Chapter 12

Summary and general discussion

TABLE OF CONTENTS

1. SUMMARY OF MAIN FINDINGS

This section summarizes the main findings per chapter. The general aim of this thesis was to study associations of depressive and anxiety disorders with markers of cellular aging. More specifically, the first aim was to investigate cross-sectional associations of telomere length with depression and several anxiety disorders. The second aim was to test associations of telomere length with established risk factors of depressive and anxiety disorders, namely childhood trauma and recent stressful life events. The third aim was to provide insights into the longitudinal trajectories of depressive and anxiety disorder and telomere length and mitochondrial DNA (mtDNA) copy number over time. Last, our fourth aim was to shed more light on possible mechanisms underlying the association between depressive and anxiety disorders and telomere length.

In **Chapter 2**, we tested cross-sectional associations between major depressive disorder (MDD) and telomere length in the Netherlands Study of Depression and Anxiety (NESDA). In this study we showed that telomere length was shorter in 1,095 persons with a current 6-month MDD diagnosis (effect size: Cohen's d=0.12; p=.027) and 802 persons with remitted MDD, who had a lifetime history of MDD but no current diagnosis (Cohen's d=0.12; p=.036), as compared to 510 never-depressed control subjects. Further, within the current MDD patients, we showed that both higher depression severity (p=.004) and longer symptom duration in the past 4 years (p=.010) were associated to shorter telomere length, suggestive of a 'dose-response' association.

Chapter 3 consists of a study with a similar cross-sectional design in NESDA. This study showed that 1,283 persons with a current anxiety disorder (social phobia, generalized anxiety disorder and panic disorder with agoraphobia) had shorter telomere length than 582 controls (Cohen's d=0.13; p=.01). Interestingly, 459 persons with a remitted anxiety disorder did not differ from controls in telomere length (p=.84), however, telomere length showed a positive association with time since remission (p=.024). A group of 225 persons who remitted up to 9 years ago had shorter telomere length than 220 persons who were remitted for 10 years or longer (Cohen's d=0.22; p=.022). Furthermore, anxiety severity scores were negatively associated with telomere length in the whole sample, but not in the current anxiety disorder sample only.

The aim of **Chapter 4** was to investigate the cross-sectional association of telomere length with late-life depression in persons older than 60 years from the Netherlands Study of Depression in Older persons (NESDO). Results of this study showed that telomere length did not differ between 355 person with late-life depression and 128 never-depressed controls (p=.59). Moreover, in this sample we found no association between telomere length and symptom severity, duration, age at onset of depression or comorbid anxiety

disorders (all p-values >.20). These null-findings are in contrast with the results of Chapter 2 which described a telomere length-depression association in younger adults.

Chapter 5 concerns a meta-analysis on the link between telomere length and psychiatric disorders, including depressive disorders, psychotic disorders, bipolar disorder, posttraumatic stress disorder (PTSD) and other anxiety disorders, with data from 32 studies (5,289 cases and 9,538 controls). The overall meta-analysis demonstrated a significant effect size (effect size: g=-0.50; p<.001), indicating that psychiatric disorders were associated with shorter LTL. More specifically, subgroup effect sizes were significant for depressive disorders (g=-0.55; p=.004), PTSD (g=-1.27; p=.003) and anxiety disorders (g=-0.53; p=.05), but not for bipolar disorder (g=-0.26) or psychotic disorders (g=-0.23). Meta-regression showed, however, no significant difference in the effect size estimates between the different disorder subgroups. This suggest that shorter telomere length may not be limited to current diagnostic constructs but may instead reflect underlying pathophysiological processes that surpass traditional diagnostic categories.

In **Chapter 6**, we examined the cross-sectional association between early and recent psychosocial life stress with telomere length in 2936 participants from NESDA. This study showed no relationship of childhood life events (p=.805) and childhood trauma (p=.205) with telomere length. However, persons had shorter telomeres if they reported more stressful life events in the past year (p=.028) or up to 5 years ago (p=.018), but not if events occurred more than 6 years ago (p>.10). These results show that the more proximate the psychosocial life stress to telomere length measurement, the more likely they were related. This might indicate that persons are to some extent able to physiologically recover from the effects of psychosocial life stress.

Chapter 7 consists of a review paper that discusses the hypothesis of reversibility of cellular aging in depression. Animal research and in vitro studies provided evidence that recovery of telomere length to some extent is possible. Further, intervention studies including lifestyle changes, stress reduction, social support and psychotropic medications may favorably impact the telomere / telomerase system, possibly by normalizing various underlying physiological dysregulations. However, the evidence is preliminary and no definitive conclusions can be drawn. Large-scale longitudinal or intervention studies that could shed more light on the possibility of reversibility are lacking.

In **Chapter 8** we examined longitudinal associations of depressive and anxiety disorders with telomere length in NESDA. This study with psychopathology and telomere length data of 2936 participants at baseline and 1883 participants at 6-year follow-up showed that persons with current (p=.017) and remitted (p=.046) diagnoses had consistently shorter telomere length compared to controls, confirming our earlier cross-sectional findings. We also showed negative associations between telomere length and the severity

of depressive (p=.007) and anxiety symptoms (p=.009), indicating that the most severe patients had the shortest telomeres. However, changes in the course of depressive or anxiety disorder characteristics over 6 years, were not associated with different telomere attrition rates over 6 years. These results indicate that psychopathology and telomere length do not dynamically change together over time.

Chapter 9 again looked at longitudinal associations, now in the Coronary Artery Risk Development in Young Adults Study (CARDIA) sample. Here, we analyzed data on depressive symptomatology, assessed with the Center for Epidemiologic Studies Depression (CES-D) scale, and two markers of cellular aging, namely telomere length and mtDNA copy number, from 977 persons over 10 years. Results showed that having long-term high levels of depression was associated with shorter average telomere length (p=.038), but no within-person associations were found between changes in depressive symptoms and concurrent changes in telomere length (p=.635). These findings, in line with Chapter 8, suggest that the relation between depression and telomere length reflects a between-person, rather than a within-person, effect. Further, in this study, we did not find evidence for a relationship between depressive symptomatology and mtDNA copy number.

In **Chapter 10**, we tested cross-sectional associations between telomere length and three major physiological stress systems in 2936 participants of NESDA. Physiological stress systems included the immune-inflammatory system, hypothalamus-pituitary adrenal-axis and autonomic nervous system. Results showed that higher levels of the inflammatory markers CRP and IL-6, higher cortisol awakening response and higher heart rate were associated with short telomere length. Next, we found that the number of such physiological dysregulations was associated with shorter telomere length, indicating a dose-response relationship (1, 2 or 3-4 dysregulations versus no dysregulations, all p-values <.01). Overall, these findings indicate that a dysregulated physiological stress response is accompanied by shortened telomere length, although cause and effect remain to be explored.

The last study of this thesis, **Chapter 11**, examined the extent to which physiological stress systems, metabolic syndrome components and lifestyle factors explain the relationship between depressive and anxiety disorders and telomere length. Mediation analyses showed that CRP, IL-6, waist circumference, triglycerides, HDL cholesterol and cigarettes smoking were significant mediators in the relation between psychopathology and telomere length. Subsequently, when all significant mediators were included in one model, the effect sizes of the relationships between telomere length and symptom severity and current diagnosis were reduced by 36.7% and 32.7%, respectively, and the remaining direct effects were no longer statistically significant.

2. DISCUSSION OF MAIN FINDINGS

In this section the main findings per aim are discussed in relation to other literature. Subsequently, clinical implications and methodological considerations are addressed, along with recommendations for future research. Finally, this chapter ends with an overall conclusion.

2.1 Are depressive and anxiety disorders cross-sectionally associated with telomere length?

At the start of this thesis only a few studies had provided preliminary, but inconsistent, evidence of a relation between depression and telomere length (1-5). In Chapter 2, we showed that 1,095 persons with major depressive disorder (MDD) had on average shorter telomeres compared to 510 never-depressed controls (effect size: Cohen's d=0.12; p=.027). Also, 802 persons with MDD in remission had shorter telomeres than controls (Cohen's d=0.12; p=.036) (6). When published in November 2013 this was the largest study to that date, which convincingly showed that persons with a lifetime depression diagnosis had shorter telomere length than their never-depressed and similarly aged counterparts. We further showed that the association between MDD and telomere length showed a 'dose–response' gradient, since the most severely and chronically depressed patients had the shortest telomeres (Cohen's d = 0.21; p=.004). Since then, a dozen of studies have looked at this association, mostly – but not all (e.g. (7)) – confirming shorter telomere length in the depressed population. In 2015, Schutte et al. (8) published a meta-analysis on the association between depression and telomere length showing a significant overall effect size (r=−.12, p<.001). This meta-analysis contained 25 studies with 21,040 participants and included studies that depression measured by clinical diagnosis and by self-report. Although there was no difference in effect size between studies that assessed depression by clinical diagnosis and those that used self-report, only a minority (3 out of 10) of individual studies using self-reported depression showed statistically significant associations, while studies with clinical diagnosis showed a more consistent picture. The in Chapter 5 presented meta-analysis (9) included 2,227 depressed patient versus 3,142 controls and again provided evidence for a significant association between clinically diagnosed depression and shorter telomere length (Hedges' *g*=-0.55, p=.004). Effect sizes of the association between depression and telomere length were overall small to medium in magnitude, which might be due to the heterogeneity of the depressed population as well as the variety of other factors that influence telomere length such as somatic health, lifestyle and heritability. Last, a recent large-scale study in the China Oxford and VCU Experimental Research on Genetic Epidemiology (CONVERGE) consortium which showed that 5,864 women with recurrent depression had shorter telomere length than 5,783

matched controls (10). Altogether, research in the past decade confirmed a cross-sectional association between clinical depression and shorter telomere length.

The association between anxiety disorders and telomere length has been less well studied. Kananen et al. (11) were to first to report on shorter telomeres in patients with panic disorder, generalized anxiety disorder (GAD), social phobia or agoraphobia, but they only confirmed this association in the older half of the anxiety disorder patients (>48 years old) and not in the whole sample of 321 patients compared with 653 controls. Further, research in non-psychiatric samples showed that higher stress appraisals (12) and high phobic anxiety (13) were negatively associated with telomere length. The study described in Chapter 3, which is the largest study to date, showed that 1,283 patients with a current anxiety disorder had on average shorter telomere length compared to 582 controls (Cohen's d=0.13; p=.01) (14). More specifically, within the current anxiety disorder group, patients with panic disorder with agoraphobia, social phobia and GAD, but not panic disorder or agoraphobia alone, had significantly shorter telomere length than the control group. More recently, Needham et al. (15) did not find a difference in 44 panic disorder or GAD patients, compared to 1058 controls. However, when stratifying by sex, they did find a telomere length difference within the female anxiety patients and controls. The meta-analysis in Chapter 5 including 1,599 anxiety disorder patients and 2,268 controls from our study and the two other studies reviewed here (11,14,15), found a significant effect size for anxiety disorders and telomere length (Hedges' *g*=-0.53, p=.05), establishing a cross-sectional association between current anxiety disorders and short telomere length.

Interestingly, and in contrast with our findings in the depressed population in Chapter 2, the study in Chapter 3 showed that 459 participants with remitted anxiety disorder resembled the 582 healthy controls in terms of telomere length (p=.67). The different findings of remitted MDD and remitted anxiety disorder patients might be due to different psychopathology-associated physiological consequences. Alternatively, the difference might be the result of relatively higher levels of residual symptoms and shorter time to remission in the remitted MDD group. Persons who have had a depressive episode often continue to have elevated depressive symptomatology after remission (16,17). In NESDA we indeed found that remitted MDD patients on average still reported mild symptomology, whereas patients with remitted anxiety disorder scored below the clinically relevant cut-off scores on self-reported symptoms inventories. Further, our cross-sectional studies showed that time since remission was positively associated with telomere length in the remitted anxiety disorder group, but not in the remitted MDD group. It should be noted that these findings might still be in line with a dose-response relationship. In Chapter 3, we found that anxiety severity scores were indeed negatively

associated with telomere length across the total study sample, including controls, remitted and current patients, suggestive of a dose-response effect (14).

In contrast with the rather convincing evidence of a cross-sectional association between depression and telomere length described above, Chapter 4 displayed a contradicting picture. In this study by Schaakxs et al. (18), we showed that telomere length did not differ between 355 individuals with current late-life depression and 128 never-depressed controls from NESDO. Persons in the study sample had an average age of 70.5 years with a range of 60 to 93 years. Telomere length was related to age, sex and the number of chronic diseases, but not to any of the clinical characteristics such as depressive symptom severity, duration of the disorder, comorbid anxiety disorder, apathy severity or antidepressant use. While this is conflicting with the substantial evidence for an association in younger adults, our results correspond with two studies that failed to find an association between self-reported depressive symptoms and telomere length in older adults (>70 years) (19,20). Further, Phillips et al. (21) found depressive symptoms to be related to telomere length only in a young cohort of 37 year-olds, but not in middle-aged (57 years) or older (76 years) adults. Wikgren et al. (22) and Hoen et al. (4), with samples that had a mean age of 59.1 years and 66.7 years respectively, did find shorter telomere length in the depressed, which might be due to their slightly younger samples and larger standard deviations (11.9 and 10.6 versus 7.3 in our study) suggesting a larger age range and consequently a higher chance of picking up the cross-sectional telomere difference in younger (<60 years) adults. In their meta-analysis, Schutte et al. (8) found a borderline significant association between mean age and effect size, indicating that the lower the mean age of the sample, the stronger the association between depression and telomere length. A possible explanation for this weakening of the depression-telomere length association with advancing age might be that as a result of increased aging-related diseases and accumulated damage over the life span in both the depressed and in controls, “cellular” effects of depression alone might not be detectable. Further, etiology and presentation of depression differs between younger and older adults (23), and consequently varies in associated physiological dysregulations. A last explanation for null-findings in NESDO might be that a study cohort especially recruiting older persons might be biased by a healthy survivor effect; that is, those with the most damaged physiology and possibly shortest telomeres might have passed away or were in such bad condition that participation in a scientific study was not feasible. Consequently, results of studies in older persons including NESDO might show an underestimation of the true effect.

Conclusion 1: MDD and anxiety disorders are cross-sectionally associated with shorter telomere length. Associations between MDD and telomere length were not similarly present in older adults (>60 years).

2.2 Is psychosocial life stress cross-sectionally associated with telomere length?

The first evidence of an association between psychological stress and cellular aging came from a collaboration of health psychologist Elissa Epel with biochemist and Nobel Prize laureate Elizabeth Blackburn at the University of California, San Francisco. In 2004 they published a study that examined telomere length in a sample of 39 stressed caregiving mothers with chronically ill children compared with 19 age-matched controls (24). They found that the years of providing care to a chronically ill child was related to shorter telomeres in the caregiving mothers, whereas the perception of stress was related to shorter telomeres across the complete sample. Researchers have since then linked psychosocial stress to shorter telomeres in a variety of samples, recently summarized in a meta-analysis by Schutte et al. (25). This meta-analysis found a significant effect size ($r=-0.25$; $p<.001$) for the association between perceived stress and telomere length in 1,143 persons from eight studies. These studies included caregivers of chronically ill children or Alzheimer's disease patients, women with a sister with breast cancer and persons with a somatic condition such as fibromyalgia, chronic pain or mastocytosis. Similarly, shorter telomere length was found in women with a history of partner violence (26) and even in relation with perceived poor neighborhood quality (27) which might be a proxy for psychosocial stressors such as vandalism or crime. In line with this, Chapter 6 showed that experiencing recent stressful life events was related to shorter telomeres in 2,936 participants of NESDA (28). Stressful life events included, among others, the loss of a friend or family member, losing a job, separation from a partner or serious financial problems. Interestingly, we found that persons with life events that happened in the past year ($p=.028$) or 1 to 5 years ago ($p=.018$) had shorter telomere length than those without any life events, while the association was no longer evident when life events happened more than 6 years ago ($p=.100$). Thus, the more proximal the stressors occurred to telomere length assessment, the more likely they were associated. Similar to the findings in remitted anxiety disorder patients in Chapter 3 – but not remitted MDD in Chapter 2 – this might suggest that people are to some extent able to physiologically recover from psychosocial stress.

Another topic of interest in recent years was whether psychosocial stress early in life, such as adverse events that happened during childhood, are associated with shorter telomeres in adulthood. This might help explain the enduring consequences of childhood adversities into adulthood, including increased risks for psychiatric disorders and several somatic conditions (29). In 2010, Tyrka et al. (30) were the first to suggest shorter telomere length in 10 persons who reported histories of childhood maltreatment compared with 21 individuals without histories of maltreatment in childhood. Since then, studies examining associations of telomere length with childhood life events, such as the death of a parent, and childhood trauma, including maltreatment or neglect, yielded

mixed results (29,31). Chapter 6 examined associations between retrospectively assessed childhood adversities and adult telomere length in 2,936 NESDA participants. We found no associations between telomere length and any of the early life events, including parental loss, divorce of parents, separation from home, emotional neglect and psychological, physical or sexual abuse. These results corresponded to research of Glass et al. (32) who found no telomere length difference between 123 persons that reported maltreatment in childhood versus 1,751 controls, and Savolainen et al. (33) who found no difference between 215 persons had had been temporarily separated from both parents in childhood and 1,271 controls. In contrast, large-scale studies by Kananen et al. (N=974) (11) en Surtees et al. (N=4,441) (34) did find shorter telomeres in those that experienced adversities during childhood. An explanation for these conflicting outcomes could be that studies with positive results actually captured enduring psychosocial stress during life as a consequence of childhood adversity, and thus might reflect an association of short telomeres with recent and early life stress together rather than early life stress alone. This suggestion is supported by two studies that found effects of childhood adversities on adult telomere length, but only in patients with current posttraumatic stress disorder (35) or persons who had also experienced traumatic events across the life span (33). Along those lines, it might be possible that mitigating psychosocial or genetic factors protect some, but not all, individuals with early trauma from durable physiological dysregulations. On the contrary, early life stress might indeed impact cellular aging, as a consequence of altered responsiveness of the nervous system and immune system (36) and some studies failed to find this association due to methodological flaws; for example, the possible inaccuracy of retrospective measures of childhood events due to errors in recall, or the use of study samples with a wide variety of psychological and somatic health statuses, thereby masking direct effects of childhood adversity on adult telomere length. Evidence of an association between early life stress and telomere length thus remains inconclusive and future research, preferably in longitudinal nationally representative samples, should provide clarification.

Interestingly, a study by Schaakxs et al. (37) in the NESDO sample again failed to find any association between measures of psychosocial stress, including childhood adversities, recent stressful life events and loneliness, with telomere length in older adults. This adds evidence to the hypothesis that accumulated (cellular) damage over the life span might make it improbable to detect effects of one sole factor, such as childhood trauma or reported loneliness, on the cellular level in the elderly. Another finding of note here is that several studies found evidence for an association between psychosocial stress during childhood and telomere length concurrently measured in children (38-40), which provides further evidence for the association of recent psychosocial stress and shorter telomere length.

Conclusion 2: Recent psychosocial life stress is associated to shorter telomere length, with indications that the more proximal to telomere length measurement, the stronger the association. Evidence of associations between early psychosocial life stress and adult telomere length remains inconclusive.

2.3.1 What are the longitudinal trajectories of depressive and anxiety disorders and telomere length over time?

Research in the past decade has established a cross-sectional association between depressive and anxiety disorders and shorter telomere length, as discussed above. An interesting question that arises from these findings is whether this indication of accelerated cellular aging in these patient groups is reversible. In other words, is telomere shortening associated with having a depressive or anxiety disorder permanent or is recovery from such disorders accompanied by a normalization of telomere length? This is the central question of Chapter 7 in which we discussed whether cellular aging in depression specifically, reflects a permanent imprint or a reversible process (41). The main mechanisms to restore telomeric DNA is the activity of the ribonucleoprotein cellular enzyme telomerase. Both animal research and *in vitro* studies have shown that upregulation of telomerase activity can restore telomere length, for example in telomerase-deficient mice (42) and in stem cells derived from dyskeratosis congenita patients (43). The extent to which these findings can be generalized to *in vivo* human cells remains, however, unclear.

Longitudinal studies in humans show that while the majority of people shorten or maintain telomere length over time, a subsample shows telomeres lengthening. As we describe in Chapter 7, it is difficult to distinguish whether this represents actual telomere lengthening or rather a "pseudo-lengthening" due to a redistribution of cell subpopulations in the blood – since telomere length is often measured in whole blood leukocytes (41,44). Either way, telomere lengthening, counteracting the normal process of shortening due to the end-replication-problem, seems to some extent possible. A consistent finding in longitudinal research, moreover, is that longer telomeres at baseline tend to shorten more over time, while shorter telomeres have a higher chance of lengthening. This effect is suggested not to be simply due to methodological 'regression to the mean' (45). Rather, it might reflect a compensatory effect, possibly resulting from an internal telomere homeostasis system, where telomerase has a selective preference for critically short telomeres, thereby protecting the chromosome from end-to-end fusion and genome instability (46).

Chapter 7 further provides a summary of intervention studies that aimed to impact telomere length or telomerase activity. Interventions range from lifestyle changes to

social support and yoga or mediation practice, and have overall yielded mixed results (41). Intervention studies in psychiatric samples specifically are very limited. One study by Wolkowitz et al. (47) examined effects of antidepressant medication on telomerase activity in 16 medication-free depressed individuals. They reported that lower baseline telomerase activity predicted a better treatment outcome after an eight-week treatment with sertraline, although telomerase activity did not significantly change with antidepressant treatment. Based on literature described up until now, which consists of cross-sectional studies in depressive and anxiety disorder patients and intervention studies aimed at impacting telomere homeostasis mostly in non-psychiatric samples, it remains unclear to what extent telomere length changes dynamically along with the course of depression or anxiety disorders. The next paragraph will further elucidate this important question by discussing evidence from two longitudinal, observational human studies described in Chapter 8 and 9 of this thesis.

In 2011, Hoen et al. (4) were the first to present a study with depressed patients and two measures of telomere length over time. Data from both time points was complete for 127 MDD patients and 481 non-depressed participants; and results showed that MDD at baseline was not predictive of five-year change in telomere length after adjustment for baseline telomere length and several other covariates. Shalev et al. (48) reported on longitudinal data from New Zealand's Dunedin study in which data on internalizing disorders (depression, GAD and PTSD) was collected on multiple time points between age 11 and 38 years, and telomere length was assessed at age 26 and 38 years. They found that the number of assessments with an internalizing disorder between age 11 and 38 years was negatively related to telomere length at 38 years in 419 men, but not in 408 women. Further, men with depression (N=80) or GAD (N=30) between 26 and 38 years had greater telomere shortening than those without a diagnosis (N=290) in that same time frame. Again, this association was not present in women with internalizing disorders. These results suggest that having a depressive or anxiety disorder is associated with faster telomere attrition in men. In Chapter 8 of this thesis we examined the course of telomere length and depressive and anxiety disorder data over time in the NESDA study. The study sample consisted of 2936 persons at baseline and 1883 at 6-year follow-up; a total of 1860 persons had complete data on both time points. First, we showed that when looking at 4819 (2936 + 1883) observations of the baseline and 6-year follow-up assessments together (while taking within-person correlations due to multiple observations per participant into account), persons with current (p=.007) or remitted (p=.021) depressive and anxiety disorders had consistently shorter telomere length than control subjects. Also, depressive (p=.007) and anxiety (p=.009) symptom severity was negatively related to telomere length over 6 years. These results replicated our cross-sectional studies described in Chapter 2 and 3. Further, resembling Hoen et al. (4), we

showed that baseline diagnosis was not related to telomere attrition rate over 6 years. Since the NESDA study contains data on psychiatric status at baseline, 2-year, 4-year and 6-year follow-up, we were able to test whether the course of depressive and anxiety disorder over 6 years was related to telomere length over the same time period. We created six groups based on diagnosis status at the four assessments: 1) a control group; 2) those with a first onset after baseline; 3) a group that had a remitted diagnosis during all four assessments; 4) a group that was remitted at baseline but relapsed over the 6-year course; 5) a group with a current diagnosis at baseline who remitted during the 6 years; and finally 6) a chronic group that had a current diagnosis at both baseline and 6-year follow-up. The group that remitted (p=.011) and the chronic group (p=.041) had significantly shorter average telomere length than the control group, but interestingly, none of the groups showed faster telomere attrition compared to the control group (group-by-time interaction p=.926). The pre-existing differences in telomere length between cases and controls did thus not become, for example, more pronounced in the chronic group or diminished when persons remitted. Also, changes in depressive or anxiety symptoms severity scores and number of months with symptoms did not correlate with changes in telomere length over six years. These results, altogether, suggest that persistence of depressive and anxiety disorders does not accelerate telomere shortening and that recovery of those disorders is not accompanied by recovering of telomere length or a deceleration of the telomere attrition rate.

Chapter 9 consists of another longitudinal study on depression and cellular aging. This study looked at the 10-year longitudinal trajectories of depressive symptoms and two markers of cellular aging, namely telomere length and mitochondrial DNA (mtDNA) copy number, in 977 participants of the Coronary Artery Risk Development in Young Adults (CARDIA) study. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression (CES-D) scale, available at follow-up evaluations from year 15, 20 and 25. Telomere length was also available at those three time points, and mtDNA copy number was measured at year 15 and 25. Replicating cross-sectional studies, we again showed that those with a CES-D score above the cutoff of 16, indicative of clinically relevant depressed mood, had shorter average telomere length (p=.015) when analyzing all observations together. Because of the multiple time points, we were further able to determine whether the relationship between depression and telomere length could be explained as stable or rather as a dynamic within-person effect. Results showed that high levels of depressive symptomatology over the ten years were associated with short average telomere length (p=.016), but within-person changes in depressive symptoms were not associated to concurrent changes in telomere length (p=.551). In line with the findings of Chapter 8, this suggests a between-person rather than a within-person relation of depression and telomere length, since a person's telomere length did

not change along with changes in that person's level of depressive symptomatology. Further, both Chapter 8 and 9 showed that the rate of telomere shortening was not a function of depressive or anxiety disorder status or level of symptomatology. This is in contrast with suggestions made by the majority of the cross-sectional studies, including our studies in Chapter 2 and 3, which argue that shorter telomere length in depressed or anxious patients is evidence of *accelerated* telomere shortening.

The inference that depressive and anxiety disorder characteristics and telomere length show a rather stable relationship but do not dynamically change together over time either suggests that 1) short telomere length antedates the onset of depressive or anxiety disorder, possibly due to a 'third factor' underlying this association; or 2) depressive and anxiety disorder-related physiological disturbances indeed accelerate telomere shortening but telomere length is, despite eventual remission, never able to recover and can thus be seen as a long-lasting cellular scar. The first suggestion may indicate the involvement of several proposed underlying 'third factors'. Genetic heritability is such a factor; since both telomere length and depressive and anxiety disorders are partly heritable there is a possibility that shared genetic effects might impact both. Twin studies estimated the heritability of depression at 37% (49). The heritability of telomere length was estimated to be as high as 64% to 70% (50,51), with a heritability for telomere attrition rate of around 28% (51). Further, recent genome-wide association studies (GWAS) have identified several Single Nucleotide Polymorphisms (SNPs) that impact telomere length of which the majority was located on genes that are known to be involved in telomere biology (TERC, TERT, NAF1, OBFC1, RTEL1) (52). Remarkably, a recent study showed that genetic variation on the human TERT (*h*TERT) gene (i.e. the SNP rs2736100, previously associated with shorter telomere length (52)), was associated with adult depression (but only in those without childhood adversity) and to the number of depressive episodes in bipolar disorder patients (53). An epidemiological study, further, found that 50 healthy young daughters (10-14 years) of mothers with recurrent depression had shorter telomeres compared to 47 girls with mothers without lifetime psychopathology; showing that those with elevated familial risk of depression might already present shorter telomeres at an early age (54). However, since GWAS in depressive and anxiety disorders have overall been inconsistent in finding associated SNPs (49), future research should further elucidate the possibility of a shared genetic risk for shorter telomeres and psychopathology.

Another possible 'third factor' is chronic inflammation. A large body of research suggests that prolonged activation of the immune system enhances vulnerability to depression. Elevated levels of pro-inflammatory cytokines and acute phase proteins, such as interleukin-6 (IL-6), c-reactive protein (CRP) (55), might directly evoke depressogenic states (56). Further, high cytokines levels in the brain might impact neurotransmitter

metabolism and reduce neurogenesis (particularly in the hippocampus), thereby contributing to the pathophysiology of depression (57). Interestingly, *in vitro* studies provided evidence that pro-inflammatory markers may also directly accelerate telomere shortening (58,59), which is further supported by similar associations *in vivo* (60,61). Possible mediating roles of inflammatory factors will be further discussed below.

The second explanation, that depressive and anxiety disorders accelerate telomere shortening "for once and for all", indicates that one depressive or anxious episode causes a long-lasting difference in telomere length. This might, as discussed before, be a consequence of considerable subthreshold symptomatology that accompanies many persons after remission – or even before first onset (16). In this sense, one could argue that the established telomere length differences reflect a difference between 1) those have a high tendency to be unhappy or anxious during their lifetime, and consequently to develop clinical disorders; and 2) those that do not have such propensities. If this was the case, short telomere length might already be found in persons who have had only one depressive or anxiety disorder episode. A recent study by Henje Blom & Han et al. (62) indeed showed that shorter telomere length was already present in 54 un-medicated adolescents with MDD (13-18 years) compared to 63 controls. This suggests that it is therefore unlikely that telomere shortening is only the result of accumulated years of depression exposure, and provides further evidence for a between-person relationship. With the current evidence, however, we cannot conclude whether telomere shortening is a direct effect of being prone to depressive or anxiety disorders and the associated physiological disturbances, or whether short telomere length antedates the onset of depressive or anxiety disorder as a consequence of heritability or other factors.

Conclusion 3.1: Depressive and anxiety disorders and telomere length show a rather stable between-person relationship, but do not dynamically change together over time. Whether short telomere length is a long-term consequence or a pre-existing risk factor for the development of depressive or anxiety disorders remains to be explored.

2.3.2 What is the longitudinal trajectory of depression and mtDNA copy number over time?

This paragraph covers the relationship between depression and mtDNA copy number, which is a relatively novel biomarker of cellular aging. The marker can be best described as the number of copies of the mitochondrial genome per cell, of which a sufficient amount is found to be essential for healthy cellular function (63). As introduced in the previous section, Chapter 9 described data on depressive symptomatology and two markers of cellular aging, telomere length and mtDNA copy number, of 977 participants

of the CARDIA study. Specifically, mtDNA copy number was measured at the year 15 and 25 follow-up evaluations of CARDIA. When looking at those with complete data on CES-D and mtDNA copy number at year 15 and 25, together providing 1954 (2 x 977) observations, we did not find evidence of an association between depressive symptomatology and mtDNA copy number (p=.610). Further, the rate of decrease in mtDNA copy number was not a consequence of depressive symptomatology. Overall, results of the CARDIA study showed no associations between depressive symptoms and mtDNA copy number, whether we examined associations averaged for the ten years, at each time point, or over time. This finding is in line with the cross-sectional study by He et al. (64) who found no difference in mtDNA copy number between 210 depressed patients and 217 controls. However, two other studies did find a negative association with the number of mtDNA copies and depressive symptomatology in ±130 elderly women (65,66). In contrast, Tyrka et al. (67) found higher mtDNA copy number for 59 persons with lifetime depression compared to 113 controls. This positive association of copy number and lifetime depression was also found by a recent large scale (N>10,000) study by Cai et al. (10). Because the sample size of the latter study greatly exceeds sample sizes of studies up until now, including our own study, this finding warrants consideration. In contrast with our study which examined depression by the self-reported CES-D scale, participants in the Cai et al. study were actually diagnosed with recurrent clinical depression. High self-reported depressive symptomatology only indicates having an actual clinical diagnosis in a subsample of persons (e.g., (68)). This might suggest that mtDNA copy number is altered in those with clinical depression, rather than in those with only elevated self-reported symptomatology, and differences in mtDNA might thus only been captured in the most severe cases.

Conclusion 3.2: Alterations in mtDNA copy number might only be present in those with clinically diagnosed depression rather than in those with elevated depressive symptomatology. Overall, it should be noted that the inconsistent results indicate that the study of mtDNA copy number in psychiatry is still in its infancy.

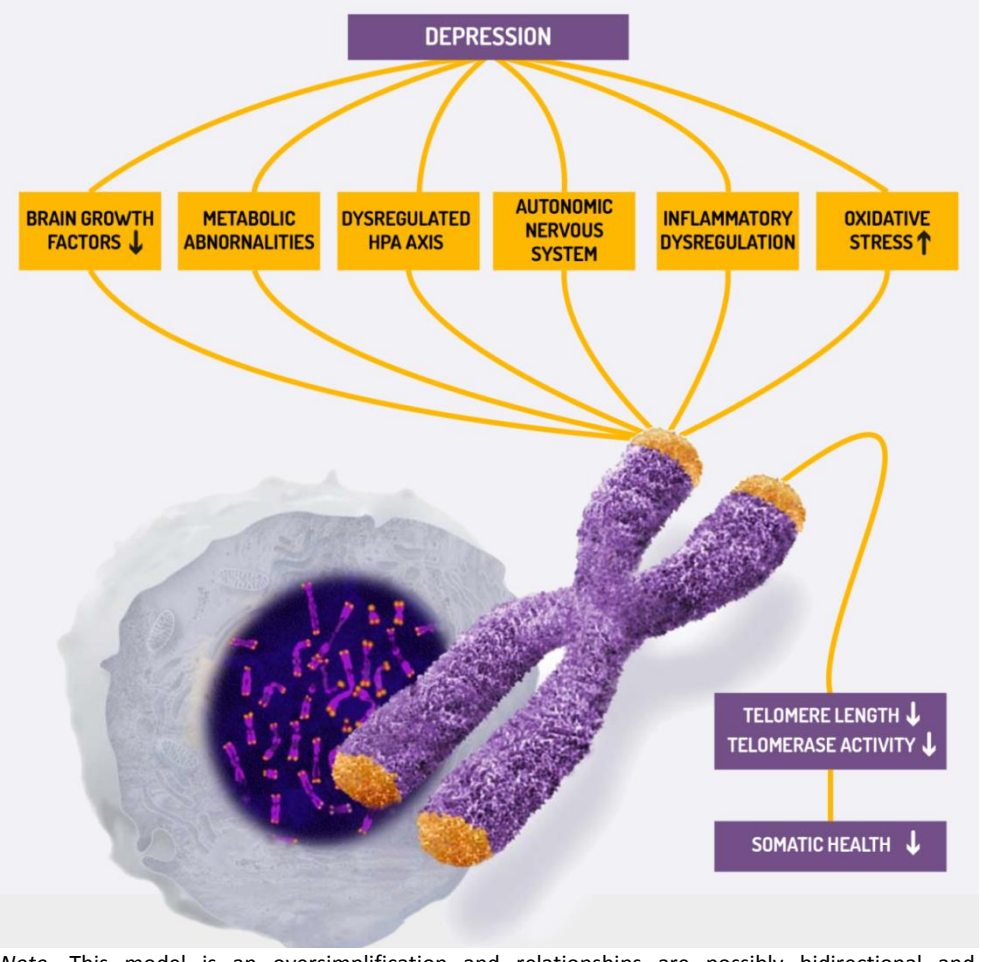
2.4 Which pathways underlie the association between depressive and anxiety disorders and telomere length?

In Chapter 7 we propose several pathophysiological mechanisms that may link depression to telomere shortening (41). These mechanisms include increased inflammation, elevated oxidative stress, dysregulations of the hypothalamus-pituitary adrenal (HPA)-axis and the autonomic nervous system, metabolic abnormalities and reduced brain-derived neurotrophic factor (BDNF) (see Figure 1), since most of these dysregulations have been

found both in association with depression (and often anxiety disorder) and short telomere length. While previous research indeed suggests associations between physiological dysregulations and short telomere length, findings remained inconsistent. In Chapter 10, we examined associations between telomere length and components of three major physiological stress systems: the immune-inflammatory system, the HPA-axis and the autonomic nervous system, in 2936 participants from NESDA (61). Inflammation markers included IL-6, CRP and tumor necrosis factor-alpha (TNF-α); HPA-axis indicators were salivary cortisol awakening curves, evening levels and 0.5 mg dexamethasone cortisol suppression ratio; and autonomic nervous system measures consisted of heart rate, respiratory sinus arrhythmia and pre-ejection period. In adjusted models, we found negative, linear associations between telomere length and CRP (p=.04), IL-6 (p=.05) and pre-ejection period (p=.001). Further, shorter telomere length was found in subjects with the highest tertiles of CRP (p=.06), IL-6 (p=.02), area under the curve with respect to the increase (AUCi), as a measure of the dynamic of the cortisol awakening response (p=.01) and pre-ejection period (p=.003). A borderline significant association was found between short telomere length and the highest tertile of heart rate (p=.07). In order to examine the impact of multiple concurrent dysregulations, a cumulative index score was calculated for the stress system indicators that showed the expected relationship with telomere length and were significant at least at p<.10. This score consisted of the number of times a subject scored in the highest tertile of CRP, IL-6, AUCi and heart rate, thus ranging from 0 to 4. We found that the more dysregulations, the shorter the telomere length (p=.002). These relationships were consistent in both healthy subjects and subjects with current and remitted depressive or anxiety disorders in NESDA. Overall, this study provided evidence that persons with more activated physiological stress systems have shorter telomere length than those with average functioning ones, which is in line with the associations of psychological stress – operationalized by chronic stress in conditions such as depressive or anxiety disorders or the experience of psychosocial stress – and telomere length. However, Chapter 10 does elucidate whether the described components actually mediate the relationship between depressive and anxiety disorders and telomere length. This question is addressed in Chapter 11 of this thesis.

In Chapter 11, we examined the extent to which physiological dysregulations and several lifestyle factors underlie the relationship between depressive and anxiety disorders and telomere length in 2750 participants of the NESDA study. To investigate this we performed mediation analyses according to the method by Hayes and Preacher (69). We first showed the cross-sectional associations between telomere length and current diagnosis (B=-63.3; p=.024), depressive symptoms severity (B=-2.4; p=.002) and anxiety symptom severity (B=-2.8; p=.009), corrected for sociodemographics and somatic health. As potential mediators we included physiological stress system markers,

Figure 1. Schematic model of pathways that explain the potential links between depression and telomere shortening.



Note. This model is an oversimplification and relationships are possibly bidirectional and interrelated. Figure adapted from Verhoeven et al. (41)

metabolic dysregulations and lifestyle factors, based on earlier associations in NESDA and other research. We selected the physiological stress system components that were associated with telomere length in Chapter 10, namely CRP, IL-6, salivary cortisol awakening response (or: AUCi) and heart rate (61). Metabolic syndrome components that were previously associated to telomere length included waist circumference, high-density lipoprotein (HDL) cholesterol, triglycerides, and fasting glucose levels (70), and lifestyle factors included alcohol use, smoking, physical activity and body mass index (BMI). Mediation analyses showed that higher CRP, IL-6, waist circumference, triglycerides and cigarettes per day and lower levels of HDL cholesterol significantly mediated the

association between depressive symptoms severity and telomere length. When those mediators were entered together in a multivariate model, they reduced the direct effect of depressive symptoms on telomere length with 36.9%, and the effect became statistically non-significant (B=-1.54; p=.06). Similarly, the mediators reduced the effect size of the association between anxiety symptoms and telomere length with 49.1% rendering the direct effect non-significant (B=-1.43; p=.20). On the association between current depressive or anxiety disorder diagnosis and telomere length, waist circumference, triglycerides and cigarettes per day were significant mediators, together reducing the effect size with 32.7%. Again, the direct effect of current diagnosis on telomere length was no longer significant (B=-42.6; p=.13). Altogether, this Chapter showed that the association between depressive and anxiety psychopathology and telomere length was partly explained by higher inflammatory markers (CRP, IL-6), less favorable metabolic profile (high waist circumference and triglycerides and low HDL cholesterol) and increased smoking behavior.

Cigarette smoking was found the be one of the largest mediators, reducing the direct effect of the psychopathology characteristics on telomere length with ~25%. As we discussed in Chapter 11, persons with depressive and anxiety disorders have a well-established increased odds of being a smoker. Smoking, in turn, is associated with several physiological alterations such as increased cortisol, oxidative stress and secretion of inflammatory markers. In a paper by Révész et al. (71), cigarette smoking was also found to be a determinant of short telomere length in NESDA (p<.001). This suggests that persons with depressive and anxiety disorders may be at additional risk of worse health outcomes if they are current smokers and emphasizes the need for smoking cessation interventions in mental health care settings. The mediating effects of metabolic dysregulations, especially waist circumference and triglycerides, further suggest that the association between depressive and anxiety disorders and telomere length may be partly explained by increased adiposity, possibly as a consequence of sedentary behavior combined with high energy intake (unhealthy diet).

It should be noted that because of the cross-sectional design in Chapter 11, we could not draw conclusions regarding the direction of the effects. The causality of the association between psychopathology and inflammation, for instance, might be from psychopathology to inflammation, inflammation to psychopathology or bidirectional. In their large meta-analysis on depression and CRP, IL-1 and IL6, Howren et al. (55) suggest that depression may lead to increased inflammation through elevated sympathetic and decreased parasympathetic nervous activity and sedentary behavior; inflammation may in turn invoke states that resemble depression such as anhedonia, sleep and appetite changes; or depression and inflammation may influence each other in a complex, bidirectional process with interplay of adiposity, the central nervous system and the HPA-

axis. Our cross-sectional mediation analysis thus shows that part of the effect of depressive and anxiety disorders on telomere length is explained by increased inflammation, but longitudinal studies are needed to clarify cause and effect. This also accounts for the mediating effects of metabolic syndrome components high waist circumference, triglycerides and low HDL cholesterol.

Finally, animal models might augment insight into the causality of pathways underlying the association of depressive and anxiety disorders with cellular aging markers. Cai et al. (10), reviewed earlier in this discussion for their work on telomere length and mtDNA copy number in humans, also presented results from a mouse model. In this model, laboratory mice were exposed to various stressors for four weeks, including tail suspension, force-swim and sleep deprivation. After these four weeks the stressed mice had more than 200% increased mtDNA copy number and a 30% decrease in telomere length compared to non-stress control mice, both in blood and saliva samples. To test what might be inducing these molecular changes, Cai et al. tested the possibility of the HPA-axis as a mediating mechanism. They administered corticosterone to mice of the same strain and showed that after four weeks, the effect of corticosterone on mtDNA copy number and telomere length was similar to the effect of the behavioral stressors (10). Interestingly when the behaviorally stressed mice were kept for another four weeks to recover, their mtDNA and telomere length levels returned to the levels of the controls. This suggests that molecular changes are indeed partly reversible. In contrast with our study in Chapter 11, this study proposes that telomere shortening and mtDNA copy number increase after stress are at least partially mediated by activation of the HPA-axis. Important to consider in this context, however, is that mice have longer telomeres than humans with complex dynamics, and extrapolating findings from laboratory models to human aging might results in problems (72). Future research should examine mediating mechanisms in both animal models and human studies to clarify to what extent they can be generalized back and forth.

Conclusion 4: The association between depressive and anxiety disorder psycho-pathology and short telomere length is partly explained by pro-inflammatory cytokines, metabolic dysregulations and cigarette smoking. Directions of the effects should be elucidated by future prospective studies.

3. CLINICAL IMPLICATIONS

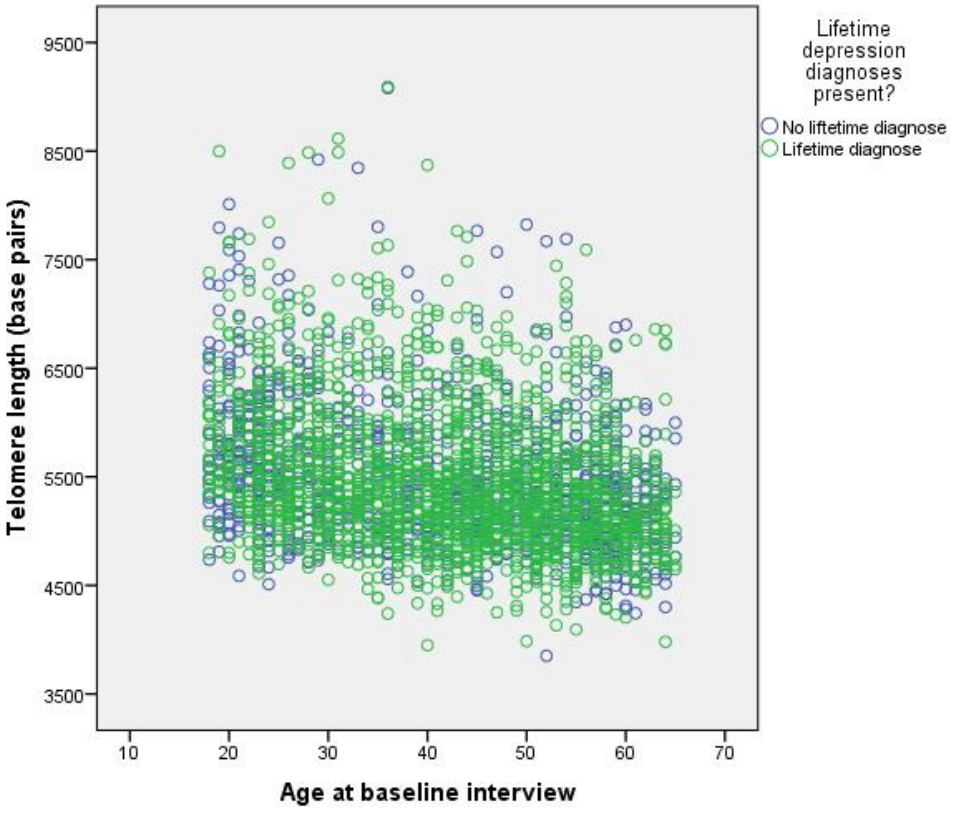
3.1 Telomere length as a biomarker in psychiatric research

The literature reviewed above shows that the associations between telomere length and depressive and anxiety disorders have been fairly well established. Persons with a lifetime diagnosis have on average shorter telomere length than those without psychopathology during their lifetime. The term 'on average' in the previous sentence is of importance in discussing possible clinical implications of telomere length. This indicates that the two groups differ in their mean telomere length, but that this telomere length difference is not necessarily translated to every individual. Visually displaying average telomere length in relation to age at NESDA's baseline assessment for those with lifetime depression versus those without shows a large inter-individual variability (Figure 2). For example, at age 65, several persons with a lifetime depression diagnoses (in green) have longer telomere length than most of the controls (in blue) at that age. Also, the person with the shortest telomeres is someone without a lifetime depression diagnosis (in blue at age 52).

This picture suggests that telomere length is not a suitable biomarker that can be applied to diagnose psychiatric disorders in individual patients. Furthermore, the meta-analysis in Chapter 5 showed was no significant difference in the effect size estimates between the different psychiatric disorder subgroups, including depressive disorders, psychotic disorders, bipolar disorder, PTSD and other anxiety disorders. This is also observed in a recent review by Lindqvist et al. (73) in which we conclude that telomere shortening "is not confined to specific traditional diagnostic categories." The evidence of shorter telomeres across different psychiatric disorders shows that short telomere length is a rather non-specific marker for conditions in which people experience chronic (psychological and physiological) stress, with therefore limited clinical properties.

The lack of specificity of telomere length to different psychiatric disorders is interesting in the context of current psychiatric research. Despite high expectations, research in the past decades did not provide many stable disorder-specific associations with biomarkers or neuroimaging correlates. This lack of robust biological underpinnings may be the consequence of high comorbidity between disorders, large heterogeneity within disorders and mechanisms that surpass the DSM-based categories. In response, psychiatric research is moving away from DSM-based categorical diagnoses to exploring whether they can be replaced by dimensional diagnoses, as proposed by the Research Domain Criteria (RDoC) initiative (74). The RDoC framework states that many biological and psychological phenomena, ranging from genetics and neural circuits to physiology and behavior, vary continuously within and between psychiatric patients and in the general population (75). RDoC's aim is to seek dimensional constructs (e.g., sustained threat) that relate to these phenomena (e.g., HPA-axis hormones, amygdala reactivity or attentional bias to threat), thereby surpassing traditional diagnostic categories. Short

Figure 2. Scatterplot telomere length and age at baseline by lifetime depression diagnosis



telomere length may be such a phenomenon, being dimensionally related to certain constructs associated with chronic stress.

3.2 Telomere length as a target for treatment

It should be noted that the evidence of a stable between-person association between depressive and anxiety disorders and telomere length presented in this thesis is largely based on data from observational studies. In our longitudinal observational studies in Chapter 8 and 9, for instance, we did not take into account whether persons recovered spontaneously or as a result of psychological or pharmacological treatment. Such treatments may actually impact telomere homeostasis, for example by increasing the activity of telomerase. Although most somatic cells have little telomerase and are consequently unable to maintain telomere length, the activity of telomerase can differ within cells. Preliminary evidence, reviewed in Chapter 7 (41), showed that increased telomerase activity was associated with e.g. decreased chronic stress and anxiety (76) or yogic mediation practice (77) in non-psychiatric samples. As reviewed earlier, in a small

study by Wolkowitz et al. (47), telomerase activity predicted a better treatment response to pharmacotherapy. In a recent review paper, we proposed the idea that telomerase activation could represent a mediating mechanism of action of certain psychopharmacological interventions (78). Preclinical evidence from animal research showed that chronic mild stress in mice led to a decrease in telomerase activity, and, interestingly, fluoxetine treatment was able to reverse this (79). Further, a study with an animal model of depression in rats showed that six weeks of lithium administration increased telomerase activity in the "depressed" rats (80). In one of the few studies in humans, however, no effect of lithium treatment on leukocyte telomerase activity was found in bipolar disorder patients (81). Although no strong conclusions can be drawn due to a lack of well-powered clinical studies, the review proposed several potential mechanisms by which psychiatric medications can modulate telomerase activity or TERT expression, such as increased BDNF expression. Increased telomerase activity may, in turn, initiate clinical effects by promoting cellular survival and/or functioning (78). It is of note, in this context, that increased telomerase activity is also found to be associated with cancer (e.g., in 80 to 90% of malignant human tumor cells, telomerase activity is up-regulated compared with normal tissue). Telomere maintenance by telomerase activity should thus balance between reducing risk of aging-related disease and avoiding less common but serious types of cancer (72).

In a currently ongoing randomized controlled trial at the VU University Medical Center, which was part of my PhD trajectory, another attempt is made to investigate the impact of psychiatric treatment on telomere biology. In this so-called MOTAR study (Mood Treatment with Antidepressants or Running; www.motar.nl), patients with MDD or anxiety disorder are either treated with pharmacotherapy (antidepressants, specifically selective serotonin reuptake inhibitors) or running therapy (vigorous exercise three times a week). Before and after these 16-week interventions, data is collected on psychological and psychiatric characteristics as well as telomere length and telomerase activity. Data of this intervention study will soon provide further insights into the potential interplay between antidepressants and running therapy and telomere homeostasis.

3.3 Telomere length as risk marker vs. risk factor

Short telomere length is associated with a wide variety of unfavorable somatic conditions, ranging from cardiovascular disease (82) and type 2 diabetes (83) to obesity (84) and poor cancer survival (85). The degree to which telomeres are causally involved in these conditions is, however, unclear. The rationale behind most psychiatric research on telomere length, is that shorter telomeres might help explain the increased risks of age-related somatic conditions found in most serious mental illnesses (86). James Coyne, professor of Health Psychology at the University Medical Center, Groningen, states that

these claims might be too far-fetched. Making such wild claims, he writes on his blog, is bad science and leads to distorted coverage in the media (87). He emphasizes the difference between risk markers, which are associated to an outcome, but do not meet the formal criteria for causality, and causal risk factors. Coyne warns for drawing exaggerated conclusions based on correlational research and argues that researchers should withhold themselves from such "biomarker porn". Indeed, with the current state of preliminary evidence, we can only speculate on the causal role of telomere length in both psychiatric and somatic health.

4. METHODOLOGICAL CONSIDERATIONS

4.1 Telomere length in leukocytes

In the three study cohorts described in this thesis, NESDA, NESDO and CARDIA, telomere length was measured in leukocytes (white blood cells). Leukocyte telomere length is the most used marker in large epidemiological studies, mainly since it can be obtained relatively non-invasive by blood draw. The three major categories of leukocytes are granulocytes, monocytes and lymphocytes. Lymphocytes are, in turn, divided into natural killer cells (innate immune system) and B-cells and T-cells (adaptive immune system). A caveat of using average leukocyte telomere length is that leukocytes thus consist of different cell types, with possibly different telomere dynamics. Since telomere length largely reflects stem cell replication, fully differentiated leukocytes might have shorter telomeres than precursor cells. Indeed, studies showed that B-cells had longer telomeres than T-cells, and within the latter group, $CD4^+$ and $CD8^+$ T-cells had similar telomere length (88). Similarly, average telomerase activity was highest in B-cells and $CD4^+$ T-cells had slightly higher telomerase activity than $CD8^+CD28^+$ T-cells. $CD8^+CD28^-$ T-cells, which are end-stage CD8+ T-cells, had the shortest telomere length and lowest telomerase activity. Rates of telomere shortening were also found to differ between leukocytes, with faster attrition rates for $CD4^+$ and $CD8^+$ T-cells than in B-cells (89). These between-leukocyte variations make it difficult to distinguish whether average telomere length differences are due to actual shortening / lengthening or rather to a redistribution of cell types. The lack of cell sorting, thus, is a limitation in most large-scale studies, including the studies presented in this thesis. However, since this is a time-consuming and expensive technique, large studies will have to consider this trade-off. Nonetheless, despite this limitation, average leukocyte telomere length has been shown to have clinical relevance as illustrated by its many associations with a variety of health outcomes.

Another issue important to consider when studying leukocytes is the extent to which leukocyte telomere length is generalizable to other types of tissues. One study measured telomere length from 87 adults (scheduled for surgery) in four different

tissues: leukocytes, skeletal muscle, skin an subcutaneous fat (90). Notably, they found a large inter-individual variation in telomere length, but strong and consistent associations between the four tissues within individuals. In other words, a persons that displayed long telomere length in one tissue typically displayed long telomere in the other tissues. Further, the four different somatic tissues showed similar age-dependent attrition rates, suggesting telomere length dynamics were similar in proliferative (blood and skin) and minimally proliferative tissues (muscle and fat) (90). Also, telomere length across tissues was thus similar regardless of their replicative activity (89). Similar within-person correlations in blood, fibroblast and buccal cell telomere length were found in 21 patients with inherited bone marrow failure syndromes (91), although correlations were less profound in a post-mortem study examining telomere length in twelve different human tissues, including leukocytes (92). In the earlier discussed mouse model by Cai et al. (10), consistent findings of decreased telomere length and increased mtDNA copy number were reported in both saliva and in blood, suggesting the generalizability of these markers between leukocytes and buccal cells. Overall, leukocyte telomere length seems generalizable to other tissues within the same person. An important question remains to what extent leukocyte telomere length correlates with certain brain tissues or functions, which are possibly relevant to mental illnesses. Two post-mortem studies of telomere length in cerebellar gray matter and the occipital cortex found no differences between MDD subjects and controls (93,94). One post-mortem study, however, found a slight trend towards a correlation between telomere length in leukocytes and the frontal lobe (92), but this remains to be replicated by future research.

4.2 Telomere length as a biomarker of aging

Telomere length is considered a biomarker of cellular aging. Its consistent relationship with age was confirmed by a large meta-analysis that included 124 cross-sectional and 5 longitudinal studies (95). The authors found an inverse correlation between telomere length and age ($r = -0.338$; $p<.001$) across cross-sectional studies and a yearly loss of 24 base pairs. Longitudinal studies reported slightly higher telomere loss rates between 32 and 46 base pairs per year (96). Further, as reviewed above, short telomere length correlates with a large variety of aging-related somatic diseases, although research from epidemiologic and clinical studies has sometimes been inconsistent (96). This could to be due to the suggestion that associations between telomere length and aging-related measures might vary across the life span (97). Two reviews that considered telomere length as a biomarker of human aging in general conclude that it remains unknown whether telomere length is a biomarker of aging for a whole organism or a biomarker of aging in specific tissues or specific periods in the life span (96,97). Future research should ideally follow humans during entire lifespans to investigate the role of telomere length in

the aging process – although this is a challenge since human lifespan often outlasts researchers or research projects.

As discussed in the introduction of this thesis, aging is a complex process that is characterized not only by telomere attrition, but also by several other markers including genomic instability, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication (see Figure 3) (98). Studies considering biological aging should thus ideally include multiple biomarkers as opposed to a single aging-indicator. This was done by Belsky et al. (99) who estimated the "Biological Age" of 954 38-year old participants of the Dunedin Study birth cohort, by applying an algorithm on 18 biomarkers. This Biological Age score included, among others, cardiorespiratory fitness, lung function, waist-hip ratio, telomere length, total cholesterol and creatinine clearance. Interestingly, despite the fact that they included relatively young and similar-aged persons, estimated Biological Age ranged from 28 years to 61 years. Further, the authors also calculated "Pace of Aging" by looking at within-individual longitudinal changes in these biomarkers across chronological ages 26 year, 32 year and 38 year. This Pace of Aging ranged from 0 years to 3 years of physiological change per chronological year. Persons with relatively high Biological Age at age 38 years, showed a faster Pace of Aging between ages 26 and 38 years (99). To further confirm the large between-person differences in Biological Age, Belsky et al. found that those with higher Biological Age perceived themselves to be in poorer health and they were perceived to be older by independent observers. Human aging should thus be considered a complex combination of multiple psychosocial, physiological and genomic exposures that differ between individuals.

4.3 Telomere length measurement

Several methods of telomere length assessment are available, each with their advantages and disadvantages. The most appropriate method might depend on the specific research question, the sample size and the required accuracy of measurements (97). The original technique to determine telomere length is Terminal Restriction Fragment (TRF) analysis by Southern Blot. In this technique genomic DNA is digested with a mix of frequent cutting restriction enzymes that are unable to bind to telomeres, hence fragmenting the genome except for telomeric DNA. The intact telomeres from all chromosomes together are then detected through either Southern blotting or in-gel hybridization. While errors of this technique are relatively small, important disadvantages are the need of substantial amounts of DNA (>10^5 cells) and the relative insensitivity to very short telomeres (100). Further, telomere length from TRF analysis might not be comparable across studies because techniques are not standardized with respect to restriction enzyme selection,

Figure 3. Hallmarks of aging

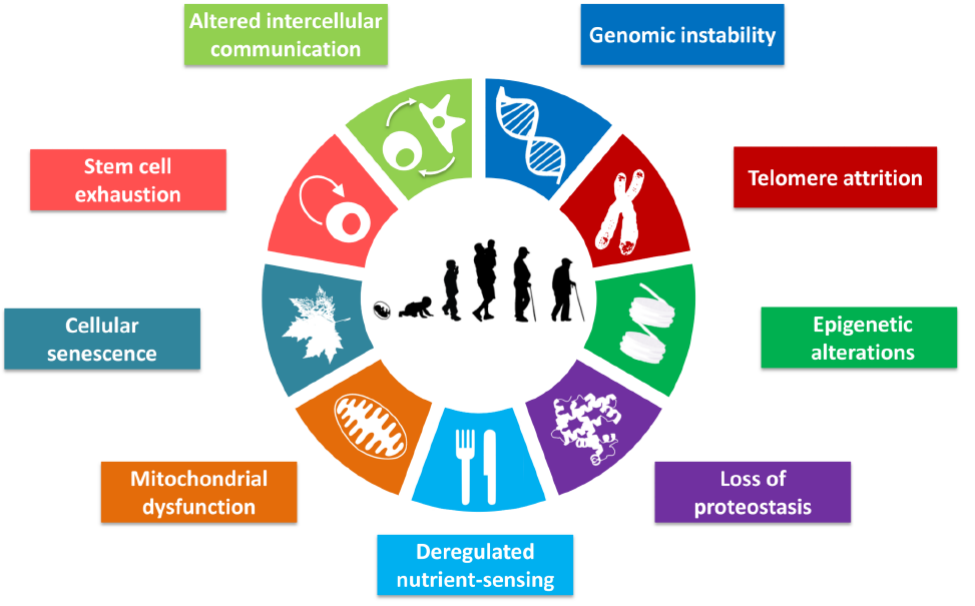


Figure adapted from López-Otín et al. (98)

starting DNA quantity and quality, and blot analysis. Single Telomere Length Analysis (STELA), on the other hand, has the ability to generate highly accurate and chromosome-specific telomere length with fewer DNA (50 cells), but this method has limited large-scale applications because it is labor intensive and technically challenging.

A more recent measurement method is quantitative (or real time) polymerase chain reaction (qPCR). In 2002, Cawthon (101) developed a protocol for measuring telomere length with qPCR which is now frequently used in epidemiology, including all studies in the current thesis. In this method, qPCR amplifies telomeric sequences over 20-30 cycles using specifically designed primers. The amount of telomeric (T) sequence is then quantified and compared to the amplification of a single copy gene (S), resulting in a T/S ratio, proportional to the average telomere length in a cell. The major advantages of qPCR are the low costs, short timeline and the high-throughput, while a concern is that the variability within and between samples is relatively high (102). TRF by Southern blot and T/S ratio from qPCR were shown to correlate rather highly ($r = 0.847$; $p<.001$) in a study comparing both measurements (103). This study further showed a lower inter-assay coefficient of variance (CV) for Southern blot (1.7%) compared to qPCR (6.5%) (103). To be able to compare T/S ratios to studies that assessed TRFs, T/S ratios is the studies of this thesis were converted into base pairs using a conversion formula. This

formula was based on an earlier comparison of T/S ratios and TRF analysis of genomic DNA samples from the human fibroblast cell line IMR90 (88). Currently, Southern blot and qPCR are thus the most frequently used measurement techniques by clinical and epidemiological studies. Other less widely used telomere length measurement techniques are Quantitative fluorescence in situ hybridization (Q-FISH) and flow FISH, which have a limited applicability to epidemiological studies since they require intact and viable cells. More details are described in an extensive review by Aubert et al. (100).

Telomere length in NESDA, NESDO and CARDIA was measured by qPCR by Lin's and Blackburn's labs, adapted from the original method by Cawthon (101). Each participants' sample was assayed three times, thus resulting in three T/S values. Subsequently, CVs were calculated, which represent the relative standard deviations (CV = mean / SD *100). In NESDA and NESDO, the inter-assay CV at baseline was 4.6% and 3.0% at six-year follow-up. Participant samples with a CV greater than 12.5% from the 3 T/S values were re-assayed to reduce the measurement error. Further, eight included quality control DNA samples on each PCR run illustrated an intra-assay CV of 5.1%. From a statistical point of view, such CVs of around 5% may be considerable if telomere length was studied at the individual level. However, they play a minor role when comparing mean telomere length between groups, since the measurement error is relatively small with respect to the within-group variance. As an example, calculating the additive effect of the variance as a result of the 5% CV showed that the total standard error on the group level increased from 11.4 to 12.4 (calculations not shown). This showed that when the variance belonging to such inter-assay CVs is added to the regular variance, the effect of these CVs boils down to a negligible amount of noise. The between-group differences in telomere length found in our studies are thus statistically meaningful despite the small telomere length measurement error.

A limitation of the longitudinal NESDA study in Chapter 8 is that the telomere length measurement was done two years apart and in different labs. This might have introduced batch-related noise between the two time points. However, to adjust for possible differences between telomere length at and baseline and 6-year follow-up, a total of 226 samples from baseline were rerun together with the follow-up samples. On average, the T/S ratios of the six-year follow-up runs were at 76% of the T/S ratios of baseline, which is likely to be caused by differential reference standards used. To convert the T/S ratios of the 6-year follow-up samples, they were divided by 0.76. It should be noted that, while it is not ideal to have data from two labs, that at both time points, cases and controls were randomly distributed over plates and wells. Therefore, it is unlikely that systematic lab differences may have masked differences in for example telomere length change between cases and controls, if they would have existed.

5. RECOMMENDATIONS FOR FUTURE RESEARCH

One of the most relevant next steps in telomere length research is to further elucidate how telomere length and telomerase activity behave over longer periods of time. The two longitudinal studies in this thesis captured telomere length over 6 and 10 years, and suggested a within-person stability of telomere length over time. However, studies with multiple assessments over different parts of the lifespan, including childhood, adolescence, old age, should shed more light on telomere length over the entire human lifespan. Factors influencing telomere length start as early as the moment of conception, where older paternal age is positively associated with the offspring's telomere length, attributed to the longer telomeres in sperm of older men (104). Further, intrauterine conditions might influence telomere length and the activity of telomerase (105) as a consequence of maternal psychosocial stress during pregnancy (105), possibly through stress-related maternal-placental-fetal processes. After birth, fast rates of telomere attrition during growth and development are observed, while attrition during adulthood (>20 years) is considered relatively small (51). In adulthood, telomere length was found to be largely genetically determined (64%), with smaller shared (22%) and individual (14%) environmental effects. Telomere attrition showed a lower heritability estimate (28%) and larger unique individual environmental effects (72%) (51). It thus remains an important question what environmental factors, including (mental) stress and lifestyle, can exert an impact on telomere length dynamics. Future research should, ideally, assess telomere length in large (birth) cohorts on a yearly basis, together with information on a wide range of physiological and psychological variables to be able to capture telomere length dynamics over the lifespan, and specifically for this thesis, provide information about cause and effect in the association with depressive and anxiety disorders.

Another interesting question that remains unanswered by the literature so far is the extent to which interventions specifically aimed at cellular aging processes can impact telomere length or mtDNA copy number. Three types of interventions might be of interest: stress-reducing interventions, interventions that impact lifestyle factors and therapies that aim to impact psychiatric health. First, while stress reduction might lead to normalization of cellular aging markers in animal models (10), it is unknown to what extent this is generalizable to humans. A recent study showed increased telomere length in 26 participants of a one-month full-time, residential, and silent meditation retreat compared to 30 controls (107), possibly by reducing psychological stress. Further, a small meta-analysis by Schutte et al. (108) provided the suggestion that mindfulness meditation leads to increased telomerase activity. It is of great interest which other stress-reducing interventions, such as relaxation therapy or yoga, might be able to impact telomere homeostasis. Second, since negative associations have been found between telomere length and smoking, alcohol use (6) and energy intake (109), interventions aiming to

change these factors might have an effect on telomere length. Further, results of a meta-analysis showed were suggestive of a positive association between physical activity and telomere length (110). Interestingly, two studies provided evidence for a moderating effect of physical exercise (111) and high levels of healthy behaviors (physical activity, healthy food and good sleep quality) (112) on the association between stress and shorter telomeres. Intervention studies should thus preferably aim at impacting multiple lifestyle factors, thereby inducing the largest possible effect on telomere homeostasis. Last, future studies should put an effort in examining interventions that might have beneficial effects on cellular aging for psychiatric patients. These interventions might also be multifaceted since this group has been found to have shorter telomeres and might therefore be at increased risk of detrimental health consequences. Since Chapter 11 showed that the association between depressive and anxiety disorders is partly explained by cigarette smoking and increased adiposity, this calls for the need of interventions stimulating smoking cessation, physical activity level or educating persons on healthy diet options in mental health care settings. Further, the role of telomerase activity is of interest as a possible mediating mechanisms in the beneficial effects of antidepressant medication (78). The previously introduced MOTAR study at the VU University Medical Center, aims to examine, among other things, the effects of running therapy or antidepressant medication on telomere length and telomerase activity in persons with depressive and anxiety disorder. Data collection is still ongoing, however, this study will soon provide more insight in the intervention-associated impact on telomere homeostasis.

6. DOES FEELING BLUE MAKE YOU GREY?

This thesis examined associations of depressive and anxiety disorders with telomere length, including their longitudinal relationship and underlying explanatory mechanisms. Overall, we provided evidence of advanced aging in those with depressive and anxiety disorders. This suggests that psychological distress, as experienced by persons with depressive or anxiety disorders, might indeed be associated with increased 'wear and tear' of a person's body. We showed that these associations were partly explained by dysregulated physiological stress systems and unhealthy lifestyle habits. Associations between telomeres and depressive and anxiety disorders were not very dynamic over the 6 and 10 years over which we followed persons, hence announcing a number of follow-up questions. The exciting challenge for future research is to determine whether feeling blue actually makes you grey, or else, what other colors - known or yet unknown to the human palette - are needed to paint a comprehensive picture.

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