35,36] DNA of participants at age 26 and 38 years was stored at −80 °C until assayed to prevent degradation of the samples. All DNA samples were assayed for LTL at the same time, independently of caseness, and all operations were carried out by a laboratory technician blind to cases or controls. LTL was measured using a validated quantitative PCR method,[37] as previously described,[38] which determines mean telomere length across all chromosomes for all cells sampled. The method involves two quantitative PCRs for each subject, one for a single-copy gene (S) and the other in the telomeric repeat region (T). All DNA samples were run in triplicate for telomere and single-copy reactions at both ages 26 and 38 years, that is, 12 reactions per Study member. Measurement artifacts (for example, differences in plate conditions) may lead to spurious results when comparing LTL measured on the same individual at different ages. To eliminate such artifacts, we assayed DNA triplicates from the same

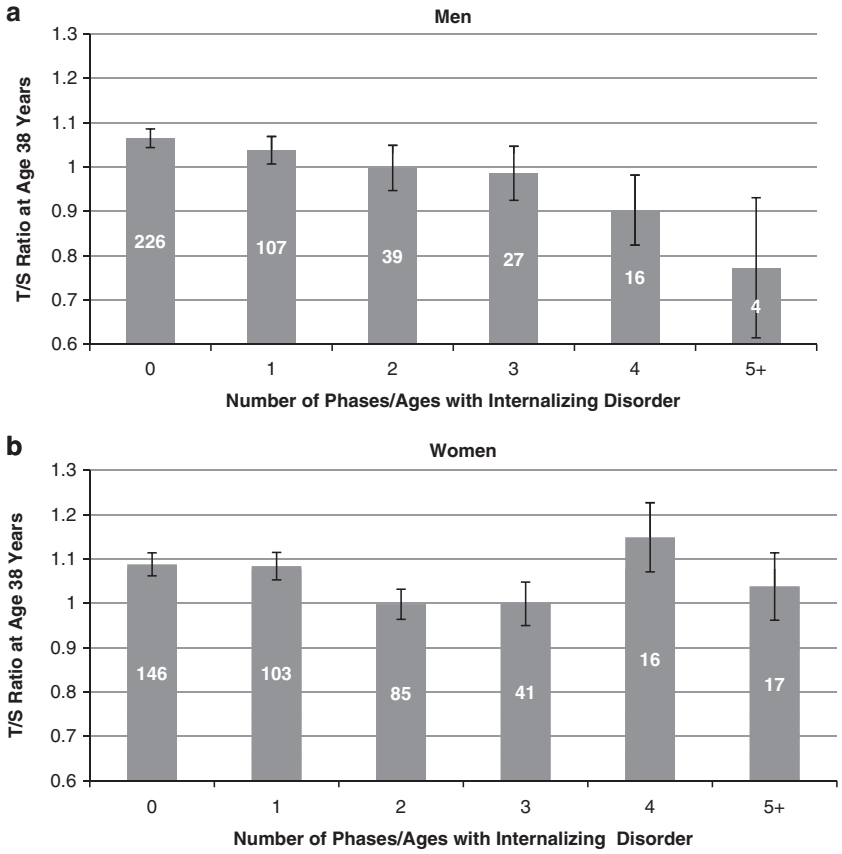

Figure 2. Telomere length. Association between internalizing disorder from age 11 to 38 years, and leukocyte telomere length (LTL) at 38 years for men (**a**) and women (**b**). Error bars reflect standard errors. Numbers within bars represent the number of Study members in each group.

individual, from both ages 26 and 38 years, on the same plate (Supplementary Figure S1). The average coefficient of variation for the triplicate Ct values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S), indicating low measurement error. LTL, as measured by T/S ratio, was normally distributed (Kolomogorov–Smirnov tests of normality), with a skew of 0.90 and kurtosis 1.59 at age 26 years, and a skew of 0.48 and kurtosis 0.38 at age 38 years.

Alternative explanatory variables

We tested for alternative explanatory variables known to be associated with both internalizing disorders and LTL. These variables have been previously published and have good reliability and validity in this cohort. These included: childhood maltreatment, lifetime cigarette consumption, substance dependence disorders between ages 18 and 38 years, psychiatric medication use between ages 20 and 38 years, poor physical health and low adult socioeconomic status at age 38 years. All alternative explanatory variables are described in Table 1.

Statistical analysis

In analysis plan 1 (Figure 1), we tested the hypothesis that persistence of internalizing disorders would predict LTL by regressing age 38 years LTL on a predictor variable indicating the number of phases/ages during which Study members met criteria for an internalizing disorder. To test for alternative explanations of the association between internalizing disorder and LTL, we statistically controlled for alternative explanatory variables in ordinary least squares multiple regression analyses. Supplementary analyses ruled out potential effects of white blood cell counts on LTL at age 38 years (Supplementary Table S3).

In analysis plan 2 (Figure 1), we tested the hypothesis that LTL erosion between ages 26 and 38 years would be associated with internalizing disorder in the interim. We compared Study members who met diagnostic criteria for internalizing disorders after the assesment at age 26 years but before the assesment at age 38 years ($n=234$) to controls who did not experience internalizing disorders during this time period ($n=524$). To estimate the statistical contribution of internalizing disorders to change in LTL, we regressed LTL at age 38 years on LTL at age 26 years (generating an estimate of LTL change), and a dummy variable indicating whether a Study member met criteria for an internalizing disorder between ages 26 and 38 years (an estimate of the association between internalizing disorder and greater LTL change).

Given the possibility of sex differences, we relaxed our significance criterion (to $\alpha=0.20$) in order to reduce the risk of making type II error when testing for sex interaction, as previously recommended[39] (p. 208). Given that in both analyses (plan 1 and 2), the sex interaction was $P\leqslant 0.20$, we present results separately for men and women. Age was not controlled statistically, as all study members were of the same age (1972-73 births).

RESULTS

Analysis plan 1: does persistence of internalizing disorder predict LTL at age 38 years?

Persistence of internalizing disorder from 11 to 38 years was significantly associated with shorter LTL at age 38 years among men in a dose–response manner ($n=419$; $\beta=-0.137$; 95% confidence interval (CI): −0.232, −0.042; $P=0.005$) (Figure 2a). In contrast, there was no significant association between internalizing disorder and shorter LTL among women, and no evidence of a dose–response association ($n=408$; $\beta=-0.066$, 95% CI: −0.163, 0.032; $P=0.185$) (Figure 2b). To be certain that the association between internalizing disorder and shorter LTL among men was not driven by the four men who had internalizing disorders at five or more phases/ages, we repeated the analysis removing these men. The association remained significant ($n=415$; $\beta=-0.116$, 95% CI: −0.212, −0.020; $P=0.018$).

Table 2. Pearson correlations and multivariate linear regression analyses of internalizing disorder from 11–38 years, predicting LTL at 38 years, controlling for alternative explanatory variables

	Bivariate Pearson correlation		*Association between internalizing disorder at age 11–38 years, and LTL at age 38 years*	
	Internalizing disorder at age 11–38 years	*LTL at age 38 years*	β *(95% CI)*	P-*value*
Internalizing disorder from age 11 to 38 years	—	—	−0.137 (−0.232, −0.042)	**0.005**
			Controlling for alternative explanatory variables:	
Childhood maltreatment	**0.173****	−0.032	−0.135 (−0.232, −0.039)	**0.006**
Lifetime cigarette consumption (pack-year) up to age 38 years	**0.245****	**−0.105***	−0.118 (−0.217, −0.020)	**0.018**
Substance dependence disorders from age 18 to 38 years	**0.335****	−0.092	−0.119 (−0.221, −0.018)	**0.021**
Psychiatric medication use from age 20 to 38 years	**0.413****	−0.019	−0.156 (−0.260, −0.051)	**0.004**
Physical health problem index at age 38 years[a]	**0.129****	**−0.135****	−0.121 (−0.217, −0.026)	**0.013**
Adult SES at age 38 years[b]	**−0.138****	0.051	−0.132 (−0.229, −0.036)	**0.007**
All alternative explanatory variables	—	—	−0.124 (−0.232, −0.016)	**0.025**

Abbreviations: CI, confidence interval; LTL, leukocyte telomere length; SES, socioeconomic status. Results are presented for men only ($n = 419$). Significant *P*-values are highlighted in boldface; *$P < 0.05$; **$P < 0.005$. [a]Analyses for the different physical health indicators are provided in Supplementary Table S2. [b]Higher scores on the scale indicate higher SES at age 38 years.

We next tested if factors that may be associated with both internalizing disorders and telomere length can account for the association between internalizing disorder and shorter LTL. Given that the positive association between internalizing disorders and LTL was limited to men, the following analyses are presented for men only. Table 2 shows that all alternative explanatory variables were significantly correlated with internalizing disorder from age 11 to 38 years. In addition, lifetime cigarette consumption, lifetime substance dependence and poorer physical health were significantly correlated with shorter age-38 LTL ($P \leqslant 0.05$). Controlling for each of the alternative explanatory variables individually neither altered the initial finding nor did controlling for all variables simultaneously ($n = 419$; $\beta = -0.124$, 95% CI: −0.232, −0.016; $P = 0.025$) (Table 2).

Analysis plan 2: do internalizing disorders predict accelerated LTL erosion from ages 26 to 38 years?

The correlation between age-26 LTL and age-38 LTL was significant, among both men ($r = 0.676$, $P < 0.001$) and women ($r = 0.678$, $P < 0.001$), indicating stability of individual differences in LTL over time; individuals with long telomeres at age 26 continued to have relatively long telomeres at age 38. In addition, there was significant decline in mean LTL from ages 26 to 38 years. The average LTL declined from 1.182T/S (s.d. = 0.40) to 1.028T/S (s.d. = 0.32) among men (General Linear Model repeated measures: $F(388,1) = 102.59$; $P < 0.001$), and from 1.196T/S (s.d. = 0.40) to 1.046T/S (s.d. = 0.30) among women ($F(368,1) = 97.25$; $P < 0.001$).

Against this background, men who experienced internalizing disorder between ages 26 and 38 years showed significantly more LTL erosion than men with no internalizing disorder ($n = 389$; $\beta = -0.111$, 95% CI: −0.184, −0.037; $P = 0.003$) (Figure 3a). In contrast, there was no significant association between internalizing disorder and accelerated LTL erosion among women ($n = 369$; $\beta = -0.031$, 95% CI: −0.106, 0.045; $P = 0.427$) (Figure 3b).

Figure 3a shows a similar effect for each type of disorder on accelerated LTL erosion among men. There was significantly accelerated LTL erosion among men who experienced depression ($\beta = -0.114$, 95% CI: −0.190, −0.039; $P = 0.003$) and GAD ($\beta = -0.100$, 95% CI: −0.181, −0.020; $P = 0.015$). The association for PTSD among men was in the same direction but did not reach significance ($\beta = -0.065$, 95% CI: −0.147, 0.017; $P = 0.120$), in part, because of lack of power. In contrast, women who experienced depression ($\beta = -0.010$, 95% CI: −0.088, 0.067; $P = 0.795$), GAD ($\beta = -0.056$, 95% CI: −0.140, 0.029; $P = 0.194$) or PTSD ($\beta = -0.007$, 95% CI: −0.095, 0.081; $P = 0.879$) did not show significantly more LTL erosion (Figure 3b). Because fewer than half of individuals in each of the three disorder groups had only one type of internalizing disorder, we lacked statistical power for testing pure cases. However, among them, the pattern of mean LTL differences decreased somewhat, suggesting more LTL erosion among comorbid cases.

Finally, given uncertainty about the interpretation of telomere lengthening (is it measurement error,[40] regression to the mean[41] or a real biological phenomenon?[42]), we retested the association between internalizing disorder and LTL erosion after excluding Study members whose telomeres lengthened. As previously described,[38] lengthening was defined as >15% increase in LTL between measurements (12.8% of this cohort, 12.9% of men and 12.8% of women). Internalizing disorder between ages 26 and 38 years remained significantly associated with accelerated LTL erosion in men ($n = 339$; $\beta = -0.075$, 95% CI: −0.142, −0.009; $P = 0.026$), and remained unassociated in women ($n = 322$; $\beta = -0.037$, 95% CI: −0.106, 0.033; $P = 0.302$).

DISCUSSION

The present study tested the hypothesis that internalizing disorders are associated with shorter telomere length and accelerated telomere erosion. After accounting for multiple alternative explanatory factors, depression, GAD and PTSD were associated with shorter LTL at age 38 years and with accelerated LTL erosion across a 12-year period among men. No significant associations were observed between internalizing disorder and LTL among women.

This study has several strengths. Although most previous telomere studies have assessed internalizing disorders at a single time point, the 4-decade prospective nature of our cohort allows reliable repeated assessments of Study members' psychiatric histories covering a period of more than 25 years. Moreover, the two DNA collection phases enabled us to test for association between internalizing disorder and LTL change over a 12-year period. Further, given the numerous factors assumed to affect both internalizing disorder and LTL, it was useful to test multiple alternative explanations of the association between internalizing disorder and LTL. We did so using measures that were both

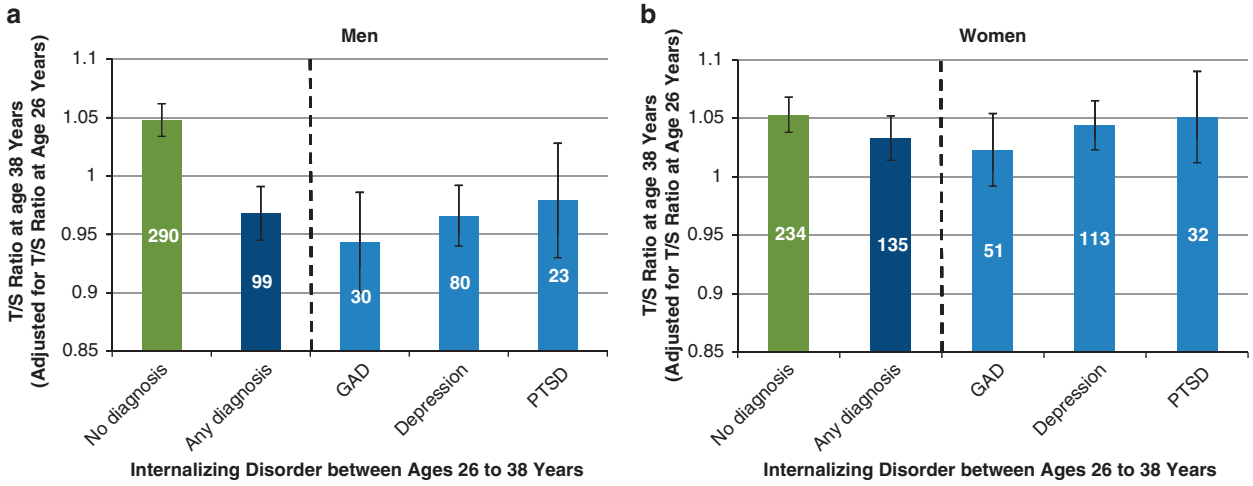

Figure 3. Telomere erosion. Association between generalized anxiety disorder (GAD), depression and post-traumatic stress disorder (PTSD) between ages 26–38 years, and leukocyte telomere length (LTL) at age 38 years (after controlling for baseline LTL at age 26 years) for men (**a**) and women (**b**). Error bars reflect standard errors. Numbers within bars represent the number of Study members in each group. The sum of each disorder (GAD, depression and PTSD) exceeds the total for any diagnosis due to comorbidity across internalizing disorders. Because fewer than half of individuals in each of the three disorder groups had only one type of internalizing disorder, we lacked statistical power for testing pure cases.

lifelong (for example, smoking) and contemporaneous (physical health). Finally, most previous studies have focused on depression. We took the opportunity to study GAD and PTSD as well as depression. Our study extends previous knowledge to suggest a potential common mechanism of accelerated cellular aging linked with all three internalizing disorders.

Our findings suggest that the association between internalizing disorder and LTL may be stronger in men or even absent in women. Despite the higher prevalence of internalizing disorder among women, there is some evidence that men who suffer from mental disorder are at higher risk of mortality than women,[9,30] which is consistent with the sex difference we observed in this study. Several physiological and biochemical processes implicated in internalizing disorders have been shown, in some studies, to be more affected among men than women, including dysregulation of the hypothalamic–pituitary–adrenal axis,[43] elevated proinflammatory cytokines[44] and elevated oxidative stress markers.[45] These sex-varying processes are also implicated in the regulation of telomere length[46–48] and could provide a basis for the stronger associations we observed here among men. Further, our study period covered the cohort womens' child-bearing years; evolutionary theory might allow sex-specific stress-protection factors for women of reproductive age. For example, animal studies suggest mitochondrial antioxidant protection among females, specifically during reproductive age,[49,50] a difference thought to be partially explained by estrogens.[49] Notably, mitochondrial dysfunction is considered a mechanism by which telomeres erode as a result of higher oxidative stress.[46,51] Moreover, the expression and activity of telomerase is increased in the presence of estrogens.[52,53] If true, women may be less susceptible to disorder-linked telomere erosion during reproductive years. Although plausible, our sex-specific finding must be replicated (prior studies have not systematically tested sex differences). Future studies may also test whether the non-association we observed is limited to women of reproductive age.

Our findings should be interpreted in light of limitations and caveats. First, as the Dunedin cohort is primarily White, future studies need to test whether the association between internalizing disorder and LTL generalizes to other populations. Second, although the Dunedin Study was one of the first cohort studies to extract DNA in the 1990s, our first LTL measure represented age 26 years, and we have no earlier LTL baseline in childhood. Third, we used an experience-sampling approach, ascertaining mental disorder in eight 1-year windows spaced across 27 years. Contiguous annual assessments would be better, but neither funders nor research participants favor this approach. Fourth, our finding of within-individual LTL change is consistent with the hypothesis that internalizing disorders affect LTL, but it is also possible that telomere erosion can be a cause of disorder, or the association between them might be brought about by an unmeasured third variable.[54] For example, theoretically individuals genetically predisposed to accelerated telomere erosion may also be at risk for disorders. Fifth, our study was ill-equipped to test whether mental health treatment use can prevent telomere erosion, because we had inadequate information about treatment type, quality, duration, compliance or response. Finally, a noteworthy caveat is that we did not find an association between childhood maltreatment and LTL at age 38 years. Although another study has reported null association,[55] a null finding contradicts our positive finding from a cohort of young children,[38] as well as other previous studies (reviewed in Price *et al.*[56] and Shalev[57]). More research is needed to understand why the link between maltreatment and LTL has emerged in some studies but not all.

The present findings have implications for basic and translational research. With regard to basic research, long-term follow-up studies are needed to test whether accelerated telomere erosion indeed mediates the link between internalizing disorders and later age-related disease outcomes. The use of LTL as a biomarker of aging remains controversial.[58] If future research shows that LTL mediates the association between internalizing disorders and age-related diseases, telomeres might become an eventual therapeutic target.[59] Moreover, research should test whether telomere erosion accompanies other persistent mental disorders such as psychoses. Furthermore, research should elucidate the molecular pathways by which the connection between internalizing disorder and LTL damage occurs. Proposed mechanisms include elevated oxidative stress, mitochondrial dysfunction and telomerase regulation. Immune system changes are considered as one potential mechanism,[19,47] as can be observed in this study with telomere length measured from immune cells. However, an indicator of elevated systemic inflammation (C-reactive protein) was unrelated

to LTL at age 38 years in this cohort (Supplementary Table S2), and thus could not mediate the association we observed. Previous research has shown that certain inflammatory markers are associated with shorter LTL.[47,60,61] However, the association between high levels of C-reactive protein and short telomere length is ambiguous. Although several studies have reported positive associations,[61–64] others have reported no association.[47,65–68] It is also noteworthy that these studies have used different methods to measure telomere length (for example, Southern blot versus quantitative PCR) and C-reactive protein (for example, high sensitivity versus not) which may have contributed to the mixed findings. Moreover, the majority of studies were of older, and mostly clinical, populations. More studies are needed to determine whether C-reactive protein is associated with telomere length in younger age groups.

With regard to translational research, our findings suggest that randomized clinical trials of treatments for internalizing disorders could incorporate before-and-after-treatment telomere length[69,70] and/or telomerase[71] measurement to ascertain whether treatments that ameliorate psychiatric disorder might also prevent or decelerate telomere erosion.[72] If yes, this would raise the possibility that effective screening and treatment for internalizing disorders in the first half of the life course might prevent or reverse processes underlying age-related disease and enhance population health expectancy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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