Expression of telomerase reverse transcriptase positively correlates with duration of lithium treatment in bipolar disorder

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Abstract

Bipolar disorder (BD) may be associated with accelerated cellular aging. However, previous studies on telomere length (TL), an important biomarker of cellular aging, have yielded mixed results in BD. We aimed to evaluate the hypothesis that BD is associated with telomere shortening and whether this is counteracted by long-term lithium treatment. We also sought to determine whether long-term lithium treatment is associated with increased expression of telomerase reverse transcriptase (\textit{TERT}), the catalytic subunit of telomerase. We determined TL and \textit{TERT} expression in 100 BD I patients and 100 healthy controls. We also genotyped three single nucleotide polymorphisms associated with TL. \textit{TERT} expression was significantly increased in BD I patients currently on lithium treatment. \textit{TERT} expression was also significantly positively correlated with duration of lithium treatment in patients treated for 24 months or more. However, we did not find any significant effect of lithium treatment on TL. Neither did we find significant differences in TL between BD patients and controls. We suggest that long-term lithium treatment is associated with an increase in the expression of \textit{TERT}. We hypothesize that an increase in

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
TERT expression may contribute to lithium’s mood stabilizing and neuroprotective properties by improving mitochondrial function and decreasing oxidative stress.

Keywords
Affective disorder; Aging; Telomere; TERT; Mitochondria; Oxidative stress

1. Introduction

Bipolar disorder (BD) is a chronic psychiatric disorder characterized by alternating states of mania and depression, affecting more than 1% of the general population. It is a leading cause of disability among young people and typically associated with a high prevalence of psychiatric and medical comorbidities. BD is divided into two main subtypes: BD I, defined as the occurrence of at least one manic episode with or without major depressive episodes; and BD II, defined as the occurrence of at least one hypomanic episode and one major depressive episode (Grande et al., 2016). In addition to the cyclic nature of BD, there is a growing body of evidence of progressive features of the disorder. Several studies have demonstrated an association between years of active disease and increased severity of symptoms, lower treatment response, worsening of neurocognitive performance, and increased risk of suicide (Fries et al., 2012; Kapczinski et al., 2014).

Lithium is the most effective long-term treatment option for BD and often the first-line mood stabilizer. It has a significant effect on reducing relapse of both mania and depression and is unique in its great reduction of suicide risk (Geddes and Miklowitz, 2013). Recently, lithium has also been identified as a potential pharmacotherapy in several neurodegenerative disorders, including Parkinson’s disease and Alzheimer’s disease, due to its neuroprotective properties (Lazzara and Kim, 2015; Morris and Berk, 2016). Lithium has widespread effects within different cellular pathways but the specific mechanisms by which lithium exerts its therapeutic effects are largely unknown (Malhi and Outhred, 2016).

The etiology and pathophysiology of BD is complex, multifactorial, and largely uncertain. Mitochondrial dysfunction has been implicated as a key element in the pathophysiology of BD through multiple lines of evidence including decreased mitochondrial respiration; altered mitochondrial morphology; increases in mitochondrial DNA polymorphisms, deletions and mutations; as well as decreased levels of high energy phosphates, elevated lactate and decreased pH in the brain. Additionally, BD is associated with changes to several cellular processes dependent on mitochondrial function including increased oxidative stress, changes in apoptosis, and calcium dysregulation (Sigitova et al., 2017). Interestingly, mitochondrial function seems to be dependent on mood state in BD with increased mitochondrial respiration and ATP production in bipolar mania contrasting with decreased mitochondrial function in bipolar euthymia and depression. Thus, it has been hypothesized that a phasic dysregulation of mitochondrial bioenergetics may be the cause of BD (Morris et al., 2017).

BD has also been associated with an increased prevalence and earlier age of onset of several age-related disorders such as cardiovascular disease, diabetes mellitus, metabolic imbalances, and dementia. Additionally, there is a significant overlap between detrimental
changes observed in BD and those occurring in normal and pathological aging including neurodegeneration, immunosenescence, and oxidative stress imbalance. Based on these findings it has been hypothesized that BD may be associated with accelerated cellular aging (Rizzo et al., 2014).

Telomere length (TL) is an important biological marker of cellular aging. Telomeres are DNA-protein structures at the ends of chromosomes consisting of non-coding hexameric tandem repeats of TTAGGG in association with several specialized proteins, among others, the protective shelterin complex (Turner et al., 2019). The main function of telomeres is to protect genomic DNA against different cellular processes, such as DNA-end joining, DNA recombination, and DNA-repair, that would otherwise cause chromosomal instability. Telomeres shorten with each cell division due to the end replication problem until a critical limit is reached, which triggers a form of DNA damage signaling, altering transcriptional profiles and causing the cell to become senescent. Telomere shortening can be accelerated by, among other processes, nuclease action, oxidative damage, and DNA replication stress (Blackburn et al., 2015). TL is also, in part, heritable, and genome-wide studies have identified several genetic variants associated with shorter TL. Single nucleotide polymorphisms (SNPs) within genes encoding for enzymes involved in either telomere lengthening or telomere maintenance constitute most of these associations (Barrett et al., 2015).

Telomere shortening can be counteracted by the activation of telomerase. Telomerase is a ribonucleoprotein complex consisting of two main components: telomerase RNA component (TERC), an RNA template used for the synthesis of telomeres; and telomerase reverse transcriptase (TERT), a catalytic subunit, together with associated proteins (Ozturk et al., 2017). The canonical function of telomerase is the lengthening of telomeres within the cell nucleus, preserving genetic integrity and promoting cell survival. TERT has also been found to perform non-canonical functions independent of TERC both within the nucleus and in other cellular compartments: influencing gene expression, signaling pathways, mitochondrial function, and resistance to cellular stress and degradation (Saretzki, 2014).

Previous studies have yielded mixed results regarding peripheral TL in BD (Squassina et al., 2017). While some studies have found shorter TL in BD (Elvsåshagen et al., 2011; Lima et al., 2015; Rizzo et al., 2013; Simon et al., 2006), others have found no difference in TL between cases and controls (Mansour et al., 2011). (Martinsson et al., 2013) found increased leukocyte TL (LTL) in BD compared to controls. Interestingly, the authors reported a positive effect of lithium treatment on LTL: longer LTL was observed in BD patients treated with lithium compared to controls and a positive correlation was found between LTL and duration of lithium treatment in patients treated for more than 30 months. Additionally, lithium responders presented with 10% longer telomeres than non-responders. Some of these findings were later replicated by (Squassina et al., 2016) that similarly found a positive correlation between LTL and lithium treatment. Authors of both studies hypothesized that an increase in TERT expression and telomerase activity may mediate the telomere-lengthening effect of lithium. This hypothesis was later supported by a study by (Wei et al., 2015) that demonstrated an increase in TERT expression and telomerase activity after 6 weeks of lithium treatment in a rat model of depression. However, in a subsequent short-term study by
(Soeiro-de-Souza et al., 2014), in which telomerase activity was quantified in a small sample of medication-free BD depressed individuals before and after 6 weeks of lithium treatment, no significant difference in telomerase activity was found. Additionally, in a recent study by (Köse Çinar, 2017) in which TERT expression was quantified in a small sample of medication-free manic BD patients before and after short term treatment with lithium and antipsychotics, no significant difference in TERT expression was found.

Thus, the association between BD and accelerated aging in terms of telomere shortening remains uncertain and further studies have been warranted. Additionally, even though recent studies have suggested that long-term lithium treatment may counteract telomere shortening in BD patients, whether this is a result of an increase in TERT expression and telomerase activity has not yet been determined.

In this study, we sought to evaluate the hypothesis that BD is associated with accelerated aging in terms of shorter TL and whether this is counteracted by long-term lithium treatment, while considering the effect of genetic variants previously associated with shorter TL. We also aimed to evaluate whether long-term lithium treatment is associated with an increase in the expression of TERT.

2. Methods

2.1. Sample

The sample comprised 100 BD I patients and 100 controls from the Mayo Clinic Bipolar Disorder Biobank (Frye et al., 2015) and the Mayo Clinic Biobank (Olson et al., 2013), respectively. Controls were matched to cases by sex and age within five years using frequency matching. All subjects were Caucasian. The BD I patients and controls in this study were recruited between 2009 and 2015.

To participate in the Mayo Clinic Bipolar Disorder Biobank, patients were required to have a clinical diagnosis of type I or II BD or schizoaffective BD according to the DSM-IV criteria, be between 18 and 80 years old, and provide written informed consent followed by a comprehension test questionnaire. Patients with active psychosis or active suicidal ideation were not included in the biobank. The Mayo Clinic Bipolar Disorder Biobank stores blood samples as well as demographic and clinical information of participants, recorded using questionnaires and in electronic medical records. When selecting cases for the present study, patients were included if they had a clinical diagnosis of BD I, ascertained using the Structured Clinical Interview for DSM-IV (SCID-I/P) and reported having at least one first-degree relative with BD.

To participate in the Mayo Clinic Biobank, subjects had to be previous or current patients at Mayo Clinic or the Mayo Clinic Health system, be 18 years old or older, and provide written informed consent. The Mayo Clinic Biobank stores blood samples as well as demographic and clinical information of participants, recorded using questionnaires and in electronic medical records. When selecting controls for the present study, participants with a history of psychiatric disorder or first-degree relatives with BD and ICD-9 codes associated with BD and/or schizophrenia were excluded.
Both biobank collections, as well as the present study, were approved by the Mayo Clinic Institutional Review Board (IRB) and the procedures were conducted according to the principles expressed in the World Medical Association Declaration of Helsinki.

### 2.2. Telomere length measurements

Total genomic DNA was extracted from frozen peripheral blood leukocyte (PBL) samples stored at −80 °C using the Maxwell® RSC Blood DNA Kit protocol (Promega, Madison, WI, USA). DNA was quantified on the Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), using the Qubit® dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). This DNA was used for both the TL and telomere-related genotyping assays. TL was measured using modified monochrome qPCR (MMQPCR) ([Cawthon, 2009](#); [Rode et al., 2015](#); [Weischer et al., 2012](#)). The MMQPCR method generates a T/S ratio (telomere/single copy gene), which represents an average telomere abundance across all chromosomes and cells in the sample. The T/S ratio is the mean cycle threshold of the telomere primer pairs as compared to a single copy gene (albumin) primer pairs, which are multiplexed in one well during the same run. All results for TL here were analyzed using the mean value of the cycle threshold (Ct) for the telomere reaction over the mean value of the Ct for the single copy gene albumin reaction to generate a T/S ratio value based on Ct. T/S ratio results of the assay are expressed as (T/S)$^{-1}$ since the cycle thresholds are inversely proportional to the quantity of template amplified. The MMQPCR assay was used here as previously described with no deviation in the sequence of primers, reagents, mastermix preparation, or cycling parameters ([Rode et al., 2015](#); [Weischer et al., 2012](#)). The assay was performed on the QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems®, Foster City, CA, USA). The MMQPCR assay for each sample was carried out in triplicate on a 384 well plate, and the inter-assay (between plate) coefficient of variation (CV) of Ct was 0.52% for the telomere primers and 0.55% for single copy gene primers. The intra-assay CVs were determined for each primer set based on a reference DNA sample from K562 cells and were 0.35% and 0.40% for the T and S primers, respectively. We determined that the telomere and albumin reaction efficiencies were equal and independent of the amount of DNA. When we plotted the ΔCt = Ct\text{albumin} - Ct\text{telomere} vs. a standard curve of DNA quantities, the linear regression had a slope of 0.07 indicating that ΔCt is constant across DNA amounts and allowed us to leave out standard curves for each plate run.

### 2.3. Genotyping

DNA extracted from PBL samples were genotyped for three SNPs: 1) rs7726159, a SNP in the *TERT* gene which encodes the telomerase reverse transcriptase; 2) rs1317082, a SNP near *TERC*, which encodes the telomerase RNA template and 3) rs2487999, a SNP near the *OBFC1* gene that is involved in the CST complex, a regulator of telomerase. Genotyping was conducted using TaqMan SNP genotyping assays (Applied Biosystems; Life Technologies, Carlsbad, CA, USA) according to manufacturers’ instructions. The assays for rs1317082 and rs2487999 were both pre-designed, whereas the assay for rs7726159 was custom designed with the following sequences from ([Rode et al., 2015](#)): *TERTF*, primer: GGATTTCTGTGCAA GGCTCTGA; *TERTR*, primer: GTCCTCATCTTTATAATCCCTTGTC TTT; *TERT* A probe: TGCCAAAGTGTTCTCTAG; *TERT* C probe: TGCCAAAGTGTTCTCTAG. The context...
sequence was: TGCCAAGTG[T/G]TCTCTAG. The reactions were performed using 10 ng of DNA (or water as negative controls) in a 5 ul total reaction volume in a 384 well plate for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C on the ViiA™ 7 Real-Time PCR System (Applied Biosystems®, Foster City, CA, USA). For analysis, an allele sum was calculated by taking the sum of the rs1317082 (TERC), rs7726159 (TERT), and rs2487999 (OBFC1) alleles previously associated with shorter TL (Rode et al., 2015). To calculate the allele sum, first a value was assigned for each sample based on its genotype: 0 = homozygous wildtype genotype (TT for OBFC1, AA for TERT, AA for TERC), 1 = heterozygous (CT for OBFC1, AC for TERT, AG for TERC), 2 = homozygous for allele previously associated with shorter TL (CC for OBFC1, CC for TERT, GG for TERC). Taking the sum of these values generates the allele sum, which ranges from 0 to 6.

2.4. Expression analysis of TERT

RNA was extracted from PBL samples using the Maxwell® RSC simplyRNA Blood Kit (Promega, Madison, WI, USA). RNA was quantified on the Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) using the Qubit® RNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). We then performed mRNA expression of TERT using the Power SYBR® Green RNA-to-Ct™ 1-Step Kit (Applied Biosystems®, Foster City, CA, USA) according to the manufacturer’s instructions. For each reaction, 40 ng of total RNA was used with 200 nM final concentrations of each primer. The sequences for the primers were obtained from (Xi and Cech, 2014). Specifically, we used the TERT2 primer set for TERT and the 18S rRNA primers as the internal control gene. RNA extracted from HeLa cells was used as a positive control/calibrator sample (Akınclılar et al., 2015; Xi and Cech, 2014) and RNase-free water as negative control. Cycling parameters were 48 °C for 30 min, 95 °C for 10 min, then 40 cycles of 95 °C for 15 secs and 60 °C for 1 min, with a melt curve performed to detect nonspecific amplification. Reactions were performed in duplicate. Calculation of TERT mRNA expression was determined by the Comparative Ct method (ΔΔCt method) and the values are expressed as the change in TERT expression of the sample normalized to 18S and relative to HeLa cells.

2.5. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Macintosh, version 24.0.

Demographic differences between cases and controls were tested using the independent samples t-test for the quantitative variable with normal distribution (age), whereas quantitative variables without normal distribution (BMI, alcohol consumption) were tested using the Mann-Whitney test, and dichotomous variables (sex, smoking history) were tested using the Chi-squared test. TERT expression was not significantly associated with any of the covariates and was thus analyzed using univariate tests. TERT expression was not normally distributed, therefore, associations with TERT expression were assessed using the Mann-Whitney test for dichotomous variables and Spearman’s correlation for continuous variables. TL was associated with some of the covariates and was thus analyzed using multivariable models that included covariates. TL was normally distributed and associations with TL as the dependent variable, adjusted for demographic confounders, were assessed using multiple linear regression. For secondary analyses, a cut-off of 24 months was chosen based on
previous studies by (Martinsson et al., 2013) and (Squassina et al., 2016) demonstrating a positive effect of lithium treatment on TL in BD patients treated for more than 30 and 24 months, respectively. The significance level was set at $p < 0.05$ (two-tailed).

3. Results

3.1. Demographics

The demographics and clinical characteristics of the study population are displayed in Table 1. There was a significant difference in BMI ($p = 0.0018$), smoking history ($p < 0.0001$) and alcohol consumption ($p = 0.00015$) between BD I and control groups. BD I and control subjects did not differ significantly in terms of age, sex or allele sum ($p > 0.64$).

3.2. TERT expression

The effect of covariates (age, sex, BMI, smoking history and alcohol consumption) on TERT expression was assessed in BD I patients and control subjects together. TERT expression was not significantly associated with any of the covariates ($p > 0.41$), hence no adjustments were made to subsequent analyses of TERT expression. There was no significant difference in TERT expression between BD I patients and controls ($p = 0.40$). BD I patients currently on lithium treatment had increased TERT expression (mean rank = 37.0) compared to BD I patients not currently on lithium treatment (mean rank = 27.3) ($p = 0.043$). TERT expression was not correlated with duration of lithium treatment when analyzing all lithium treated patients ($\rho = 0.18$, $p = 0.17$). There was, however, a strong positive correlation between TERT expression and duration of lithium treatment in patients treated for 24 months or more ($\rho = 0.53$, $p = 0.042$).

3.3. Telomere length

The effect of covariates (age, sex, BMI, smoking history, alcohol consumption, and allele sum) on TL was assessed in BD I patients and control subjects together. TL was negatively associated with age ($\beta = -0.00072$, $p < 0.0001$). After adjusting for age, there was a negative association between TL and smoking history ($\beta = -0.0088$, $p = 0.0015$), whereas none of the other covariates (sex, BMI, alcohol consumption, and allele sum) were associated with TL. After adjusting for age and smoking history, there was no significant difference in TL between BD I patients and controls ($p = 0.35$). TERT expression was not significantly associated with TL ($p = 0.62$). After adjusting for age and smoking history, there was no significant difference in TL between BD I patients currently on lithium treatment and BD I patients not currently on lithium treatment ($p = 0.80$). TL was not associated with duration of lithium treatment when analyzing all lithium treated patients ($p = 0.98$). Neither was TL correlated with duration of lithium treatment for patients treated for 24 months or more ($p = 0.75$).

4. Discussion

In this study, we evaluated whether long-term lithium treatment in BD patients was associated with an increase in the expression of TERT. We found that BD I patients currently treated with lithium had higher TERT expression than BD I patients not on lithium
We also found that TERT expression was positively correlated with duration of lithium treatment in patients treated for 24 months or more.

The observed increase in TERT expression in lithium treated patients may be interpreted either as caused by the lithium treatment or as a trait marker of BD pathophysiology in the subpopulation of patients undergoing lithium treatment or both. Both current lithium treatment and especially long-term lithium treatment could be proxies for both favorable clinical response, as the likelihood of being treated with lithium increases the better treatment response patients have; and a more stable illness, as the likelihood of being considered a lithium responder increases the fewer mood episodes patients have.

The short-term effect of lithium treatment on TERT expression in BD I patients has been investigated in one previous study. (Köse Çinar, 2017) measured whole blood TERT expression, using qPCR, in 21 medication-free manic BD I patients, before treatment and at remission after treatment with lithium and antipsychotics, and compared that to TERT levels in 20 healthy controls. The author found that TERT expression was significantly increased in BD I patients both before and after treatment with lithium and antipsychotics compared to controls. A relative increase in TERT expression after pharmacological treatment was observed, however, the author did not report any statistically significant difference. The author hypothesized that an increase in TERT expression may constitute a novel aspect of BD pathophysiology, possibly in order to counteract telomere shortening. However, as the effect of change in mood state and that of pharmacological treatment on TERT expression are inseparable in the study it may also be that increased TERT expression is a state dependent marker of mania which decreases as the patients recovers while lithium and/or antipsychotics at the same time independently increases it. It could also be that the sample size of 21 BD patients was too small or that the follow-up period of approximately 8 weeks was too short to detect significant treatment-associated changes in TERT expression.

The interpretation that lithium treatment may be responsible for the increase in TERT expression is supported by a study by (Wei et al., 2015) who investigated the effect of lithium treatment on TL, TERT expression, telomerase activity, and potential mediators of telomerase activity in the hippocampus of a genetic rat model of depression, the Flinders Sensitive Line (FSL), compared to the Flinders Resistant Line (FRL). The FSL rats had shorter TL, decreased TERT expression, decreased telomerase activity and decreased BDNF expression compared to FRL rats at baseline. After 6 weeks of lithium treatment, TERT expression and telomerase activity were significantly increased and β-catenin was upregulated. An increase in β-catenin provides a possible mechanistic explanation for lithium’s effect on TERT expression. Lithium indirectly increases levels of β-catenin, a central protein of the Wnt pathway, through inhibition of glycogen synthase kinase 3 (GSK3)–β (Bersani et al., 2015). In a study by (Zhang et al., 2012), β-catenin was, in turn, shown to activate TERT expression and telomerase activity through removal of the repressor T-cell factor 4 (TCF4) from the TERT promoter in human cancer cells.

One previous study investigating the effects of lithium treatment on telomerase activity in BD patients has been published. (Soeiro-de-Souza et al., 2014) measured peripheral blood mononuclear cell (PBMC) telomerase activity in 28 medication-free BD I and II
patients with depression compared to 23 healthy controls and assessed the effect of 6 weeks lithium treatment on telomerase activity in 21 of the BD patients, using the Telomeric Amplification Protocol (TRAP). They found no significant difference in telomerase activity between BD patients and controls, or before and after 6 weeks of lithium treatment in BD patients. The difference in results between our study and the study by Soeiro-de-Souza et al. may be explained by several methodological differences. The duration of lithium treatment was 6 weeks in the study by Soeiro-de-Souza et al., while several years on average in our study. Soeiro-de-Souza et al. analyzed telomerase activity using TRAP in PBMCs while we analyzed TERT expression in leukocytes. As opposed to a leukocyte sample, a PBMC sample does not include granulocytes and granulocytes differ in terms of telomere dynamics from other leukocytes (Rufer et al., 1999). Moreover, TRAP measures telomerase’s telomere-lengthening effect while TERT expression, although the rate-limiting step of telomerase activity, can also reflect several non-canonical functions of TERT (Saretzki, 2014).

Apart from the telomere-lengthening effect of telomerase, of which TERT is the catalytic subunit, TERT performs several additional functions in the nucleus and other subcellular locations such as the cytoplasm and mitochondria. Interestingly, TERT has been shown to translocate from the nucleus to mitochondria during increases in cellular oxidative stress. Mitochondrial localization of TERT has, in turn, been shown to improve mitochondrial function, decrease oxidative stress, lower nuclear and mitochondrial DNA damage, and prevent apoptosis (Saretzki, 2014). This non-canonical function of TERT may be of key importance to brain energy metabolism. While TERC, the RNA component of telomerase, is expressed in most adult human cells, TERT expression is generally not detectable, with major exceptions including most cancer cells, some adult stem cells, and some proliferating cells such as T and B cells (Zheng et al., 2019). Interestingly, even though telomerase activity in the brain is downregulated early during human development, possibly due to a downregulation of TERC, TERT expression has been shown to persists in adult neurons (Ishaq et al., 2016; Spilsbury et al., 2015). The reason for this may be that the brain is a very energy-demanding organ, highly dependent on mitochondrial respiration, while neurons at the same time are very sensitive to oxidative stress (Grimm and Eckert, 2017). A persisting expression of TERT may serve to decrease the generation of oxidative stress by mitochondrial respiration, preventing neuronal damage. Indeed, (Spilsbury et al., 2015) found that, in cultivated primary mouse embryonic neurons, an increase in oxidative stress caused TERT to translocate to mitochondria, resulting in decreased mitochondrial production of reactive oxygen species. Moreover, they found that TERT-expressing neurons in the hippocampus of Alzheimer’s disease brains did not contain hyperphosphorylated tau and vice versa and that TERT was colocalized with mitochondria in later stages of the disease.

Thus, we hypothesize that the anti-oxidative properties of TERT in mitochondria may constitute an important mechanism by which lithium protects against the progressive features of BD. Additionally, in line with the theory that alternating mood episodes may be caused by a phasic dysregulation of mitochondrial bioenergetics, we hypothesize that translocation of TERT to mitochondria may contribute to lithium’s effect on mood stabilization by improving mitochondrial function.
In this study, we also investigated whether long-term lithium treatment in BD patients was associated with an increase in TL. After adjusting for age and smoking history, we did not find a significant association between TL and long-term lithium treatment.

Several previous studies have reported a positive effect of long-term lithium treatment on TL. (Martinsson et al., 2013) measured LTL in 256 BD patients characterized for lithium response and 139 healthy controls. They found longer LTL in BD patients treated with lithium overall, as well as those on lithium monotherapy, compared with controls. They also found a positive correlation between LTL and duration of lithium treatment in patients treated for more than 30 months. (Squassina et al., 2016) investigated LTL in 200 BD patients characterized for lithium response. Like Martinsson et al., they found that LTL was positively correlated to duration of lithium treatment in patients treated for more than 24 months. As we only had reliable data on lithium treatment for 63 patients, we may have been underpowered to detect lithium’s impact on TL. It has been hypothesized that oxidative stress generated by recurrent mood episodes may be the cause of telomere shortening in BD (Rizzo et al., 2014). Considering this, important clinical factors influencing TL may include the number, duration, and severity of mood episodes. This hypothesis is supported by studies by (Elvsåshagen et al., 2011) and (Martinsson et al., 2013), in which the number of depressive episodes was found to have a negative impact on TL. As we were not able to adjust for this factor due to a lack of data on number of episodes, the difference in results between this and previous studies may be due to a difference in illness burden between study populations.

In this study, we also investigated a possible association between BD and accelerated aging in terms of shorter TL. After adjusting for age and smoking history, we found no significant differences in TL between BD I patients and control subjects.

Several previous studies investigating peripheral blood TL in BD patients and controls have been published, yielding mixed results (Elvsåshagen et al., 2011; Lima et al., 2015; Mansour et al., 2011; Martinsson et al., 2013; Rizzo et al., 2013; Simon et al., 2006; Squassina et al., 2016). These studies have differed in terms of sample size and methodology. Sample sizes have ranged from 22 BD patients and 17 controls to 286 BD patients and 139 controls and TL has been measured in either leukocytes or PBMCs and quantified using either quantitative polymerase chain reaction (qPCR), terminal restriction fragment (TRF) analysis, or high throughput quantitative fluorescence in situ hybridization (HT Q-FISH).

There are several different methods available to measure TL in cells and tissues, each with different strengths and limitations. qPCR measures a telomere signal (T) in relation to a single-copy gene signal (S), allowing the calculation of a T/S ratio which is proportional to average TL. qPCR has the advantage of being relatively easy to perform, requiring a relatively small amount of starting DNA and there are many published studies available for comparison. However, the method only provides information on average TL, it does not provide information about the shortest telomeres, and results depend on the single-copy gene used. TRF analysis utilizes a modified Southern blot analysis to measure the intensity of telomere smears to determine average TL. TRF was the original method used to determine TL and is often described as the gold standard and there are many published
studies available for comparison. However, it mainly provides information on average TL, it requires large amounts of starting DNA, is labor intensive, and depending on the choice of restriction enzymes used there can be large variations in results. HT Q-FISH uses automated high throughput microscopy in large plates to measure telomere fluorescence intensity after hybridization with a fluorescent peptide nucleic acid probe against telomeric repeats. It has the advantage of being applicable to fixed tissues and cells, it produces very reliable and reproducible results, and there are many published studies for comparison. However, it does not recognize telomere-free ends and as the probe may bind to some interstitial telomeric sequences it may generate false-positive results (Lai et al., 2018). In this study, we used qPCR for TL measurements due to its methodological advantages and since it was the preferred method in the majority of previous studies of TL in BD.

Three previous studies and the present study analyzed LTL in relatively large samples using qPCR. (Mansour et al., 2011) found no significant difference in LTL between 108 BD patients and 114 control subjects after adjusting for age and sex. (Martinsson et al., 2013) reported significantly higher mean LTL in 286 lithium treated BD patients compared to 139 controls adjusted for age and sex. (Lima et al., 2015) found significantly shorter LTL in 85 BD patients compared to 95 control subjects matched by age, sex and educational level.

TL in BD is likely a product of several demographic, clinical, and pharmacological factors, sometimes with opposite effects on TL, as in the case of illness burden and lithium treatment, that may vary between study populations. As these factors were not all accounted for, neither in this nor in previous studies, results regarding TL may be expected to vary. Smoking is one such important factor which was adjusted for in our sample but not in many of the previous studies including the studies by (Mansour et al., 2011; Martinsson et al., 2013; Lima et al., 2015), which may contribute to the lack of replication.

The results of this study must be interpreted taking into account its limitations. TL and TERT expression were measured in peripheral blood cells and not in the brain where lithium is considered to exert its therapeutic effect. The impact of illness burden, estimated by number of depressive and manic episodes, and possible effects of other pharmacological treatments besides lithium were not considered. The sample size was small, especially that with an ALDA score, and we were thus underpowered to assess the association of lithium response with TL and TERT expression. Finally, our retrospective study design allowed us to assess associations, but not causality. In this study, we didn’t correct for multiple testing. However, all tests were hypothesis-driven.

To our knowledge, this study provides a novel finding of a positive correlation between lithium treatment and TERT expression in BD patients. This constitutes a possible mechanistic explanation for the previously reported positive correlation between lithium treatment and TL. Additionally, we hypothesize that an increase in TERT expression may contribute to lithium’s mood stabilizing and neuroprotective properties by improving mitochondrial function and decreasing oxidative stress. If so, the expression of TERT and mechanisms involved in its subcellular translocation could become targets of future pharmacological interventions, both in BD and in other disorders where mitochondrial dysfunction, oxidative stress, and accelerated cellular aging plays a major role.
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References

Akınçilar SC, Low KC, Liu CY, Yan TD, Oji A, Ikawa M, Li S, Tergaonkar V, 2015. Quantitative assessment of telomerase components in cancer cell lines. FEBS Lett. 589, 974–984. 10.1016/j.febslet.2015.02.035. [PubMed: 25749370]

Barrett JH, Iles MM, Dunning AM, Pooley KA, 2015. Telomere length and common disease: study design and analytical challenges. Hum. Genet 134, 679–689. 10.1007/s00439-015-1563-4. [PubMed: 25986438]

Bersani FS, Lindqvist D, Mellon SH, Penninx BWJH, Verhoeven JE, Révész D, Reus VI, Wolkowitz OM, 2015. Telomerase activation as a possible mechanism of action for psychopharmacological interventions. Drug Discov. Today 20, 1305–1309. 10.1016/j.drudis.2015.06.016. [PubMed: 26166813]

Blackburn EH, Epel ES, Lin J, 2015. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. Science 350, 1193–1198. 10.1126/science.aab3389. [PubMed: 26785477]

Cawthon RM, 2009. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 37, e21. 10.1093/nar/gkn1027.

Elvsåshagen T, Vera E, Bøen E, Bratlie J, Andreassen OA, Josefsen D, Malt UF, Blasco MA, Boye B, 2011. The load of short telomeres is increased and associated with lifetime number of depressive episodes in bipolar II disorder. J. Affect. Disord 135, 43–50. 10.1016/j.jad.2011.08.006. [PubMed: 21880373]

Fries GR, Pfaffenseller B, Sertz L, Paz AVC, Dargél AA, Kunz M, Kapczinski F, 2012. Staging and neuroprogression in bipolar disorder. Curr. Psychiatry Rep 14, 667–675. 10.1007/s11920-012-0391-2. [PubMed: 23090632]

Frye MA, McElroy SL, Fuentes M, Sutor B, Schak KM, Galardy CW, Palmer BA, Prieto ML, Kung S, Sola CL, Ryu E, Veldic M, Geske J, Cuellar-Barboza A, Seymour LR, Mori N, Crowe S, Rummans TA, Biernacka JM, 2015. Development of a bipolar disorder biobank: differential phenotyping for subsequent biomarker analyses. Int. J. Bipolar Disord 3, 30. 10.1186/s40345-015-0030-4. [PubMed: 26105627]

Geddes JR, Miklowitz DJ, 2013. Treatment of bipolar disorder. Lancet 381, 1672–1682. 10.1016/S0140-6736(13)60857-0. [PubMed: 23663953]

Grande I, Berk M, Birmaher B, Vieta E, 2016. Bipolar disorder. Lancet 387, 1561–1572. 10.1016/S0140-6736(15)00241-X. [PubMed: 26388529]

Grimm A, Eckert A, 2017. Brain aging and neurodegeneration: from a mitochondrial point of view. J. Neurochem 143, 418–431. 10.1111/jnc.14037. [PubMed: 28397282]

Ishaq A, Hanson PS, Morris CM, Sareztki G, 2016. Telomerase activity is downregulated early during human brain development. Genes (Basel) 7, 27. 10.3390/genes7060027.

Kapczinski F, Magalhães PVS, Balanzá-Martínez V, Dias Vv, Frangou S, Gama CS, Gonzalez-Pinto A, Grande I, Ha K, Kauer-Sant’Anna M, Kunz M, Kupka R, Leboyer M, Lopez-Jaramillo C, Post...
RM, Rybakowski JK, Scott J, Streijilevitch S, Tohen M, Vazquez G, Yatham L, Vieta E, Berk M, 2014. Staging systems in bipolar disorder: an international society for bipolar disorders task force report. Acta Psychiatr. Scand 130, 354–363. 10.1111/acps.12305. [PubMed: 24961757]

Köse Çinar R, 2017. Telomere length and hTERT in mania and subsequent remission. Revista brasileira de psiquiatria (Sao Paulo, Brazil : 1999) 40, 19–25. 10.1590/1516-4446-2017-2216.

Lai TP, Wright WE, Shaw JY, 2018. Comparison of telomere length measurement methods. Philos. Trans. Royal Soc. B 10.1098/rstb.2016.0451.

Lazzara CA, Kim Y–H, 2015. Potential application of lithium in Parkinson’s and other neurodegenerative diseases. Front. Neurosci 9. 10.3389/fnins.2015.00403.

Lima IMM, Barros A, Rosa DV, Albuquerque M, Malloy-Diniz L, Neves FS, Romano-Silva MA, de Miranda DM, 2015. Analysis of telomere attrition in bipolar disorder. J. Affect. Disord 172, 43–47. 10.1016/j.jad.2014.09.043. [PubMed: 25451394]

Malhi GS, Outhred T, 2016. Therapeutic mechanisms of lithium in bipolar disorder: recent advances and current understanding. CNS Drugs 30, 931–949. 10.1007/s40263-016-0380-1. [PubMed: 27638546]

Mansour H, Chowdari K, Fathi W, Elsayy M, Ibrahim I, Wood J, Banme M, Tobar S, Yassin A, Salah H, Elsayed H, Eissa A, El-Bahaei W, Gomaa Z, El-Chennawi F, Nimgaonkar VL, 2011. Does telomere length mediate associations between inbreeding and increased risk for bipolar I disorder and schizophrenia? Psychiatry Res. 188, 129–132. 10.1016/j.psychres.2011.01.010. [PubMed: 21300409]

Martinsson L, Wei Y, Xu D, Melas PA, Mathé AA, Schalling M, Lavebratt C, Backlund L, 2013. Long-term lithium treatment in bipolar disorder is associated with longer leukocyte telomeres. Transl. Psychiatry 3, e261. 10.1038/tp.2013.37.

Morris G, Berk M, 2016. The putative use of lithium in Alzheimer’s disease. Curr. Alzheimer Res 13, 853–861. [PubMed: 26982287]

Morris G, Walder K, McGie SL, Dean OM, Tye SJ, Maes M, Berk M, 2017. A model of the mitochondrial basis of bipolar disorder. Neurosci. Biobehav. Rev 74, 1–20. 10.1016/j.neubiorev.2017.01.014. [PubMed: 28093238]

Olson JE, Ryu E, Johnson KJ, Koenig BA, Maschke KJ, Morrisette JA, Liebow M, Takahashi PY, Fredericksen ZS, Sharma RG, Anderson KS, Hatchcock MA, Carnahan JA, Pathak J, Lindor NM, Beebe TJ, Thibodeau SN, Cerhan JR, 2013. The mayo clinic biobank: a building block for individualized medicine. Mayo Clin. Proc 88, 952–962. 10.1016/j.mayocp.2013.06.006. [PubMed: 24001487]

Ozturk MB, Li Y, Tergaonkar V, 2017. Current insights to regulation and role of telomerase in human diseases. Antioxidants (Basel, Switzerland) 6, 17. 10.3390/antiox6010017.

Rizzo LB, Costa LG, Mansur RB, Swardfager W, Belanger SI, Grassi-Oliveira R, McIntyre RS, Bauer ME, Brietzke E, 2014. The theory of bipolar disorder as an illness of accelerated aging: implications for clinical care and research. Neurosci. Biobehav. Rev 42, 157–169. 10.1016/j.neubiorev.2014.02.004. [PubMed: 24548785]

Rizzo LB, do Prado CH, Grassi-Oliveira R, Wieck A, Correa BL, Teixeira AL, Bauer ME, 2013. Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. Bipolar Disord. 15, 832–838. 10.1111/bdi.12121. [PubMed: 24021055]

Rode L, Nordestgaard BG, Bojesen SE, 2015. Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. J. Natl. Cancer Inst 107 10.1093/jnci/djv074.djv074.

Rufer N, Brümmendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM, 1999. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. J. Exp. Med 190, 157–167. 10.1084/jem.190.2.157. [PubMed: 10432279]

Saretzki G, 2014. Extra-telomeric functions of human telomerase: cancer, mitochondria and oxidative stress. Curr. Pharm. Des 20, 6386–6403. 10.2174/1381612820666140630095606. [PubMed: 24975608]
Sigitova E, Fišar Z, Hroudová J, Cikánková T, Raboch J, 2017. Biological hypotheses and biomarkers of bipolar disorder. Psychiatry Clin. Neurosci 71, 77–103. 10.1111/pcn.12476. [PubMed: 27800654]

Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, Nierenberg AA, Fava M, Wong K–K, 2006. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol. Psychiatry 60, 432–435. 10.1016/j.biopsych.2006.02.004. [PubMed: 16581033]

Soeiro-de-Souza MG, Teixeira AL, Mateo EC, Zanetti M.v., Rodrigues FG, de Paula VJ, Bezerra JF, Moreno RA, Gattaz WF, Machado-Vieira R, 2014. Leukocyte telomerase activity and antidepressant efficacy in bipolar disorder. Eur. Neuropsychopharmacol 24, 1139–1143. 10.1016/j.euroneuro.2014.03.005. [PubMed: 24731723]

Spilisbury A, Miwa S, Attems J, Saretzki G, 2015. The role of telomerase protein TERT in Alzheimer’s disease and in tau-related pathology in vitro. J. Neurosci 35, 1659–1674. 10.1523/JNEUROSCI.2925-14.2015. [PubMed: 25632141]

Squassina A, Pisanu C, Corbitt N, Alda M, 2017. Telomere length in bipolar disorder and lithium response. Eur. Neuropsychopharmacol 27, 560–567. 10.1016/j.euroneuro.2015.10.008. [PubMed: 26621262]

Turner KJ, Vasu V, Griffin DK, 2019. Telomere biology and human phenotype. Cells 8, 73. 10.3390/cells8010073.

Wei Y., Backlund L, Vegener G, Mathé AA, Lavebratt C, 2015. Telomerase dysregulation in the hippocampus of a rat model of depression: normalization by lithium. Int. J. Neuropsychopharmacol 18 10.1093/ijnp/pyv002.

Weischer M, Bojesen SE, Cawthon RM, Freiberg JJ, Tybjærg-Hansen A, Nordestgaard BG, 2012. Short telomere length, myocardial infarction, ischemic heart disease, and early death. Arterioscler. Thromb. Vasc. Biol 32, 822–829. 10.1161/ATVBAHA.111.237271. [PubMed: 22199369]

Xi L, Cecc TR, 2014. Inventory of telomerase components in human cells reveals multiple subpopulations of hTR and hTERT. Nucleic Acids Res. 42, 8565–8577. 10.1093/nar/gku560. [PubMed: 24990373]

Zhang Y, Toh L, Lau P, Wang X, 2012. Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/B-catenin pathway in human cancer. J. Biol. Chem 287, 32494–32511. 10.1074/jbc.M112.368282. [PubMed: 22854964]

Zheng Q, Huang J, Wang G, 2019. Mitochondria, telomeres and telomerase subunits. Front. Cell Dev. Biol 10.3389/fcell.2019.00274.
### Table 1
Demographics and clinical characteristics of the study population.

|                          | BD I N (% ) or mean (SD) | Controls N (% ) or mean (SD) | p-value |
|--------------------------|--------------------------|-----------------------------|---------|
| Age [years]              | 44.8 (15.4)              | 44.9 (15.3)                 | 0.97\(^a\) |
| Sex, male                | 35 (35%)                 | 35 (35%)                    | 1.0\(^b\) |
| BMI [kg/m\(^2\)]        | 31.9 (7.26)              | 28.8 (6.57)                 | 0.0018\(^c\) |
| Ever smoker, yes\(^1\)  | 51 (52%)                 | 21 (21%)                    | < 0.0001\(^b\) |
| Alcohol [g/week]\(^2\)   | 43.9 (93)                | 56.6 (64.1)                 | 0.00015\(^c\) |
| Allele sum               | 3.74 (0.93)              | 3.66 (1.09)                 | 0.64\(^c\) |
| Ever lithium treatment, yes\(^3\) | 28 (44.4%)            |                             |         |
| Current lithium treatment, yes\(^3\) | 22 (34.9%)             |                             |         |
| Duration of lithium treatment [months]\(^3\) | 41.2 (86.3)           |                             |         |
| Duration of lithium treatment ≥ 24 months [months], 17 patients | 151 (106)               |                             |         |

\(^1\)Ever smoker is defined as having smoked 100 cigarettes or more.

\(^2\)An estimate of grams of alcohol per week was achieved by multiplying the average number of days of alcohol consumption per week by the average number of standard drinks of alcohol per day of alcohol consumption by 14 (grams of alcohol contained within a standard drink in the United States).

\(^3\)There was only reliable data on lithium treatment for 63 patients.

\(^a\)Independent samples \(t\)-test,

\(^b\)Chi-square test,

\(^c\)Mann-Whitney test.