Telomere Attrition as a Potential Biomarker of Accelerated Aging in Severe Affective Disorders

A Comparative Study

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Summary

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Title: Telomere Attrition as a Potential Biomarker of Accelerated Aging in Severe Affective Disorders. A Comparative Study.

Author statement: The current study was conducted with data from the Norwegian Centre for Mental Disorders Research (NORMENT). My primary supervisor, senior scientist Monica Aas, Ph.D., and my co-supervisor, Prof. Lars Tjelta Westlye, are both working with the NORMENT project. Hypothesis development, data processing, and analyses were conducted independently.

Background: Severe affective disorders are associated with increased risk of somatic disease, cognitive decline, and premature death. A proposed model of accelerated aging suggests that pathophysiological mechanisms may be intrinsic to the disease itself. Telomere length (TL) attrition is one proposed biomarker of such processes. Evidence suggest that affective illness may be related to accelerated aging represented by shorter TL compared to age-matched controls.

Aims: To explore if the occurrence and progression of affective disorders are related to shorter TL in a naturalistic sample of patients compared to age-matched healthy controls.

Methods: This cross-sectional study compared TL in blood samples from relatively young patients with DSM-IV severe affective disorders (n = 248) and healthy controls (n = 401). Analyses were performed to investigate whether TL was related to illness duration or number of lifetime affective episodes in the total patient sample. Additional comparisons were made between diagnostic sub-groups of bipolar disorder I (n = 159), bipolar disorder II (n = 67), and major depressive disorder (n = 22).

Results: Shorter TL was observed in patients compared to controls (d = 0.18, p = 0.02). Duration of affective illness was negatively associated with TL (b^* = -0.18, p = 0.046). No other findings were significant. However, these associations did not remain significant after stringent correction for multiple testing.

Conclusions: Trend level variations of TL were potentially due to the young age of participants. Still, the present findings suggest that increased telomere attrition may be linked to both the occurrence and duration of affective disorder in patients relative to healthy controls.

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List of abbreviations

MDD	Major depressive disorder
BD I	Bipolar disorder type 1
BD II	Bipolar disorder type 2
CVD	Cardiovascular disease
TL	Telomere length
qPCR	Qualitative polymerase chain reaction
SCID	Structural Clinical Interview for DSM
IDS-C	Inventory of Depressive Symptoms – Clinician rated Scale
YMRS	Young Mania Rating Scale

Table of contents

1 Introduction 1

1.1 Diagnostic considerations 1

1.2 Mortality and life expectancy 2

1.3 Accelerated aging in severe affective disorders 4

1.4 Telomeres 6

1.5 Current research objectives 10

2 Method 11

2.1 Organizational structure and study coordination 11

2.2 Participants 12

2.3 Measures and procedures 13

2.4 Ethical considerations 16

2.5 Statistical analyses 17

3 Results 21

3.1 Descriptive statistics 21

3.2 Main analyses 26

4 Discussion 30

4.1 Presentation of findings 30

4.2 Methodological considerations 33

4.3 Strengths 35

4.4 Limitations 36

4.5 Interpretation of findings 40

4.6 Future directions 43

4.7 Concluding remarks 43

References 45

1 Introduction

Patients with severe affective disorders, such as major depressive disorder (MDD) and bipolar disorder (BD), have mortality rates more than twice that of the general population (Chesney et al., 2014; Cuijpers et al., 2014; Laursen, 2011). Some of this reduction in life expectancy is related to a higher frequency of suicide and accidents, but severe affective disorders are also consistently linked with age-related somatic disease and dementia (e.g., Da Silva et al., 2013; Walker et al., 2015). Furthermore, a progressive illness course, characterized by more persistent episodes, treatment non-response, and cognitive and functional impairments, is suggested (Berk et al., 2017; Kessing & Andersen, 2017). As a result, researchers have hypothesized that patients with severe affective disorders might be characterized by accelerated aging relative to healthy individuals (Berk et al., 2017; Vance et al., 2018). However, the specific mechanisms driving these associations are still poorly understood.

1.1 Diagnostic considerations

The Diagnostic Statistical Manual, edition IV (DSM-IV; Guze, 1995) characterizes MDD as the occurrence of at least one major depressive episode lasting two weeks or more and involving distinct changes in mood, interest, and energy levels, cognitive dysfunction, and vegetative symptoms. BD is characterized by episodes of depression and elevated mood. These fluctuations are accompanied by changes in energy and activity and are associated with characteristic cognitive, physical, and behavioral symptoms. Mania refers to severe and sustained elevated or irritable mood that results in a marked disturbance of behavior and functioning. Hypomania is characterized by less severe elevations in mood and lower levels of disturbance (Goodwin et al., 2007; Johnson et al., 2011; Tondo et al., 2017). Mania, as well as hypomania, may be associated with a surplus of positive emotions and productivity. Even so, both hypomanic, manic, and depressive episodes typically have a substantial negative impact on the social, occupational, and financial situation of the individual (Goodwin et al., 2007). The DSM-IV classifies BD into type I, in which individuals experience at least one manic episode, and type II, in which individuals only experience hypomanic and depressive episodes. Some individuals also experience so-called mixed state episodes, in which manic and depressive symptoms co-occur (Johnson et al., 2011).

Cognitive deficits, in domains such as executive functioning, information processing, and verbal memory, are a common characteristic of depressive episodes and can be present even in euthymic phases, resulting in impaired general functioning in both bipolar and unipolar depression (Bora et al., 2013). Comorbid psychiatric disorders, such as anxiety and alcohol or drug abuse, are also common in severe affective disorders, making diagnosis and correct treatment challenging (Merikangas et al., 2011; Otte et al., 2016). About 20% of the general population experience at least one major depressive episode during their lifetime (Moffitt et al., 2010; Patten, 2009). Among patients hospitalized for MDD, 80% experience at least one serious lifetime recurrent episode (Otte et al., 2016). For BD, lifetime prevalence across 11 countries has been estimated at 0.6% for BD I, 0.4% for BD II, and 1.4% for bipolarity at a subthreshold level (Merikangas et al., 2011). However, these estimates have been contested and could be higher, especially for BD II and BD with atypical or sub-threshold symptoms (Benazzi & Akiskal, 2003).

Specific issues should be taken into consideration when reading the present thesis. First, MDD and BD show significant diagnostic overlap, as well as substantial individual heterogeneity (Akiskal, 2003; Ghaemi et al., 2002; Perlis et al., 2011). Besides, both disorders represent a broad spectrum of symptoms (Merikangas et al., 2011), and there is controversy over where the distinction between diagnoses should be made for individuals experiencing mild or atypical symptoms (Benazzi, 2007; Malhi et al., 2010). Thus, clearly defined diagnostic boundaries are difficult to make, posing an important challenge in research. Second, most studies do not specify whether included patients have the recurrent form of MDD or not, which may confound the interpretation of results. Third, many studies with BD samples do not distinguish between diagnostic sub-types BD I, BD II, and BD not otherwise specified (NOS). This lack of specificity represents a substantial limitation in the current literature and makes assessment of previous observations rather challenging.

1.2 Mortality and life expectancy

Clinical reports of increased mortality in patients with severe mental illness were published as early as in the 17th century (Graunt, 1662). Since then, the understanding and treatment of mental illnesses have radically improved. However, a considerable gap in life expectancy between the general population and individuals with severe mental disorders is still a serious matter of concern.

In modern research, standardized mortality ratios (SMRs) are commonly used to measure differences in mortality between populations. The ratios are calculated by dividing the observed mortality of a cohort of patients by the expected mortality of a sex- and age-matched cohort from the general population. For patients with severe affective disorders, studies have indicated a standard mortality ratio more than twice as high as that of the general population (Chesney et al., 2014; Cuijpers et al., 2014; Laursen, 2011; Hällgren et al., 2019). Reduced overall lifespan in patients with mood disorders is partly due to an increased risk of death by accident or suicide. People experiencing severe affective episodes are almost 20 times more likely to die by suicide than individuals from the general population. Even so, since suicide is rare, this only accounts for a small portion of the total reported deaths (Chesney et al., 2014). An increased prevalence of somatic disease, in particular cardiovascular disease (CVD), is the predominant cause for the increased mortality rates in severe mental disorders (Gilman et al., 2017; Laursen et al., 2016a; Lomholt et al., 2019; Ösby et al., 2016).

Evidence suggests a multifactorial reason for this increase in SMRs. Deleterious health-related behaviors may be one essential driving force. Multiple studies have linked depression with a sedentary lifestyle, unhealthy diet, smoking, and excessive alcohol use (Appelhans et al., 2012; Chen et al., 2011; Kingsbury et al., 2015; Roshanaei-Moghaddam et al., 2009). Moreover, evidence has emerged over the past twenty years or so, which identifies side-effects of pharmacological treatment as a major contributor to mortality risk in individuals with mental illness. Obesity-related cardiovascular risk factors, such as the metabolic syndrome, are among these targeted side-effects (for a comprehensive review, see: Correll, Detraux, De Lepeleire, & De Hert, 2015). Furthermore, disparities in health care provision, as well as health care access and utilization, may also play an important role (Lawrence & Kisely, 2010). Studies have reported that people with serious mental illnesses have a reduced ability to seek medical help and are more frequently overlooked in the somatic health care system (Newcomer & Hennekens, 2007; Vigo et al., 2016).

For some time now, researchers have proposed that the somatic and psychological ailments associated with severe affective disorders might share a similar etiology (Cardoso et al., 2015; Martino et al., 2016; Muneer, 2016). In the current scientific climate, there is rising interest in the underlying disease-related mechanisms that might connect psychological symptoms to somatic factors in severe mental disorders. Subsequently, growing evidence supports the hypothesis of pathophysiological mechanisms intrinsic to the disorder itself (e.g., Lindqvist et al., 2017; Penninx, 2017).

1. 3 Accelerated aging in severe affective disorders

A progressive course of illness, characterized by accelerated episodes, treatment non-response, and functional impairment, is frequently reported in severe mood disorders (Berk et al., 2017; Kessing & Andersen, 2017). This phenomenon has given rise to the proposed framework of disease staging, which implies a division of illness into temporal stages such as a prodrome, onset, progression, and an end-stage. Stages entail a progressive disease process with a marked deterioration and treatment resistance in later stages of the disorder when not correctly treated (Grande et al., 2016; Post et al., 1986). This framework model may help us understand some of the therapeutic difficulties in late-stage affective disorders regarding treatment non-response and reduced general functioning (Berk et al., 2017).

1.3.1 Progressive illness with successive episodes

Since the time of Kraepelin, recurrent affective episodes are known to be followed by shortened periods of euthymia (Kessing, Andersen, & Vinberg, 2018; Kessing & Andersen, 2017; Kraepelin, 1896). In addition, more severe symptoms, lower functioning, and reduced overall quality of life are also associated with successive episodes (Dols & Beekman, 2018). Three recent studies did not find age at onset or duration of illness to be a predictor of poor outcome. However, more frequent mood episodes were associated with an increase in illness severity and a decrease in functioning (Bonnín et al., 2010; Reinares et al., 2013; Tabarés-Seisdedos et al., 2008). Other studies have observed more pervasive structural brain alterations in patients with successive affective episodes, particularly manic, relative to early-stage patients (Javadapour et al., 2010; Lavagnino et al., 2015). Most prior evidence is, however, cross-sectional and cannot suggest causality.

Mood disorders are highly heterogeneous, both in clinical manifestation and trajectories, which complicates research. A recent comprehensive review took this heterogeneity into consideration and gave evidence for illness progression in both BD and MDD. The review reported that a higher number of affective episodes was associated with an elevated risk of recurrence, longer duration of episodes, higher severity of symptoms, decreased threshold for developing new episodes, as well as an elevated risk of dementia (Kessing & Andersen, 2017).

1.3.2 Cognitive decline and structural brain changes

In addition to age-related somatic disorders, premature cognitive decline and dementia are more frequently observed in individuals with severe affective disorders relative to healthy individuals (Gildengers et al., 2007; James et al., 2018; Seelye et al., 2019). Evidence has demonstrated progressive cognitive impairment in both BD and MDD (Cherbuin et al., 2015; James et al., 2018; Stella et al., 2014), and multiple studies have indicated a strong association between cognitive alterations and the number of prior affective episodes (El-Badri et al., 2001; Kozicky et al., 2014). While early-stage patients typically show preserved cognitive functioning, patients in later stages of the disorder often exhibit significantly reduced cognitive functioning compared to healthy controls (López-Jaramillo et al., 2010; Rosa et al., 2014). This progressive cognitive decline may be a key contributing factor to the increase in general disability in affective disorders (Bora et al., 2009; Gildengers et al., 2007; James et al., 2018; Seelye et al., 2019).

Disease progression is believed to influence structural brain changes, giving rise to the construct of neuroprogression. Several research authors have used this concept in order to explain how biological mechanisms might influence the dynamic trajectory and outcome of mental illness (Macneil et al., 2012; Reinares et al., 2010). Neuroprogression can be understood as the pathological brain rewiring that occurs as a consequence of, or parallel to, recurring affective episodes (Passos et al., 2016). Although with small effects, recent MR brain studies have also observed accelerated structural changes, commonly associated with aging, in individuals with affective disorders. These include ventricular enlargement, as well as cerebellar, hippocampal, and prefrontal cortex grey matter reduction in patients relative to age-matched controls. Morphology in the hippocampus, a part of the brain intricately linked to memory function and acutely vulnerable to both normal aging and dementia, may be particularly at risk in major affective disorders (Han et al., 2020; Kaufmann et al., 2019; Roda et al., 2014; Schmaal et al., 2016). This structural deterioration fits into a staging framework as later-stage disease is associated with more extensive alterations (Lavagnino et al., 2015; Schmaal et al., 2016). However, structural patterns of brain aging are often exceedingly subtle, with large within-group variance and considerable overlap between diagnostic sub-groups.

1.3.3 Disease mechanisms might explain accelerated aging

The research discussed implies that severe affective disorders may be associated with an acceleration of processes underlying biological aging. The forces driving this acceleration are hypothesized to result from multiple biological systems interacting with individual environmental factors (Fries et al., 2020; James et al., 2018; Laursen et al., 2016). The impact of aging-related biological processes may, to some extent, help explain the poorer health outcomes, treatment non-responsiveness, and enhanced mortality in late-stage affective disorders (Berk et al., 2017). Hence, it is of great interest to explore whether, and perhaps how, severe affective disorders are related to accelerated aging. A better understanding of this relationship may again help uncover whether mechanisms related to biological aging also contribute to a disorder-related progressive decline. Telomere length has been proposed as one potential biomarker of accelerated aging in mood disorders.

1.4 Telomeres

Telomeres are structures comprised of repeating 5´-TTAGGG-3´ base pair sequences and protective proteins at the ends of the DNA-strand (Aubert & Lansdorp, 2008; Blackburn, 2001). They are essential in maintaining and protecting the cells' genetic material. However, during each division, the cell fails to replicate a small portion of the telomere strand, leading to a gradual shortening over time. As telomeres become critically short, the risk of cell senescence and apoptosis is increased. Thus, telomere length is age-dependent and has been proposed as a biomarker of aging in humans (Allsopp & Harley, 1995; Collins & Mitchell, 2002). Greater overall telomere attrition is associated with age-related illnesses, such as CVD, cancer, and dementia, and overall mortality (Fani et al., 2020; Haycock et al., 2014; Wentzensen et al., 2011; Zhao et al., 2013), and telomeric shortening is roughly proportional to the risk of common diseases of old age. Moreover, the maintenance of telomeres is partly determined by genetics while also shaped by non-genetic factors throughout the lifespan (Blackburn et al., 2006). Because of this, researchers have proposed that telomere shortening may both be part of disease etiology and the result of progressive illness (for a comprehensive review, see Blackburn, Epel, & Lin, 2015).

Evidence has implied a dose-dependent effect of exposure to chronic life-stress on telomere shortening in the general population (Price et al., 2013a; Puterman et al., 2015; Verhoeven et al., 2015). Shorter telomeres are related to higher cortisol levels in humans (Gotlib et al., 2015), and evidence suggest that excessive levels of glucocorticoids may

dampen the activity of telomerase, an enzyme that adds telomere repeat sequences and thus replenishes damaged telomere strands (Choi et al., 2008). Chronic exposure to stress may, therefore, play a causal role in telomere shortening.

In patient populations, studies imply that normal telomeric erosion may be amplified through lifetime exposure to affective episodes (Elvsåshagen et al., 2011; Phillips et al., 2013; Wolkowitz et al., 2011). Previous research has suggested shorter telomere length either as a long-term consequence of affective disorders or as an underlying vulnerability factor contributing to the risk of disease (Kapczinski et al., 2010a; Verhoeven et al., 2016). Hence, accelerated telomere shortening might occur in a dose-dependent manner as a consequence of episode-related stress. Alternatively, increased telomere erosion may be provoked by an accumulation of chronic disease-related stress. A third explanation would be that shorter telomeres in patients simply represent a risk factor for severe affective disorders, as well as age-related disease.

1.4.1 Telomere length in major depressive disorder

Research on telomere attrition in severe depressive disorders is inconsistent and non-conclusive (Darrow et al., 2016). The first study on telomeres in MDD found patients to have significantly shorter telomere length compared to healthy controls (Simon et al., 2006). Other cross-sectional studies have indicated a negative association between telomere length and the severity and duration of depressive episodes (Gillis et al., 2019; Schutte & Malouff, 2015; Wolkowitz et al., 2011). The authors of a 6-year longitudinal cohort study comparing patients with age-matched controls found telomere length to be inversely related to severity of depression (Verhoeven et al., 2016*).* Another longitudinal study demonstrated the same inverse relationship between depressive symptom severity and shorter telomere length, but this time only in younger adults (Phillips et al., 2013). Other studies have failed to replicate a negative correlation between depressive episodes and telomere length (Hartmann, Boehner, Groenen, & Kalb, 2010). For instance, Schaakxs and colleagues (2015) noticed an inverse relationship between age and telomere length in a late-life patient sample. However, the authors did not observe an association between telomere length and clinical depression, severity of affective symptoms, or the number of prior depressive episodes. Nevertheless, although non-conclusive, the current findings generally point towards a negative association between major depressive episodes and telomere length (Darrow et al., 2016; Muneer & Minhas, 2019).

1.4.2 Telomere length in bipolar disorder

Likewise, in BD, telomeres as a potential biomarker for accelerated aging is still controversial. Several studies have indicated significant telomere shortening in bipolar patients compared to healthy individuals (Barbé-Tuana et al., 2016a; Darrow et al., 2016; Elvsåshagen et al., 2011; Lima et al., 2015; Rizzo et al., 2014; Vasconcelos-Moreno et al., 2017), while others have failed to observe a marked group difference (Fries et al., 2017a; Mamdani et al., 2015; Palmos et al., 2018). One meta-analysis, including a total of 1115 BD patients and healthy controls, reported no significant group differences (Colpo et al., 2015), weakening the evidence for telomeres as a robust biomarker in BD. Nevertheless, the generalizability of this study may be limited. Only seven studies were included, most of them with small sample sizes ($n < 100$), and with reported substantial heterogeneity between studies.

This heterogeneity is primarily due to a substantial difference in subject age across studies, inclusion criteria regarding diagnostic sub-groups and patient characteristics, different telomere length measurement techniques, and a lack of sex-matching. Studies with younger subjects (mean age under 35 years) more often failed to report significant differences between patients and controls relative to studies with older samples (e.g., Elvsåshagen et al., 2011; Fries et al., 2017; Mansour et al., 2011). Although speculatively, this pattern of observations may indicate that differences in telomere length between patients and healthy controls advance with age, supporting the notion of progressive biological aging in BD.

Heterogeneity between studies can also partly be explained by confounding factors associated with telomere erosion, including: age (Müezzinler et al., 2013), sex (Barrett & Richardson, 2011), heritability (Kim et al., 2020), ethnicity (Ly et al., 2019), employment status (Parks et al., 2011), obesity, smoking (Valdes et al., 2005), alcohol consumption (Dixit et al., 2019), medication use (Squassina et al., 2017), physical health status (Barrett et al., 2015), and comorbid psychiatric issues (Huang et al., 2018). The largest meta-analysis on telomere length in BD to date addressed heterogeneity in the existing data (Huang et al., 2018). The authors included four of the latest studies on leukocyte telomere length in BD, in addition to the studies comprising the Colpo and colleagues paper (2015). Following this inclusion, the new meta-analysis reported significantly shorter telomere length in patients with BD relative to healthy controls. However, the analysis did not report statistically significant effects of illness duration on the overall effect size (Huang et al., 2018). To

summarize, there are large inconsistencies in the literature. The latest meta-analysis does, however, indicate an association between BD and augmented telomere erosion.

Telomere length and illness duration in BD

To date, only a few studies have tested for associations between illness duration and telomere length in BD. Elvsåshagen and colleagues (2011) reported no significant correlation between duration of illness and the length of telomeres in BD type II patients. However, the authors reported a significant negative association between number of depressive episodes and telomere length (Elvsåshagen et al., 2011). Since then, two more studies have investigated potential differences in telomere length between early-stage BD and late-stage BD patients compared to controls. One of these studies found telomere length to be significantly shorter in late-stage BD relative to early-stage BD (Köse Çinar, 2018). A more substantial difference between patients and controls was also evident within the late-stage group. Here, the authors operationalized early-stage BD as those with less than five previous episodes and late-stage BD as those with more than ten prior episodes (Köse Çinar, 2018). The second study also reported shorter telomere length in both patient groups compared to controls. However, due to a small sample size, they were unable to perform comparisons between the early- and late-stage patient groups (Barbé-Tuana et al., 2016b). A preliminary implication of previous findings may be that telomere shortening is accelerated with the progression of illness. Huang et al. (2018) proposed that the total number of lifetime affective episodes may play a crucial part in this acceleration.

In summary, a progressive course of illness may be accompanied by accelerated aging-related processes in affective disorder compared to the general population (Berk et al., 2017; Vance et al., 2018). Although non-conclusive, current evidence suggests that a longer duration of illness and a higher number of lifetime affective episodes may be related to accelerated aging. However, findings are inconsistent, and the research is still largely underdeveloped. Most studies on telomere length in BD are underpowered, and at present, only Martinsson and colleagues (2013) have published a study with a patient group of over 200 individuals. In general, low-powered studies are not only associated with increased probability of false negatives but are also severely prone to produce inflated effect sizes. Studies on MDD are more extensive, with both cross-sectional and longitudinal designs. Still, samples are often heterogeneous, and many studies lack consideration of relevant confounding factors (e.g., sex, ethnicity, employment status, medication status, smoking, exercise). Furthermore, only one study (Lima et al., 2015) has, to my knowledge, compared

telomere length in BD sub-groups BD I and BD II. The authors reported no significant differences. Crucial differences or similarities between sub-groups of affective disorders, for example, those with mania versus those without, might cast light on underlying etiological mechanisms.

1.5 Current research objectives

The present study provides much needed statistical power (controls, $n = 401$; patients, $n = 247$), novel sub-group comparisons, and hypotheses that have been tested only a handful of times before. Only a few previous studies have investigated the relationship between duration of illness and telomere length in patients with mood disorders. Each of the past studies of illness duration consisted of relatively small samples ($n < 50$) (e.g., Elvsåshagen et al., 2011; Wolkowitz et al., 2011). Moreover, the current study is the first to compare affective sub-groups MDD with psychotic symptoms, BD I, and BD II on telomere length. To my knowledge, no study of telomere length in severe affective disorders has previously included a subset of MDD patients with psychotic symptoms. Potentially critical confounding factors, such as age, sex, ethnicity, education, occupational status, weekly physical activity, nicotine use, and BMI, were considered and explored. Combined, this may help to bring light on the potential acceleration of aging in severe affective disorders.

Aims

1. To explore differences in telomere length between patients with severe affective disorders and healthy controls.
2. To explore whether telomere length is associated with duration of illness in the complete patient group.
3. To investigate whether telomere length is associated with the number of lifetime affective episodes in the complete patient group.
4. To investigate whether telomere length is associated with illness duration or number of lifetime affective episodes in the separate diagnostic sub-groups.

2 Method

2.1 Organizational structure and study coordination

The current study was conducted using data from the Norwegian Centre for Mental Disorders Research (NORMENT), a continuation of the Thematically Organized Psychosis (TOP) Study, established in 2001 as a collaboration between Ullevål University Hospital and the University of Oslo. The project was granted the title and privileges of Centre of Excellence in 2013 and now runs a large, multi-center translational study project. An essential aim of NORMENT is to investigate the underlying mechanisms of severe mental disorders, such as schizophrenia, bipolar disorder, and major depressive disorder with psychosis. NORMENT is currently divided into several thematically organized sub-groups. The present study is part of the biological psychiatry sub-group, led by Professor MD Nils Eiel Steen, and the South-Eastern Norway Health Authority (HSØ) project Stress Under Skin (SUS), led by Monica Aas, Ph.D. The SUS-project was funded by a research grant given to Aas, as well as the general NORMENT research grant from the Research Council of Norway and the KG Jebsen Foundation.

2.1.2 Design

My study is a continuation of a previous study published in 2019 as part of the SUS-project (Aas et al., 2019). Aas and colleagues explored average leukocyte telomere length in a cohort of patients with bipolar disorder and schizophrenia compared with controls. In the present study, a cross-sectional, between-groups design was applied to compare patients with severe affective disorders to healthy controls, in addition to separate, diagnostic sub-group comparisons. Within-group analyses of clinical characteristics were performed on the combined patient group. The patient samples in the previous and present study were partly overlapping. However, in the present study, schizophrenia patients were excluded, and patients diagnosed with MDD with psychotic features were instead added. The previous study by Aas and colleagues made no distinction between types of mood episodes, whereas, at present, analyses for prior depressive, hypomanic, and manic episodes were completed separately. Moreover, the current study comprised novel comparisons of diagnostic sub-groups BD I, BD II, and MDD with psychotic symptoms.

2.2 Participants

Participants in the SUS-project were enrolled between 2007 and 2016. Both patients and healthy controls were in the age range of 18 to 64 years old. Exclusion criteria for both groups were an Intelligence Quotient (IQ) below 70, pregnancy, any neurological disorders, a history of organic psychosis, and any unstable or uncontrolled medical condition that may interfere with brain functioning.

2.2.1 Patients

Both outpatients and inpatients were recruited from psychiatric units of four hospitals in the Oslo region. In this particular SUS-project sub-study, only patients diagnosed with severe affective disorders (n = 248) were included. The sample consisted of patients with bipolar disorder type I (BD I, n = 159), bipolar disorder type II (BD II, n = 67), and major depressive disorder with psychotic symptoms (MDD, n = 22). The MDD group contained individuals with recurrent (n = 16) and first-time (n = 6) depressive episodes. Patients received no financial compensation for participating.

2.2.2 Healthy controls

Healthy controls (n = 401) were invited at random from the population registers of Oslo (Folkeregisteret, via information technology company EVRY). Information about the study and request to participate was given by letter. Initial screening consisted of the Primary Care Evaluation of Mental Disorders (Tamburrino et al., 2009). Additional exclusion criteria for healthy controls were any personal or close family history of severe mental disorder (e.g., schizophrenia, BD, or MDD). Controls were financially compensated with 500 NOK.

2.3 Measures and procedures

2.3.1 Socio-demographic and lifestyle variables

Self-reports on age, sex, ethnicity, and years of education were gathered from both patients and healthy controls. In patients, additional self-report data were collected on occupational status, hours of weekly physical activity (light activity or exercise), daily nicotine use, and body mass index (BMI). Reports of these lifestyle variables lacked for the control group. In the final dataset, ethnicity was split into European ancestry and non-European ancestry. Occupational status was dichotomized and defined as working or studying, either part-time or full time, or being currently disabled. Daily nicotine use, in the form of smoking or snuff, was similarly split into daily use and no daily use.

Mean age of the total patient (n = 248) and control groups (n = 401) was 31.6 years (± 11.3) and 31.4 years (± 7.6), respectively. Age ranged between 18 and 64 years with a median of 28.0 years for patients, and 18 and 46 years with a median of 31.0 years for controls. The percentage of males in the whole patient group was 44.8%, while the percentage of males in the control group was 56.9%. Out of the patients, 84.3 % were of European ancestry, while the proportion was 98.3 % for healthy controls. The range of years in education was 9 to 24 years, mean 14.5 (± 2.3) years, and median 14.0 years for patients, whilst 9 to 25 years, mean 14.5 (± 2.2) years, and median 15.0 years for controls. In the total patient group, 52.2% were able to work or study, either part-time or full-time, and 54.0% reported daily nicotine use. Weekly physical activity in patients varied between 0 and 21 hours, with a mean of 4.1 (± 4.4) hours, and a median of 3.0 hours. The average BMI for patients was 25.7 (± 4.3), ranging from 15 to 42, with a median of 25.4.

2.3.2 Clinical assessment

All clinical assessments were completed by trained physicians, psychiatrists, or clinical psychologists. Diagnosis and lifetime number of mood episodes were based on the Structural Clinical Interview for DSM-IV (SCID, First et al., 1995). All clinical personnel completed a training program in diagnostics and symptoms rating. The training was based on a UCLA program (Los Angeles, California) (Ventura et al., 1998) comprising SCID 101 training videos and videos with reliability testing between SCID scorers (http://www.scid4.org/index.html). Overall agreement on the DSM-IV diagnostic categories, derived from the latest information available from the total NORMENT sample, was

satisfactory with diagnostic reliability of 82% and an overall *k* of .77 (95% CI: .60-.94). Fasting blood samples were collected in both patients and healthy controls.

2.3.3 Clinical characteristics

The following clinical data were collected from patients: duration of illness, lifetime number of affective episodes, and current depressive and elevated mood status. Illness duration was defined as current age minus age at first SCID verified mood episode. Current depressive and elevated mood symptoms were measured using the Inventory of Depressive Symptoms – Clinician rated Scale (IDS-C; Rush, Gullion, Basco, Jarrett, 1996) and Young Mania Rating Scale (YMRS; Young, Biggs, Ziegler, & Meyer, 2004), respectively.

Duration of affective illness ranged from 0 to 43 years, with a mean of 10.6 years (± 9.3), and a median of 8.0 years. Eighteen patients were included in the year of their first confirmed mood episode. The mean number of lifetime depressive episodes was 6.4 (± 10.8), while mean lifetime number of manic and hypomanic episodes was 1.8 (± 5.9) and 4.3 (± 17.8), respectively. Number of lifetime depressive episodes ranged from 0 to 120, with a median of 4.0, lifetime hypomanic episodes ranged from 0 to 245, with a median of .1, and lifetime manic episodes ranged from 0 to 85, with a median of 1.0. IDS-C sum score varied from 0 to 58, while YMRS sum score varied from 0 to 28. Mean and median IDS-C sum score was 18.0 (± 11.6) and 17.0. Mean and median YMRS sum score was 3.4 (± 4.7) and 2.0.

2.3.4 Measurement of telomere length

Peripheral leukocyte telomere length of both patients and healthy controls was measured using qualitative polymerase chain reaction (qPCR) at the Newcastle Institute of Aging and Institute for Cell and Molecular Bioscience.

qPCR

Regular polymerase chain reaction is a method in which the heat-stable enzyme DNA-polymerase is used to replicate strands of DNA. The replication process is accomplished through a sequence of cycles, each comprising three steps: thermal denaturation, annealing (or hybridization), and extension (or elongation). Thermal denaturation involves a heating process in which the hydrogen bonds connecting the DNA double helix are broken, separating the two strands. In the second step, annealing allows

primers to attach to specific sites at each strand. Polymerase will, in the presence of four deoxyribonucleotide triphosphates (DNA building blocks), finally synthesize the complementary DNA strand of each original strand. These three steps are repeated manifold to attain the desired amount of amplified DNA (Evrard, Boulle, & Lutfalla, 2010, p. 842).

Qualitative PCR is an approach based on the monitoring of PCR by detecting and measuring fluorophore (a fluorescent chemical substance that re-emits light upon light excitation) merged into the amplified DNA product. Fluorescent signals measured cycle by cycle are directly proportional to the number of amplicons generated. An amplicon is a piece of DNA that is the source or product of the amplification process in regular PCR (Evrard et al., 2010, p. 845).

How are telomeres measured using qPCR?

Quantification of genetic material using qPCR involves demonstrating the number of cycles beyond which the amplification process becomes detectable (i.e., significantly stronger than the background noise). A higher concentration of genetic material in the initial reaction medium amounts to a quicker start to the exponential and quantitative (i.e., detectable) PCR amplification phase. Therefore, as the initial concentration rises, the number of PCR cycles needed to detect amplification becomes lower (Evrard et al., 2010, p. 845). This process makes the comparison of two samples of telomere PCR possible – a sample A and a sample B. If the threshold cycle for sample A appears at an earlier cycle than B, it would suggest that it had more "starting material," or telomeric sequences, and therefore longer telomeres than B. Sample A must then be normalized against a single-copy gene for which the starting material is a unique sequence and not the repetitive telomeric sequences. Provided that sample A also has more "starting material" than the single-copy gene (i.e., $2^{3.5}$ times more DNA), the telomeric outcomes in both samples must be corrected against this difference in DNA.

Specific qPCR instruments and procedures

In this project, the amount of telomeric template versus a single copy gene (36B4) was estimated by PCR on 10 ng of DNA, with 5 µl SYBR® Green JumpStart Taq Ready Mix and .25 µl of ROX fluorescent reference dye (Sigma-Aldrich, Gillingham, UK) and the following primers: 300nM TelA (5´-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3´); 900 nM TelB (5´-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3´) for telomeric reaction and 200 nM 36B4F (5´-CAG CAA GTG GGA AGG TGT AAT CC 3´) and 400 nM 36B4R (5´-CCC ATT CTA TCA ACG GGT ACA A-3´) for the

single-copy gene. All samples were assessed in triplicate, and all PCRs were executed using an Applied Biosystems 7900HT Fast Real Time qPCR system with 384-well plate capacity. Three control DNA samples of known telomere length (10.4 kb, 3.9 kb, and 2 kb) were run within each plate to correct for possible variation between plates. Revaluation of samples in the top 5% or the bottom 5% of the distribution, as well as samples with no valid data on first run, were performed as additional verification. The intra-assay coefficient of variation was 6.07%, while the inter-assay coefficient of variation was 6.08%. Telomeres were defined as the ratio between telomere template and amount of single-copy gene template in the direction of smaller numbers indicating shorter average telomere length. Telomere length was measured in blood samples stored and extracted from the Biobank in Oslo, Norway.

2.4 Ethical considerations

Both biological material and sensitive personal information were gathered from patients with severe psychiatric disorders. Ethical considerations were, therefore, of particular relevance and considered accordingly. The issue of confidentiality and informed consent was a central concern, and all participants were given both oral and written information about how their data would be stored and used. Patients were informed that participation was voluntary and that ending their participation would not affect further treatment. Then, prior to participation, all participants gave oral and written consent. The clinical assessment lasted for several hours but could be spread out over a couple of days to limit the risk of fatigue. All assessments could be completed in the research facility or a clinic office following participant preference. Travel expenses were compensated for all participants, and transportation by taxi was offered when needed.

Collection and storing of data were approved by the Regional Committee for Medical Research Ethics (2009/2485/REK sør-øst) and the Norwegian Data Inspectorate as part of the larger project. Moreover, all data used in the present study were anonymized and password protected. As an external contributor to the NORMENT-project, I, the author of this thesis, signed an agreement of non-disclosure before being granted data access.

2.5 Statistical analyses

Statistical analyses were completed using the IBM SPSS software, version 26 for Windows (IBM Corp., 2019). Mean telomere length and lifetime number of depressive, hypomanic, and manic episodes were non-normally distributed. The skewness was .72 (*SE* = .96) for average telomere length, which can be described as moderate. The skewness was 5.92 (*SE* = .16) for number of lifetime depressive episodes, 10.98 (*SE* = .16) for number of lifetime hypomanic episodes, and 11.76 (*SE* = .16) for number of lifetime manic episodes, indicating a high degree of skewness (Joanes & Gill, 1998). Thus, these respective variables were log-transformed in order to normalize the data. Log-transformed variables were included in post hoc statistics and hypothesis testing. Other included variables did not have a substantial skewness, defined as either a moderate or high degree, and were thus not log-transformed. Raw data on demographic variables are presented in Table 1 in the results section.

2.5.1 Descriptive analyses

One-way analysis of variance (ANOVA) and Chi-squared tests were used for continuous and categorical variables, respectively. These tests were implemented to assess demographic and clinical variables in the complete patient group and control group. Bonferroni post hoc tests were then performed to assess possible group differences in descriptive data between diagnostic sub-groups.

Furthermore, Pearson's r correlation and one-way ANOVA were used to evaluate correlations between telomere length and potentially confounding factors in the complete sample on continuous and categorical variables, respectively. All socio-demographic and lifestyle variables reported in Table 1 (see Results) were included in this inquiry. Variables with a significant or trending correlation with telomere length were added into the primary statistical model to control for confounding effects (see Results: 3.1.3 Potential confounding variables). Selection of control variables was also based on the existing literature on factors believed to be related to telomere length (i.e., Barrett & Richardson, 2011; Ly et al., 2019; Müezzinler et al., 2013; Parks et al., 2011; Valdes et al., 2005).

2.5.2 Inferential analyses

The first research aim was investigated using a one-way ANOVA analysis. Here, average telomere length between the complete patient group and healthy controls was compared. Variables were examined one by one in all inferential analyses, with a Bonferroni corrected *p*-value threshold at .008. As there were six statistical tests in total, this new threshold value was based on a .05 level with a six-factor adjustment. Post hoc Bonferroni is known as a conservative method of correction (Moran, 2003) and was thus selected to counteract the problem of multiple comparisons.

Multiple linear regression analyses were performed to investigate associations between telomere length and illness duration and number of lifetime depressive, manic, and hypomanic episodes in the complete patient sample. Age, sex, ethnicity, and occupational status were added as control variables in the regression model following the assessment of potential confounding factors (see Results 3.1.3).

Standardized effect sizes for any significant group differences were calculated by hand using the Cohen's *d* equation (Cohen, 1988; Durlak, 2009):

$$d = \frac{M_1 - M_2}{\text{Sample SD pooled}}$$

This effect size measure can be interpreted using Cohen's rule of thumb, where .20 represents a small effect, .50 a medium effect, and .80 a large effect size (Durlak, 2009). Within-group results from regression analyses were reported as the standardized coefficient beta. The standardized beta coefficient can be interpreted using similar guidelines to that of Cohen's *d* (Durlak, 2009).

Sub-group comparisons

Diagnostic sub-groups were compared using between-groups analysis of covariance (ANCOVA). Sub-group comparisons were only performed on variables showing significant or trending effects in the total patient group to account for the issue of multiple testing. Analyses were performed with mean telomere length as the dependent variable and clinical measures as independent variables. Age, sex, ethnicity, and occupational status were set as covariates. Separate analyses were performed for each clinical variable.

2.5.3 Outlier influence and assumptions for multiple linear regression

In linear regression, outliers that impose strong influence (or high leverage) on the fitted slope and intercept may result in a poor fit to the overall bulk of data points. The method of least squares involves minimizing the sum of the squared vertical distances between each data point and the fitted line. Multiple linear regression is consequently sensitive to outlier effects. A high-leverage outlier may result in the dataset not meeting the assumption of linearity because the goodness of fit test might not be able to detect a linear fit (Field, 2013).

Detection of outliers in linear regression can be achieved by examining residuals, with a general definition being a data point that falls outside a standardized residual of ± 3. A second detection method is Cook's distance, which measures the amount of influence a given value has on the fitted linear regression. Here, >1 is a common cut off score (Cook, 2000). A third detection method is Mahalanobi's distance. This measure gives an estimate of the number of standard deviations a data point is diverging from the dependent variable in question. Greater distance to the rest of the sample signifies greater influence on the slope of the regression equation and, thereby, higher leverage. One can estimate the probability of this representing a significant divergence, resulting in a *p*-value (Schinka, Velicer & Weiner, 2013). In the current dataset, one data point was found to have a standardized residual of -7.0 and an unstandardized residual of -4.0. Cook's distance of the same data point was 1.03, and the probability of the Mahalanobi's distance was $p = .01$. These measures combined indicated the specific value point to exhibit a large degree of influence on the estimated coefficient. In regression, an approach to dealing with outliers is the exclusion of data points displaying high leverage on the estimated coefficient. Still, exclusion of outliers is a controversial approach in statistical method (Field, 2013). All inferential analyses in this study were thus executed both with and without the outlier. Notes under Table 4 and Table 5 in the results section show the effect of outlier inclusion. The outlier was not included in the descriptive statistics.

Furthermore, tests showed a degree of covariance between illness duration and age (r (649) = .697, $p < .001$). Several studies suggest that multicollinearity exists when correlation between predictor variables is above $r = .80$ (Franke, 2010; Shreshta, 2020). However, because of the observed covariance, some caution may be warranted when interpreting the standardized beta coefficients and *p*-values from the regression model. All

other assumptions for multiple linear regression (as described in Chapter 5 of Tabachnick & Fisdell, 2013) were tested and found to be satisfactory.

2.5.5 Missing data

Three subjects were removed from the dataset due to missing values in illness duration, the main independent variable. Tables 1 and 3 in the Results section give a complete overview of missing demographic and clinical data, respectively. Evaluation through separate variance *t*-tests showed values missing from the dataset to be missing at random. Hence, the probability of a data point missing given the observed data did not depend on the unobserved data. Missing data may reduce power and introduce bias in estimates, hence generating unreliable results. Imputation is generally accepted as a robust approach to handling values missing at random. Nevertheless, the degree to which imputation is required will, in part, depend on the number of observations relative to the number of missing values. A dataset with a large sample size and relatively few missing values would essentially make listwise deletion unproblematic (Allison, 2002; Barandi & Enders, 2010; Collins et al., 2001). Relatively few values were missing from the current dataset (see Table 1 and 3), and the study is currently one of the largest of its kind in the literature. Listwise deletion was thus implemented in all statistical analyses, and no imputed variables were applied.

3 Results

3.1 Descriptive statistics

3.1.1 Socio-demographic and lifestyle variables

Table 1 gives an overview of demographic and lifestyle variables for the complete patient group and healthy controls. Patients and controls did not differ in average age, although the age distribution varied considerably (see Method section: 2.3.1 Socio-demographic and lifestyle variables). There were significantly more men in the control group compared to the patient group. Healthy controls were significantly more ethnically homogenous, with a greater majority of individuals with European ancestry. Total years in education did not differ between patients and controls. Data for the remaining variables (occupational status, physical activity, nicotine use, BMI) lacked in the control group, and further comparisons were unfeasible.

Table 2 shows socio-demographic and lifestyle data for the separate diagnostic sub-groups. Post hoc Bonferroni corrected analyses revealed significantly more females in the BD II group compared with BD I and MDD. Although not statistically significant, the MDD group appeared to be more ethnically diverse and less able to work. No significant difference was found for age, number of years in education, average hours of weekly physical activity, daily tobacco use, or BMI.

Table 1

Socio-demographic and Lifestyle Data of Patients and Healthy Controls

	Patients *n* = 248	Healthy controls *n* = 401
Age, mean ± *SD*, y	31.6 ± 11.3	31.4 ± 7.6
Males, *n* (%)	111 (44.8)	228 (56.9)*
European, *n* (%)	209 (84.3)	394 (98.3)*
Education, mean ± *SD*, y[a]	14.5 ± 2.9	14.5 ± 2.2
Employed, *n* (%)	118 (47.6)	-
Physical activity, mean ± *SD*, h[b]	4.1 ± 4.4	-
Nicotine use, *n* (%)	134 (54.0)	-
BMI, mean ± *SD*, kg/m^2 [c]	25.7 ± 4.3	-

Note. Descriptive analyses executed using one-way ANOVA and Chi-squared tests. Raw data are presented. Abbreviations: X^2 = Pearson's Chi-square; y = years; h = hours per week; employed = fulltime or part-time employment; nicotine use = daily cigarette / snuff use; BMI = body mass index.

* $p < .001$

[a] Missing for 9 patients (3.6% of *n)*

[b] Missing for 5 patients (2% of *n)*

[c] Missing for 9 patients (3.6% of *n)*

Table 2

Socio-demographic and Lifestyle Data of Diagnostic Sub-groups

	BD I	BD II	MDD	Statistics	Post hoc
	n = 159	*n* = 67	*n* = 22		
Age, mean ± *SD*, y	31.3 ± 10.9	33.1 ± 11.8	28.9 ± 11.8	F = 1.3, df= 2, p= .29	N.S.
Males, *n* (%)	74 (46.5)	21 (31.3)	16 (72.7)	X^2 = 20.9, df= 2, p = .002	MDD > BD I, BD II
European, *n* (%)	136 (85.5)	58 (86.6)	15 (68.2)	X^2 = 7.7, df= 2, p = .093	N.S.
Education, mean ± SD, y	14.5 ± 2.9	15.0 ± 3.0	13.5 ± 3.3	F = 1.9, df= 2, p= .123	N.S.
Employed, *n* (%)	71 (44.7)	39 (58.2)	8 (36.4)	X^2 = 4.6, df= 2, p= .10	N.S.
Physical activity, mean ± *SD*, h	4.0 ± 3.3	3.8 ± 3.5	2.8 ± 2.5	F = 1.1, df= 2, p= .33	N.S.
Nicotine use, *n* (%)	92 (57.9)	31 (46.3)	11 (5.0)	X^2 = 2.7, df= 2, p= .26	N.S.
BMI, mean ± *SD* , kg/m^2	25.9 ± 4.3	25.3 ± 4.4	24.8 ± 3.1	F = 1.6, df= 2, p= .20	N.S.

Note. Descriptive analyses executed using one-way ANOVA and Chi-squared tests. Raw data are presented. N.S = not significant; BD I = bipolar disorder type 1; BD II = bipolar disorder type 2; MDD = Major depressive disorder; BMI = body mass index; X^2 = Pearson's Chi-square; y = years; h = hours per week; employed = fulltime or part-time employment; nicotine use = daily cigarette / snuff use; BMI = body mass index.

3.1.2 Clinical characteristics

Table 3 shows clinical variables in the entire patient group and separate diagnostic sub-groups. Post hoc sub-group comparison revealed BD II patients to have significantly longer average duration of illness, as well as a greater number of lifetime depressive episodes relative to BD I and MDD individuals. This difference was also highly statistically significant for number of hypomanic episodes. Moreover, MDD patients reported a significantly higher IDS sum score. No significant group difference was found for the YMRS sum score.

Table 3

Clinical Data of Patients with Severe Affective Disorders

	Patients, total	BD I	BD II	MDD	Statistics	Post hoc
	n = 248	*n* = 159	*n* = 67	*n* = 22		
Illness duration	10.6 ± 9.3	9.5 ± 8.8	14.5 ± 9.9	6.9 ± 6.5	*F*= 9.2, *df*= 2, *p* < .001	BD II > BD I, MDD
Depressive episodes[a]	6.4 ± 10.8	5.6 ± 11.4	9.3 ± 10.1	2.8 ± 2.1	*F*= 14.2, *df*= 2, *p* < .001	BD II > BD I, MDD
Manic episodes[b]	-	2.8 ± 7.2	-	-	-	-
Hypomanic episodes[c]	4.3 ± 17.8	1.8 ± 5.5	11.77 ± 32.7	-	*F*= 52.3, *df*= 1, *p* < .001	BD II > BD I
IDS[d]	18.1 ± 11.6	16.2 ± 11.2	20.9 ± 10.5	24.2 ± 14.4	*F*= 5.8, *df*= 2, *p* = .003	MDD > BD I, BD II
YMRS	3.4 ± 4.7	3.8 ± 5.4	2.5 ± 2.6	3.1 ± 3.6	*F*= 1.7, *df*= 2, *p* = .19	N.S.

Note. Descriptive analyses executed using one-way ANOVA and Chi-squared tests. Values are mean ± *SD*. IDS = Inventory of Depressive Symptomatology (at time of inclusion); YMRS = Young Mania Rating Scale (at time of inclusion).

[a] Missing for 11 patients (4.4% of *n)*

[b] Missing for 4 patients (2.5% of *n)*

[c] Missing for 13 patients (5.7% of *n)*

[d] Missing for 18 patients (7.2% of *n)*

3.1.3 Potential confounding variables

Pearson's r tests showed no statistically significant correlations between continuous demographic variables and telomere length. Of particular interest, chronological age was unrelated to telomere length in the complete cohort, consisting of both patients and controls: $r(647) = -.05$, $p = .22$. However, based on the evaluation of existing literature on chronological age and telomere length (e.g., Müezzinler et al., 2013), and large age range in the present data set (see Method section: 2.3.1 Socio-demographic and lifestyle variables), age was still included as a control variable.

A one-way ANOVA test of sex on telomere length indicated a trend level difference between men and women in the combined cohort: $F(1, 646) = 3.45$, $p = .06$. Daily nicotine use was not correlated with telomere length: $F(1, 245) = .02$, $p = .893$. However, ethnicity: $F(1, 645) = 4.08$, $p = .044$, and ability to work: $F(1, 244) = 5.20$, $p = .023$, did correlate with telomere length. Hence, being unemployed and of non-European ancestry was associated with shorter telomere length, although only at a trend level with the new adjusted .008 significance level. Based on these results and the assessment of prior research (i.e., Barrett & Richardson, 2011; Ly et al., 2019; Müezzinler et al., 2013; Parks et al., 2011; Valdes et al., 2005), age, sex, ethnicity, and occupational status were selected as control variables.

3.2 Main analyses

3.2.1 Difference in telomere length between the complete patient group and healthy controls

Patients with severe affective disorders had shorter average telomere length relative to the control group, as demonstrated by one-way ANCOVA with adjustments for age, sex, and ethnicity: $F(1, 646) = 6.05$, $d = 0.18$. $p = .012$. However, with the Bonferroni corrected threshold level of .008, this did not qualify as statistically significant.
Figure 1 gives a visual presentation of the results.

A one-way ANOVA Bonferroni post hoc test showed no statistically significant difference in telomere length between the distinct diagnostic sub-groups:
$F(2, 246) = 0.67$, $p = .51$.

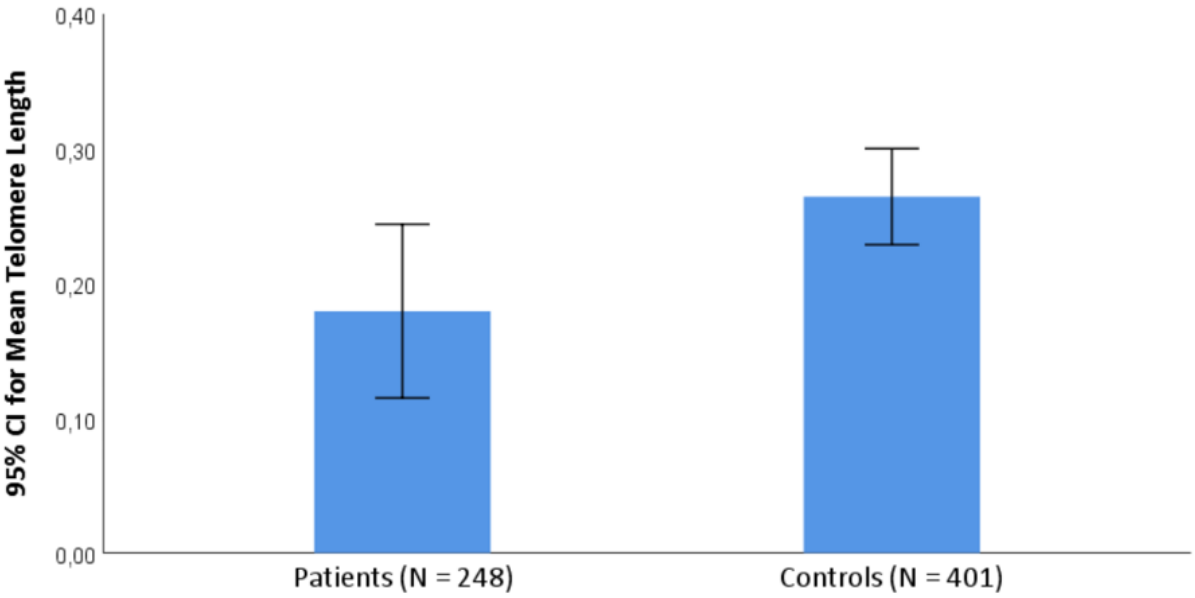

Figure 1. Mean Leukocyte Telomere Length in Patients with Severe Affective Disorders Compared to Healthy Controls. A one-way ANOVA was performed in order to compare the mean telomere length between the two groups. Age, sex, and ethnicity were set as confounders. Error bars represent the 95% confidence interval (*CI*). Patients with affective disorders had shorter average telomere length relative to the control group ($F(1, 646) = 6.05$, $d = 0.18$, $p = .012$). This was not significant with the adjusted threshold.

3.2.2 Telomere Length – association with illness duration and number of episodes

Average leukocyte telomere length was negatively associated with duration of illness (b^* = -0.18; p = .046). Figure 2 gives a visual illustration of this association. However, this p-value was statistically non-significant with the new adjusted .008 significance level. Table 4 shows the associations between telomere length and duration of illness and number of affective episodes in the complete patient cohort, with adjustments for age, sex, ethnicity, and occupational status.

No significant associations were found between telomere length and number of affective episodes.

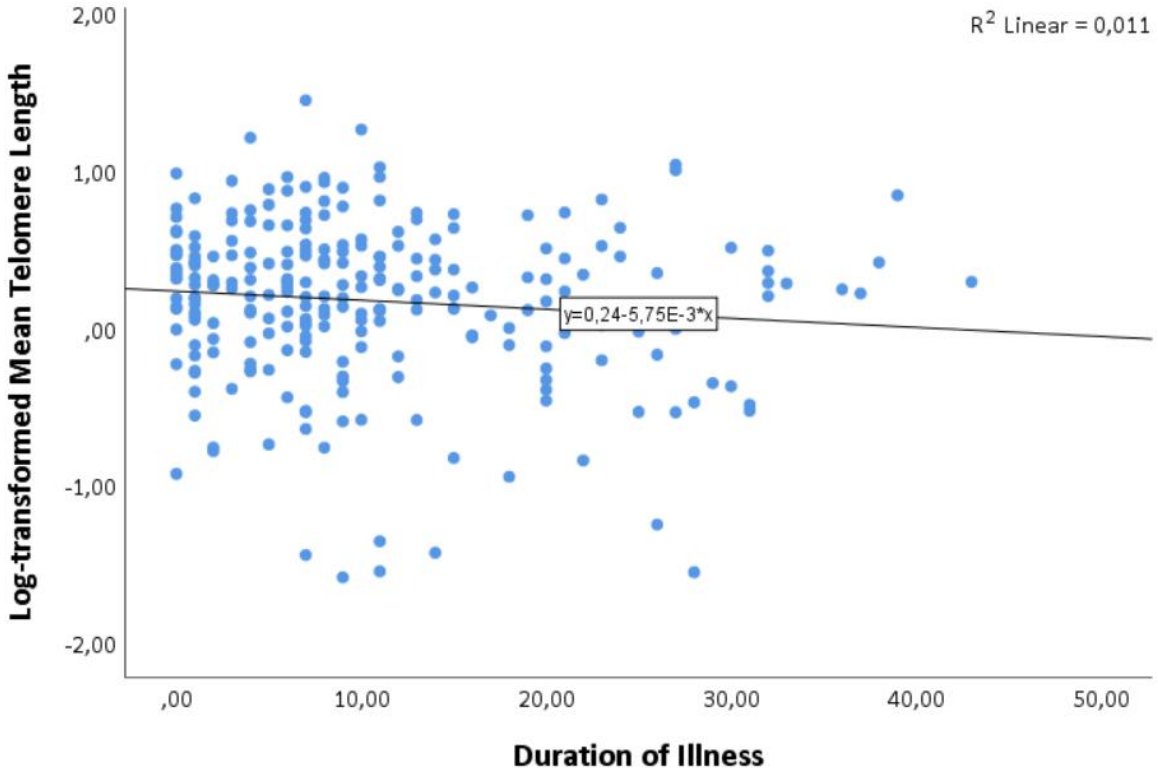

Figure 2. Association Between Duration of Illness and Average Telomere Length in Patients with Affective Illness. Data were analyzed using linear regression with adjustments for age, sex, ethnicity, and occupational status. Due to skewness, log-transformed values for the mean leukocyte telomere length were computed and used in the final analysis. Mean leukocyte telomere length was negatively associated with illness duration (b^* = -0.18; p = .046), although this was not statistically significant with the new, corrected .008 threshold level.

Table 4

Associations Between Clinical Variables and Mean Leukocyte Telomere Length

	*b**	Standardized error	*t*	95% CI for *b**	*p*-value
Illness duration[a]	-.18	.005	-2.01	[-.02, .00]	.046
Depressive episodes	-.04	.04	-.65	[-.09, .05]	.51
Manic episodes	.01	.05	.18	[-.08, .10]	.86
Hypomanic episodes	-.13	.03	-1.94	[-.13, .001]	.054

Note. Linear regression analyses with mean telomere length as the dependent variable and clinical measures as independent variables, adjusted for age, sex, ethnicity, and occupational status, were performed. Separate analyses were performed for each clinical variable. CI = confidence interval; *b** = standardized regression coefficients Beta. [a] *P*-value was altered with inclusion of the outlier variable (*b** = -.06, *p* = .051).

3.2.3 Sub-group comparison

Table 5 gives a detailed description of the diagnostic group comparison. Sensitivity analyses were performed to explore possible sub-group differences in the hypothesized association between telomere length and illness duration. Sub-group differences were only explored between telomere length and illness duration because of the close-to-significant result in the complete patient group. No significant differences were found.

Table 5

Sub-group Comparison of the Association Between Telomere Length and Illness Duration

	*b**	Standardized error	*t*	95 % CI for *b**	*p*-value
Illness duration					
BD I	-.19	.05	-1.80	[-.02, .001]	.07
BD 2	-.15	.14	-.63	[-.04, .02]	.53
MDD	-.11	.103	-.45	[-.04, .26]	.66

Note. Analyses were performed with mean telomere length as the dependent variable and age, sex, and duration of illness as independent variables. CI = confidence interval; *b** = standardized regression coefficients Beta. The negative trend between telomere length and illness duration was non-significant with inclusion of the outlier variable (*b** = -.003, *p* = .702).

4 Discussion

In the present study, I tested whether telomere length, a proposed biomarker of aging, was associated with the occurrence and duration of affective disorders in a patient sample consisting of relatively young individuals diagnosed with BD I, BD II, and MDD compared with healthy controls. The main finding was shorter telomeres in the complete patient sample compared to the control group. Moreover, the results indicated a negative correlation between telomere length and illness duration, but not between telomere length and number of lifetime affective episodes, in the total patient sample. Among the separate diagnostic sub-groups, no association was found between telomere length and duration of illness, nor between telomere length and lifetime number of depressive, manic, or hypomanic episodes.

4.1 Presentation of findings

4.1.1 Difference in telomere length between patients and controls

The current findings correspond with previous observations by suggesting shorter telomeres in patients with mood disorders relative to healthy controls, although admittedly with a small effect size (Cohens' $d = 0.18$). Recent meta-analyses have given similar conclusions, although with some dispute (for a comprehensive review, see: Muneer & Minhas, 2019). These studies have reported shorter telomere length in patients with affective disorders relative to healthy controls, with Cohens' *d* effect size of around 0.20 (Darrow et al., 2016; Huang et al., 2018; Lin et al., 2016).

The present study provided a comparatively large sample consisting primarily of individuals with BD. No similar studies have included a sample of this size, and most previous BD studies have examined less than 100 patients. Notably, the whole patient group and control group were matched regarding average age and years of education, factors which are believed to be associated with telomere length (Canela et al., 2007; McGrath et al., 2007; Puterman et al., 2010). However, there were a few differences between patients and controls, namely in the distribution of males versus females and in distribution of European versus Non-European individuals. All socio-demographic and lifestyle variables were explored to investigate potential confounding factors. No correlation was reported between telomere length and age, years in education, hours of weekly physical activity, daily nicotine use, or BMI. However, marginally significant correlations were found between telomere length and

sex, ethnicity, and employability. These variables were controlled for in the main analyses and deemed not to represent substantial confounding factors.

4.1.2 Association between telomere length and duration of illness in the complete patient sample

The present study reported a negative association between telomere length and duration of illness in the total patient sample. To my knowledge, only two prior studies have investigated the possibility of such an association in patients with mood disorders (Elvsåshagen et al., 2011; Wolkowitz et al., 2011). Both studies included relatively small samples, meaning fewer than 30 patients each, with MDD subjects in the Wolkowitz study and BD II subjects in the Elvsåshagen study. The present outcome is in accordance with the findings of Wolkowitz et al. (2011) by showing a negative association between telomere length and illness duration, while Elvsåshagen did not observe any association. Both the current and the prior studies consisted of participants with a relatively low average age. Mean age for patients in the present study was 31.6 (±11.3) years, with 36.8 (±11.0) years in the Wolkowitz-study, and 34.8 (±7.7) years in the Elvsåshagen-study. In the current data set, mean duration of illness was 10.6 (± 9.3) years, while the mean was 13.0 (± 11.2) years in the Wolkowitz sample, and 19.0 (± 6.5) years in the Elvsåshagen sample.

As telomere length is believed to be inversely related to chronological age, low average cohort age may partly account for the non-robust results in all three studies. As expected in my study, there was a high degree of co-linearity between chronological age and illness duration. Interestingly, age did not in itself correlate with telomere length, while duration of illness showed a significant correlation before adjustments for multiple testing. Neither the previous nor the present studies provide any conclusive evidence for the hypothesis of accelerated telomere attrition with the progression of illness in severe affective disorders. However, the current results provide some further credence to the proposed inverse relation between telomere length and duration of illness.

4.1.3 Association between telomere length and number of affective episodes in the complete patient sample

The present study did not report any significant correlation between telomere length and lifetime number of depressive, manic, or hypomanic episodes. These findings are in accord with Hartmann et al. (2010) and Schaakxs et al. (2015), who reported no significant association between number of depressive episodes and telomere attrition in two cohorts of MDD individuals, and with Martinsson et al. (2013) and Elvsåshagen et al. (2011) who reported no association regarding number of lifetime manic or hypomanic episodes in BDI and BDII patients. On the contrary, a few prior studies have demonstrated a significant association between number of depressive episodes and shorter telomere length in severe affective disorders, although primarily in MDD patients (Gillis et al., 2019; Schutte & Malouff, 2015b; Wolkowitz et al., 2012).

There is considerable variation in sample size, age, distribution of females to males, medication status, and clinical diagnoses included in the studies mentioned above. Prior studies have also lacked reports of the association between telomere length and different types of affective episodes in combination. Significant discrepancies in methodology, sample characteristics, and design may account for some of the heterogeneity in recent findings and make comparisons with the present study results unreliable.

4.1.4 Sub-group comparisons

Among the diagnostic sub-groups within the present cohort, no significant associations were observed between telomere length and illness duration or lifetime number of affective episodes. To my knowledge, only one previous study (Lima et al., 2015) has compared telomere length in sub-groups of bipolar individuals. The authors reported results corresponding to the present findings, in that no significant difference between BD I and BD II patients was found regarding telomere length. The present sub-group comparison differs by including BD I and BD II, as well as MDD patients with psychotic symptoms. One potential explanation for the absence of a statistically significant difference might be the relatively small number of patients in the MDD group (n = 22). Consequently, there may not have been sufficient power to detect any real difference between the BD and MDD subjects. Small but essential differences may become visible only in samples with larger group sizes. Still, severe affective disorders are highly heterogeneous syndrome disorders, and the separate diagnostic sub-groups might not show significant variation amongst themselves.

4.2 Methodological considerations

4.2.1 Representability

My investigation was performed on out-patients and in-patients living in Oslo and receiving "treatment as usual" under real-life conditions. Healthy controls were included from the same geographical area within a corresponding time interval of nine years, thus minimizing socio-cultural and time-related differences. However, due to the Norwegian person data security act, information on individuals declining to participate was unavailable, and it was not feasible to estimate the rate of participation. Study participation is typically fraught with selection bias and self-selection, which is known to be intricately linked to socio-demographic differences and may have influenced the attendance of controls (Bordens & Abbott, 2018). Regarding patients, cognitive deficits or severe affective or psychotic symptoms would probably have prevented the more severely affected subjects from participation. Besides, patients lacking the capacity to give informed consent would not be approached. Therefore, it seems sound to conclude that the patient group, as well as the controls, were representative of comparatively young, ethnically homogenous, and somatically healthy individuals living under approximately equal socio-demographic conditions. This was also confirmed by exploring the descriptive data.

4.2.2 Generalizability of findings

The first aim of this thesis was to compare patients with severe affective disorders and individuals from the general population regarding telomere length. In Norway, health care and social security services are publicly funded and provided at a low cost to all citizens, thereby securing equal health and social services to the whole population. Thus, the chance that patients and controls differ greatly in terms of socio-economic factors that may affect telomere erosion is minimized, and the difference in telomere length found is likely to be the result of disease-specific factors. The observations of this study showed a small difference in telomere length between patients and controls. However, the current patient sample was relatively young and otherwise healthy, and as telomere length is believed to be a measure of biological aging, telomere attrition would probably be even more pronounced in older and more chronically ill patients.

4.2.3 Measurement of leukocyte telomere length

Sub-group analyses in the Colpo and colleagues' meta-analysis (2015) suggested that the type of assay used to measure telomere length might explain some of the heterogeneity in the literature. Expressly, the study indicated that the qPCR method more often fails to find short telomeres in patients relative to controls compared to other measurement methods. Another meta-analytic review found that the choice of assay had a significant moderating effect on the relationship between telomere length and severe affective disorders. Studies using Southern blot and fluorescent in situ hybridization assays (FISH) showed larger effect sizes relative to the studies using qPCR (Schutte & Malouff, 2015). Hence, qPCR, the assay utilized in the current study, may not be as sensitive to telomere length differences relative to other measurement methods. Evidence also implies that disagreements between specific laboratory standards may account for some variation from one study to another (Martin-Ruiz et al., 2015). The fact that studies with smaller cohorts are more likely to use Southern Blot or FISH may also impact results (Schutte & Malouff, 2015). Aubert and colleagues (2012) propose that the preferred choice of assay may depend on the purpose of the study and the characteristics of the sample.

Studies of live humans typically measure telomere length in peripheral blood samples, serving as a proxy for telomeres in brain tissue. There might be significant differences between telomere length in the brain versus in the blood. These differences may again influence the validity of my results. Howbeit, recent exploration has demonstrated a positive correlation between leukocyte telomere length and telomere length in other tissues, and regulation of telomere length appears to be overall tissue independent (Daniali et al., 2013; Dlouha, Maluskova, Lesna, Lanska, & Hubacek, 2014). Nevertheless, potential tissue-specific differences in telomere length are still not fully elucidated. This limitation must be acknowledged when interpreting the results.

4.2.4 Statistical issues

When reviewing the current study, a couple of statistical issues should be considered. First, the problem of multiple comparisons, or multiple testing. Multiple testing can be defined as such: When several, simultaneous statistical inferences are made, erroneous inferences are more likely to occur. As more hypotheses are tested, the probability of detecting a false positive rises. Consequently, the chance of falsely rejecting the null hypothesis increases (Hothorn et al., 2008). To address this problem, I employed the

Bonferroni correction method. This method operates by re-calculating the probabilities obtained from a repeated test and compensating for the increased chance of an incorrect inference (Dunn, 1961). Bonferroni adjustment for six inferential tests resulted in a new statistical significance threshold level of .008. As mentioned, Bonferroni is often considered to be conservative, in particular, when the various statistical tests are not independent and may underestimate the true significance value (Moran, 2003). Thus, the non-corrected results ($p < .05$) could well indicate relevant associations that should be further tested in future studies. Moreover, pairwise comparisons between the diagnostic sub-groups were only made on variables with results at a $p < .05$ significance level in the complete sample analyses. I chose to do this as an additional way to cope with the issue of multiple testing.

The issue of missing data and outlier influence is described in depth in the method section. Missing data was determined not to be a major problem in a study cohort of the current size. Due to extreme values on the telomere length variable, one subject was considered for removal from the final dataset. Cook's and Mahalanobi's distance measures were used to identify outliers. All analyses were executed twice, once with and once without the outlier. Inclusion of the outlier subject affected the *p*-value of the illness duration and telomere length analysis in the complete and BD I cohort. No other results were affected by the addition. A possible reason for this extreme value may be telomere length measurement error. However, this type of error is unlikely, given that the assay measurement was completed multiple times to control for potential missteps. The outlier may also be due to a typing error. Nevertheless, this extreme value may also represent a true, naturalistic telomere length value and witness the large variability in telomeres between people.

4.3 Strengths

The present study consisted of a comparatively large sample of patients ($n = 248$), studied together, and compared according to diagnostic sub-groups. No similar study, with a predominantly BD sample, has to date consisted of this many participants. The majority of studies on telomere length in BD patients have comprised less than 100 patients. In the current literature, there is also a large caveat of studies comparing diagnostic sub-groups. The inclusion of three well-characterized diagnostic sub-groups is unique compared with studies of similar design. The patient and control group were age-matched and similar concerning essential factors that may influence telomere length, reducing the risk of statistical noise. In

addition, reliability testing was completed for all essential items, and potential confounding factors were assessed and controlled for in all inferential analyses.

4.4 Limitations

As previously discussed, results in this field of study are often inconsistent and non-conclusive. Several limitations should, therefore, be considered when interpreting the present results.

4.4.1 The "age problem"

Research suggests that age-related processes, such as cerebral cortical thinning and telomere attrition, may not become pronounced before *after* the age of 50. For instance, King and colleagues (2014) demonstrated more pronounced structural changes in the brain and a more significant drop in telomere length in participants above 50. The King et al. study sample was a large population-based male cohort. This finding is in accordance with the Belsky and colleagues' study (2018), which failed to give a strong prediction of age-related characteristics in the midlife Dunedin cohort. In affective illnesses, evidence suggests that while early-stage patients show preserved cognitive faculties, late-stage patients often exhibit significantly reduced cognitive functioning relative to healthy controls (López-Jaramillo et al., 2010; Rosa et al., 2014). As previously noted, studies with younger subjects more frequently fail to observe a significant association between affective disorders and telomere length compared to studies with older samples (e.g., Elvsåshagen et al., 2011; Fries et al., 2017; Mansour et al., 2011).

Following these observations, it is essential but difficult to disentangle the differences in telomere length between patients and controls that are not just due to chronological age. Regarding the current data set, there was no statistically significant age difference between the groups, and age was added as a control variable in the statistical analyses. It is important to bear in mind, however, that many individuals were in their early twenties, and only 24 patients were 50 years old or older, thereby limiting the patient age distribution. Given the literature discussed, it may not be feasible to observe marked accelerated aging in studies of young individuals. The comparably low age of the present patient sample might disguise a drop in telomere length that becomes visible only after a certain age. Moreover, a generally young cohort may also explain why telomere length was found not to correlate significantly with chronological age. As a result of disease progression, patients might experience a more

sudden plunge in telomere length relative to healthy individuals. Future research should explore this potential drop in telomere length using age-stratified groups, preferably with a longitudinal design.

4.4.2 Diagnostic reliability and clinical validity

A significant number of individuals wait up to ten years for a proper BD diagnosis, as hypomanic and manic episodes may not be present during the first years of disease and can be atypical and tough to identify. In particular, the BD II diagnosis is often challenging to make and is frequently misdiagnosed as MDD (Ghaemi et al., 2002). Mild or atypical episodes of hypomania may often go unnoticed by patients, as well as clinicians (Benazzi & Akiskal, 2003; Vöhringer & Perlis, 2016). High comorbidity with other psychiatric illnesses, such as anxiety and personality disorders, also contribute to inaccurate diagnoses in BD (Barroilhet et al., 2013; Pavlova et al., 2015). Thus, the diagnostic validity of a bipolar versus a unipolar disorder can be weak, and only a careful, systematic assessment of current, as well as past, hypomanic or manic episodes allows for accurate diagnosis (Mitchell et al., 2011; Vöhringer & Perlis, 2016).

BD and MDD are heterogeneous disorders. However, because of their many commonalities, BD and MDD have been proposed as representing different parts of the same diagnostic spectrum (Akiskal, 2003; Ghaemi et al., 2002; Perlis et al., 2011). This proposal may be particularly relevant for the more severe manifestations of unipolar depression, such as MDD with psychotic symptoms. Given the high prevalence of comorbidity between psychiatric disorders (Kessler et al., 2005), heterogeneity within disorders, and the lack of robust biological underpinnings for diagnoses, modern psychiatric research is moving away from disorder-based constructs. A target objective is to elucidate new transdiagnostic criteria (Insel et al., 2010). The Research Domain Criteria is a recent initiative with the aim of creating a transdiagnostic classification system, contrasting that of the DSM. The basis of this new framework is the many commonalities in core features, mechanisms, and influences underlying different mental illness syndromes. Influenced by this, the past ten years have seen a rise in studies comprised of several diagnostic groups (Cuthbert & Insel, 2013). At present, a primary focus is given to explore shared mechanisms and how these mechanisms manifest in dynamic and interactive processes, rather than in established and immobile traits. In the current data set, analyses were conducted on a combined patient group with the goal of exploring the general diagnostic category of severe affective disorder. In addition,

comparisons between controls and patients were also made based on the DSM-system of diagnoses and sub-diagnoses, thereby integrating both the transdiagnostic and traditional diagnostic thinking into my research design.

In affective disorders, depression is usually more detrimental to the general functioning and quality of life than elevated episodes. Depressive episodes tend to be more frequent and last longer, with sub-depressive symptoms such as loss of energy and cognitive difficulties that often linger into the euthymic phase (Ghaemi et al., 2002). Besides, medication strategies are usually much more efficient for mania and hypomania than for depression in BD (Smith et al., 2012; Vohringer & Ghaemi, 2011). Therefore, of relevance to the present study, depression may be more related to chronic stress and telomere attrition than elevated mood episodes.

Theories of progression in mood disorders, such as the staging model, are often concerned with the effect of lifetime *number* of affective episodes. They propose that the advancing impairment and treatment resistance seen in late-stage illness might be generated by the accumulated number of lived episodes (de la Fuente-Tomás et al., 2020; Reinares et al., 2010; Verduijn et al., 2015). However, variation in individual episode length is sizable (Ghaemi et al., 2002), and it might be reasonable to suggest that comparing a few long-lasting episodes with many short episodes would be problematic in regard to research. Thus, considerable variability in presentation of symptoms may put the validity of a variable measuring the lifetime number of affective episodes into question.

4.4.3 Diagnostic considerations in the current sample

Because of the diagnostic issues discussed above, BD II is still a somewhat controversial diagnostic entity. In addition, the MDD patient sub-group in my study was small and consisted of first-episode patients (n = 6), as well as patients with recurrent depressive episodes (n = 16). This distinction is important when exploring the effect of illness duration and lifetime number of affective episodes on telomere length. Furthermore, all MDD patients had suffered psychotic episodes and could well be in the early stages of bipolar disorder. On the other hand, in BD I, the occurrence of at least one manic or mixed episode is a prerequisite for the diagnosis, and this diagnosis can therefore be considered the most robust and homogenous clinical entity in my sample (Malhi et al., 2010). The BD I sub-group was also by far the most numerous (n = 159), and the study might have profited from having a more consistent sample of research subjects. Further arguments to support this notion is the

noticeable clinical and demographic differences between the three diagnostic sub-groups. BD II patients had significantly longer average illness duration, as well as significantly more lifetime affective episodes, although similar in age to the rest of the sample. Including two diagnostically more uncertain sub-groups may thus perhaps have altered the study outcome.

4.4.4 Comorbid psychiatric disorders

Patients with mood disorders often report high rates of anxiety, and a majority classify for an anxiety spectrum diagnosis (Freeman et al., 2002; Gorman, 1996; Keller, 2006). Some authors have suggested anxiety disorders to be indicative of shorter telomere length (Hoen et al., 2013; Wang et al., 2017). However, anxiety is often so prevalent in affective disorders that it may represent an essential characteristic of the disease itself (Akiskal, 2003). Many patients report severe anxiety to be even more taxing and damaging to their quality of life than the affective symptoms per se (Goes et al., 2012). Since affective disorders can be considered to be "syndromic" rather than distinct illnesses, it is at this stage impossible to untangle how different elements of the disorders may affect telomere attrition independently.

4.4.5 Possible confounding factors

As mentioned, subject-specific variables may introduce bias into the analyses of telomere length in humans. To date, no study has been able to control for all these factors. For instance, a 2015 meta-analysis suggested that various sex-distribution among studies could explain a considerable amount of heterogeneity in the results as women seem to have an overall longer average telomere length than males (Colpo et al., 2015). This proposal fits with the fact that women tend to live longer and healthier lives, with fewer years of disease in old age (Naghavi et al., 2015; Seifarth et al., 2012).

To assess potential confounding factors, I explored the correlation between telomere length and socio-demographic and lifestyle variables. My choice of control variables for the statistical analyses was also based on a review of relevant literature (i.e., Barrett & Richardson, 2011; Ly et al., 2019; Müezzinler et al., 2013; Parks et al., 2011; Valdes et al., 2005). Per previous observations, differences in telomere length between men and women were observed at a trend level, indicating somewhat longer telomeres in women. However, telomere length seemed to be unrelated to chronological age in both patients and controls. This somewhat unexpected finding is most probably explained by the considerable cohort age

skewness, with few participants aged above 50. As previously discussed, telomere length might not be predictive of age and aging before after a certain age.

On the other hand, both ethnicity and working status showed associations with telomere length. As in the present study, Ly and colleagues (2019) previously reported a negative association between non-European ethnicity and telomere length. The authors argued that the stress related to being part of an ethnic minority might provoke accelerated telomeric attrition. Furthermore, as expected, negative working status was associated with telomere erosion. The ability to work or study, either full-time or part-time, gives a good indication of general functioning. A low percentage of employed patients indicates an overall lower level of functioning within this group. Nevertheless, the inclusion of control variables showed no sizable effect on my statistical model and may not represent a substantial influence. Data were not collected on working status, physical activity, nicotine use, or BMI in the control group, making group comparisons on these variables unfeasible. However, most analyses were performed only in patients, making potential differences with controls less relevant.

An important limitation of the current study is the lack of reports on psychotropic medication use. Preliminary research indicates that lithium, a mood-stabilizing medication, may *protect* against telomere attrition (Martinsson et al., 2013; Squassina et al., 2016). Moreover, the mechanism behind this could be a direct effect on telomerase activity (Wei et al., 2015). Growing research also implies a potential protective effect of antidepressant medication (Rasgon et al., 2016; Zhou et al., 2011). Consequently, future studies should include reports on psychotropic medication use when investigating telomere length in a patient sample.

4.5 Interpretation of findings

4.5.1 Telomere length and the effects of chronic stress

It may be reasonable to assume that the duration of illness and the number of lifetime affective episodes would vary in concordance with one another. The somewhat surprising finding of telomere length to be dependent on illness duration but not on the number of affective episodes may tell us something about the overall burden of stress in affective illness. The aftermath of an affective episode has the potential of being just as devastating as the episode itself, both psychologically, socially, and economically (Ghaemi et al., 2002). Telomere shortening might not result directly from episodic or state toxicity, as previously

proposed (Kapczinski et al., 2010), but rather from the accumulated burden of a chronic disorder.

Previous research has convincingly demonstrated telomeric attrition to be associated with chronic stress in the general population (Price et al., 2013a; Puterman et al., 2015; Verhoeven et al., 2015). As a consequence of living with a serious and often debilitating psychiatric condition, patients with mood disorders are believed to experience large amounts of chronic stress (Kapczinski et al., 2010). For many of these individuals, the burden of disease represents a progressive functional impairment with substantial occupational and social consequences. Few individuals are able to hold a full-time job up until retirement (Schoeyen et al., 2011). As a result, daily life stress is hypothesized to augment throughout the years. In the present study, the finding of shorter telomeres with longer illness duration, but not with affective episodes, is consistent with a chronic stress hypothesis of telomere attrition. This observation entails that the crucial factor for accelerated aging may be the long-term strain of illness, more than the dose-dependent effect of episodes.

Allostatic load is a construct that might help explain the process by which telomere shortening occurs. This construct refers to the individuals' accumulated exposure to stress represented as the "wear and tear on the body" (McEwen & Stellar, 1993). Compensatory adaptions made to re-establish homeostasis are generated at a cost to the organism, further increasing vulnerability to stress. In affective disorders, the allostatic load is increased. With prolonged exposure to high levels of stress, a vicious cycle may be created. This cycle may further increase susceptibility to recurrent episodes, exacerbate symptom severity, and result in poorer general functioning (Berk et al., 2017).

4.5.2 Biological mechanisms

Research implicates various dynamic bio-mechanisms in telomere shortening. These mechanisms lead to a decrease in neurotrophins and, consequently, to deficient neurogenesis and increased cell death in the central nervous system (Fries et al., 2014; Mondello & Scovassi, 2004). Some proposed underlying processes include epigenetics, inflammation, and oxidative and nitrosative stress. For instance, oxidative stress resulting from chronic overactivation of the automatic stress response may speed up telomere attrition (Andreazza et al., 2008; Epel et al., 2004). Furthermore, chronic stress is associated with reduced levels of telomerase activity, an enzyme that works to replenish the telomere sequence, thereby protecting the cell (Epel et al., 2004). Hence, increased exposure to prolonged stress may

cause an increase in telomere attrition. Chronic overactivation of the neuroendocrine and automatic stress response is commonly observed in affective disorders (Kapczinski et al., 2008).

Various endogenous and exogenous factors may interact in an infinitely complex manner to either mediate or moderate the progression of an individual illness course. For example, early childhood abuse may induce vulnerability to dysfunction in biological systems, while day-to-day factors, like sleep disturbances, may exacerbate an already ongoing disease process (McGorry et al., 2010).

4.5.3 Directionality

An important question remains: Does shorter telomere length represent a risk factor, a consequence, or a driving force of illness progression and accelerated aging in affective disorder? Evidence suggests that shorter telomeres might both precede and be the product of disease. Powell et al. (2017) found telomere length to be shorter in first-degree relatives of BD patients compared to unrelated healthy controls, indicating a component of heritability. Research data from experimental studies propose that shorter telomere length may incite cessation of cell division (Herbig et al., 2004), apoptosis, and cell death (Mondello & Scovassi, 2004). These findings are consistent with evidence of the increased risk of age-related disorders in mood disorders (Fries et al., 2014; Verhoeven et al., 2014; Fries et al., 2020). In conclusion, several lines of research have suggested telomere shortening as both preceding and leading to diseases of aging (Blackburn et al., 2015). However, it is still unclear what constitutes the specific interactions between telomeres and other age-related biological factors.

Leukocyte telomere length is intended as a proxy measure of biological aging, which varies between individuals of the same chronological age. However, it remains to be determined whether telomere length constitutes a reliant measure of biological aging. Somewhat surprisingly, a study of the now midlife Dunedin Study cohort recently reported low rates of agreement between eleven different measures of biological aging, including telomere length (Belsky et al., 2018). The authors suggested that various approaches to measuring biological aging might assess different facets of the aging process. Additionally, Belsky and colleagues reported only a low to moderate association between various proposed biomarkers of aging and clinical characteristics related to health span, such as physical and cognitive capacity, in this segment of individuals from the general population. While age

related biomarkers, such as telomere length, are shown to predict disease and mortality in old age (Blackburn et al., 2015), it is still unknown whether the assessment of biological aging in young age or midlife has a comparable predictive power (Belsky et al., 2018).

4.6 Future directions

Affective disorders are associated with accelerated aging and premature death, but the precise underlying mechanisms for this remain opaque. Augmented telomere attrition represents a potential biomarker of accelerated aging as multiple studies have linked shorter leukocyte telomere length with age-related illness progression and reduced life expectancy in the general population (Blackburn et al., 2015). Future research, using experimental study designs, will need to address the specific mechanisms through which telomere shortening is linked to biological aging in order to elucidate a causal relationship. In this endeavor, the role of telomerase activity might be of specific interest. A better understanding of the underlying mechanisms might lead to the discovery of new methods for preventing and treating age-related diseases in vulnerable parts of the population.

Further clinical studies on accelerated aging in patients with severe mental disorders would preferably include more age-diverse samples with stratified patient and control groups. Furthermore, patients and controls should be matched, as far as possible, on socio-demographic factors that could potentially influence telomere shortening. Prospective clinical studies should also include reports of psychotropic medication use and comorbid psychiatric illnesses.

To date, the majority of studies have been cross-sectional. However, the disease course in mood disorders is highly heterogeneous, and the underlying etiology and illness processes remain undecided. Consequently, clinical studies with a longitudinal design are warranted.

4.7 Concluding remarks

This research aimed to investigate whether affective disorders are related to accelerated aging, represented by shorter telomere length. The results suggest shorter telomere length in patients with severe affective disorders relative to healthy controls. In the patient group, longer duration of illness was associated with shorter telomeres. These associations did not remain significant after strict correction for multiple testing. However, given the conservative nature of these corrections, the nominally significant associations ($p < .05$) could well indicate relevant observations that should be tested in future studies. No associations

between affective episodes and telomere length were found, and diagnostic sub-group comparisons were non-significant. Nevertheless, the present observations complement the existing literature by indicating that augmented telomere attrition may be linked to both the occurrence and progression of severe affective illness in patients. These results do, in turn, suggest accelerated aging in patients, and are a valuable addition to the growing understanding of health and wellbeing in severe mental disorders.

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