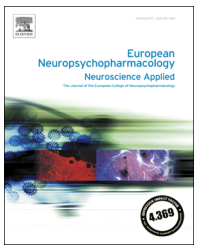
European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro



Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients

Alessio Squassina[a,*,1], Claudia Pisanu[a,1], Donatella Congiu[a], Paola Caria[b], Daniela Frau[b], Paola Niola[a], Carla Melis[a], Gioia Baggiani[c], Juan Pablo Lopez[d], Cristiana Cruceanu[d], Gustavo Turecki[d], Giovanni Severino[a], Alberto Bocchetta[c], Roberta Vanni[b], Caterina Chillotti[c], Maria Del Zompo[a,c]

[a]*Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy*
[b]*Department of Biomedical Sciences, Unit of Biology and Genetics, University of Cagliari, Italy*
[c]*Unit of Clinical Pharmacology of the University Hospital of Cagliari, Italy*
[d]*McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada*

Received 22 December 2015; received in revised form 12 February 2016; accepted 24 March 2016

KEYWORDS
Leukocyte telomere length;
Bipolar disorder;
Lithium;
Telomerase;
Quantitative real time PCR;
Neural progenitor cells

Abstract
Bipolar disorder (BD) has been suggested to be associated with accelerated aging and premature cell senescence. While findings on shorter telomeres in BD are controversial, a recent study showed that long-term lithium treatment correlates with longer telomeres in BD. In our study, we sought to investigate the correlation between leukocyte telomere length (LTL) and long-term lithium treatment in a sample of 200 BD patients characterized for lithium response. We also compared data from two different methods commonly used to measure telomere length, quantitative PCR (qPCR) and quantitative fluorescence in situ hybridization (Q-FISH). We also measured, for the first time, the effect of lithium in vitro on the expression of the telomerase gene in human-derived neural progenitor cells (NPCs). Our findings showed that LTL correlated negatively with age ($p=0.0002$) and was independent of sex, diagnosis, age at onset, suicidal behavior, number of mood episodes, response to lithium and use of other psychotropic medications. After correcting for age, LTL was positively correlated with lithium treatment duration in patients treated for more than two years ($n=150$, $R=0.17$, $p=0.037$). There was a significant correlation between data measured with qPCR and Q-FISH ($p=0.012$,

*Correspondence to: Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Cittadella Universitaria di Monserrato, sp 6, Sestu-Monserrato, Km 0.700, 09042 Cagliari, Italy.
E-mail address: squassina@unica.it (A. Squassina).
[1]These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.euroneuro.2016.03.020

Please cite this article as: Squassina, A., et al., Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.03.020

R=0.826). Lithium treatment increased telomerase expression in NPCs, though this effect was not statistically significant.
Our data support previous findings showing that long-term lithium treatment associates with longer telomeres in BD, though this effect appeared to be independent from clinical response to the treatment. Moreover, we suggested for the first time that lithium increases the expression of telomerase gene in human neural progenitor cells.

1. Introduction

Bipolar disorder (BD) is a disabling psychiatric illness characterized by alternating manic and depressive episodes, with a prevalence of 0.8–1.25 in the general population (Merikangas et al., 2007). Lithium is one of the most effective long-term treatments for BD. However, only 30% of patients treated with lithium present an excellent response with complete remission (Rybakowski, 2011). Despite many years of intensive research, the specific mechanisms by which lithium exerts its effects are not well understood. A large body of evidence has shown that lithium has neuroprotective effects through regulation of multiple signaling pathways (Rowe and Chuang, 2004). In particular, lithium has been reported to inhibit glycogen synthesis kinase-3β (GSK-3β) (Zhang et al., 2003), which, among others, is involved in apoptotic cell death (Beurel and Jope, 2006), and to up-regulate anti-apoptotic factors such as B-cell lymphoma protein-2 (Bcl-2), as well as brain-derived neurotrophic factor (BDNF) and β-catenin (Angelucci et al., 2000; Chen et al., 1999).

A recent study showed that long-term lithium treatment is associated with longer leukocyte telomere length (LTL) in BD (Martinsson et al., 2013). Even though several studies reported shorter telomeres in BD compared to healthy controls (Simon et al., 2006; Elvsåshagen et al., 2011; Rizzo et al., 2013; Lima et al., 2014), this finding has been put into question by a recent meta-analysis (Colpo et al., 2015). Nevertheless, BD seems to be associated with accelerated aging and cell senescence (Rizzo et al., 2014), a marker of which is telomere shortening. Interestingly, a recent study showed that lithium increases the expression of the gene codifying for the catalytic subunit of telomerase (*hTERT*) as well as its enzymatic activity in the hippocampus of a rat model of depression (Wei et al., 2015), further supporting the involvement of telomeres in mood disorders.

Telomeres consist of protective DNA-protein complexes containing TTAGGG tandem repeats located at the ends of chromosomes (Blackburn et al., 2006). This specialized end is crucial for integrity of the genome, preventing chromosome fusion and genomic instability (O'Sullivan and Karlseder, 2010). In most human cells, telomere repeats progressively shorten after each round of DNA replication and when they become critically short cells stop dividing or die for apoptosis (Calado, 2009). Longer telomere length (TL) has been associated to better survival (Bakaysa et al., 2007), family history of longevity (Atzmon et al., 2010) and healthy ageing (Njajou et al., 2009). In contrast, shorter TL has been reported in psychological stress and in age-related disorders, such as cardiovascular diseases and diabetes (Salpea et al., 2010; Saliques et al., 2010). In proliferative tissues, such as in germ cells, activated lymphocytes, developing neurons and in adult progenitor cells in general, where TL is essential for prolonged persistence and genetic stability (Flores et al., 2006, 2008), telomere shortening is counteracted by telomerase, a specialized reverse transcriptase (Osterhage and Friedman, 2009). In the human central nervous system, telomerase is abundant in neural progenitor cells in the developing (Klapper et al., 2001; Cai et al., 2002) and adult brain (Caporaso et al., 2003). However, in the other human tissues, telomerase is normally not expressed and telomeres shorten with age.

The majority of studies investigating TL in psychiatric disorders have focused on leukocyte telomere length (LTL), as this is recognized as a valuable and easily accessible marker of cellular aging, and is correlated with TL in other somatic cells (Bodelon et al., 2014).

Considering the paucity of findings on the effect of lithium on telomere dynamics in BD, we carried out a study aimed to investigate (a) the effect of long-term lithium treatment on LTL in BD, (b) the potential difference in LTL between patients responding and not responding to lithium as well as the impact of other clinical modifiers on LTL, and, for the first time, (c) the effect of lithium treatment on the expression levels of the catalytic subunit of human telomerase (hTERT) in human neuronal progenitor cells (NPCs).

2. Experimental procedures

2.1. Sample

The sample comprised 200 patients with BD (Table 1) of Sardinian ancestry for at least four generations, recruited at the Lithium Clinic of the Clinical Psychopharmacology Centre of the University Hospital of Cagliari, Italy from 1993 to 2012. Diagnosis was carried out by trained clinical psychopharmacologists according to DSM-IV criteria and the Schedule for Affective Disorder and Schizophrenia-LifetimeVersion (SADS-L) (Endicott and Spitzer, 1978). Age at onset, number of depressive, manic and hypomanic episodes, duration of illness, duration of lithium treatment and suicidal behavior were assessed. Use of other mood stabilizers, antidepressants, antipsychotics and benzodiazepines was also evaluated. Patients were characterized for lithium response using the “Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder” as described previously (Grof et al., 2002; Manchia et al., 2013). This scale quantifies the degree of improvement in the course of treatment with a score from 0 to 10 (total score, TS). Patients with a TS equal to 7 or higher are considered lithium responders (LiRs). Our sample included 59 LiRs and 141 partial or non-responders (non-LiRs). We also measured TL using the quantitative fluorescence in situ hybridization (Q-FISH)

Please cite this article as: Squassina, A., et al., Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.03.020

Table 1 Demographic and clinical characteristics of the sample (n=200).

Variables	
Age[a]	46 (37, 57)
Sex (M/F)	64/136
Diagnosis (BD I/BDII)	150/50
Lithium response (LiR/Non-LiR)	59/141
Age at onset[a]	24 (18, 32)
Years of illness[a]	18 (12, 26)
Years of lithium treatment[a]	6 (3, 10)
Number of depressive episodes[a]	3 (1, 7)
Number of manic episodes[a]	1 (0, 4)
Number of hypomanic episodes[a]	0 (0, 1)
Suicidal behavior (%)	37 (19%)
Use of antidepressants (%)	31 (16%)
Use of benzodiazepines (%)	102 (53%)
Use of other mood stabilizers (%)	31 (16%)
Use of antipsychotics (%)	68 (36%)

BD I, bipolar disorder type 1; BD II, bipolar disorder type 2; LiRs, lithium responders; Non-LiRs, lithium non-responders.
[a]Median (25th, 75th percentile).

technique in a small sub-sample of eight patients for whom new blood samples were collected in 2015. The main purpose of this step was to test the correlation between Q-FISH and quantitative PCR (qPCR) data, two different approaches commonly used to measure TL that present different sensitivities, costs and advantages, as discussed elsewhere (Squassina et al., 2015). The research protocol was approved by the local Ethics Committee, and after a detailed description of the study procedures all participants signed informed written consent. Procedures are in accordance with the Helsinki Declaration of 1975.

2.2. Neuronal progenitor cells (NPCs)

Human NPCs were obtained from wild type induced pluripotent stem cell (iPSC) line 8330-8 as described elsewhere (Lopez et al., 2014; Cruceanu et al., 2015). The cell line was divided into three replicates, each replicate separated into two aliquots, and one cultured with lithium 1 mM for one week while the other was cultured in regular medium.

2.3. Determination of LTL by quantitative PCR

Relative LTL was assessed according to the method described by Cawthon (2002). DNA samples were processed in triplicates both for the telomere (Tel) and for the single-copy gene (hemoglobin-b, Hgb) in separate PCR plates using Precision Mastermix 2x with ROX (Primerdesign). The primers (10 nM) were (Tel-1: 5′-CGGTTTG-TTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′,Tel-2: 5′GGCTTGCC-TTACCCTTACCCTTACCCTTACCCTTACCCT-3′) for the telomere PCR and (Hgb1: 5′-GCTTCTGACACAACTGTGTTCACTAGC-3′, Hgb2 (5′-CAC-CAACTTCATCCACGTTCACC-3′) for the Hgb PCR. The PCR temperature conditions were 95 °C for 5 min followed by 28 cycles of 95 °C for 15 s and 62 °C for 1 min for Tel gene; 95 °C for 2 min followed by 35 cycles of 95 °C for 15 s and 62 °C for 1 min for Hgb gene. A dissociation curve was included in each plate to assess specificity. The reactions were carried out on an ABI PRISM 7000 instrument (Applied Biosystems). A control sample (calibrator) was included in each plate and LTL was calculated using the $2^{-\Delta\Delta CT}$ method ($\Delta\Delta CT = \Delta CT$ sample-ΔCT calibrator; ΔCT sample=CT Tel gene - CT Hgb gene). A dilution series of a pooling of samples was included in each plate to establish efficiency.

2.4. Cell culturing, PNA-FISH and Q-FISH

Short term cultures of phytohemagglutinin-M (GIBCO, Milan, Italy) stimulated T-lymphocytes, metaphase preparations, telomere peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) and Quantitative-FISH (Q-FISH) analysis were carried out as previously described (Cantara et al., 2012). Metaphase chromosomes were hybridized using a FITC-labeled $(CCCTAA)_3$ PNA probe (DAKO, Glostrup, Denmark) according to the manufacturer's instructions. Telomere hybridization signals of 20 complete metaphases per subject were evaluated and telomere fluorescence signals were quantified by the ImageJ software, version 1.43u (National Institutes of Health, http://rsbweb.nih.gov/) according to the provided guidelines. The software measures fluorescence intensity of individual telomeres, expressed as the product of the telomere area and the average gray value within the selected telomere. The method provides excellent quantitation as the fluorescence intensity directly correlates to the length of the telomeres.

2.5. Determination of TERT expression in NPCs

qPCRs were run in triplicate using TaqMan® assay on demand (Hs00972656_m1; Applied Biosystems, Foster City, CA, USA) in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). GAPDH was used as a housekeeping gene to normalize target gene. Relative expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical analyses

Correlation between LTL and age was assessed using nonparametric Spearman's correlation test. Correlation between LTL and continuous variables was determined using the partial correlation test, controlled for age. Dependence of LTL on sex, diagnosis (BD I or BD II), suicidal behavior, use of psychotropic medications other than lithium (other mood stabilizers, antidepressants, antipsychotics, benzodiazepines), and response to lithium treatment were tested using analysis of covariance (ANCOVA), corrected for age.

In the subsample of eight subjects, correlation between Q-FISH and qPCR was tested with Spearman's correlation test. Effect of lithium on *hTERT* expression in NPCs was assessed with t-test for paired data. A p-value<0.05 was considered statistically significant. Statistical analyses were conducted using IBM SPSS Statistics v. 20 (IBM Corporation, Armonk, NY, USA) and Graphpad Prism V. 6 (GraphPad Software, San Diego, CA USA).

3. Results

The demographic and clinical characteristics of the sample are shown in Table 1. LTL correlated negatively with age (p=0.0002, Spearman's rho=−0.256) and was independent of sex ($p>0.05$). After correcting for age, LTL was not significantly different according to any of the tested variables. However, similarly to Martinsson and colleagues (2013), partial correlation analysis showed that LTL was positively correlated with lithium treatment duration in patients treated for more than two years (n=150, correlation coefficient=0.17, p=0.037, Figure 1).

LiRs and non-LiRs did not significantly differ for any of the tested variables, with the exception of age (p=0.021).

When considering age as covariate, LTL was not significantly different between response groups.

There was a significant correlation between measures of TL with qPCR and Q-FISH in the subset of eight patients ($p=0.012$, Correlation coefficient=0.826, Figure 2A). Q-FISH and qPCR data showed a positive though not statistically significant correlation with duration of lithium treatment when corrected for age (Figure 2B and C).

Lithium treatment increased *hTERT* expression in NPCs, though the effect was not statistically significant (Fold Change=1.67; $p>0.05$, Figure 3).

4. Discussion

In this study, we tested the correlation between lithium treatment and other clinical modifiers on LTL in BD patients characterized for lithium response. Similarly to a previous investigation (Martinsson et al., 2013), LTL was positively correlated with years of lithium treatment in patients treated for more than two years. This effect persisted even when correcting for the effect of other psychotropic medications. Our results add to the hypothesis that long-term lithium treatment exerts a protective effect against telomere shortening in BD patients. The mechanism through which lithium may counteract telomeres shortening and the correlation between TL in blood cells and the brain are still unknown. Interestingly, the study by Wei et al. (2015) suggested that lithium influences telomerase expression and activity in the rat brain, but this was never explored in human derived neuronal cells.

In our study, we tested for the first time the effect of lithium on human NPCs, showing that 1 week of treatment upregulates *hTERT* expression. Although the increase in *hTERT* expression was non-significant, possibly because of the small number of samples included, these findings support the hypothesis that telomerase could be one of the mechanisms through which lithium may counteract telomeres shortening, at least in tissues where telomerase is active. Another mechanism through which lithium could exert an effect on

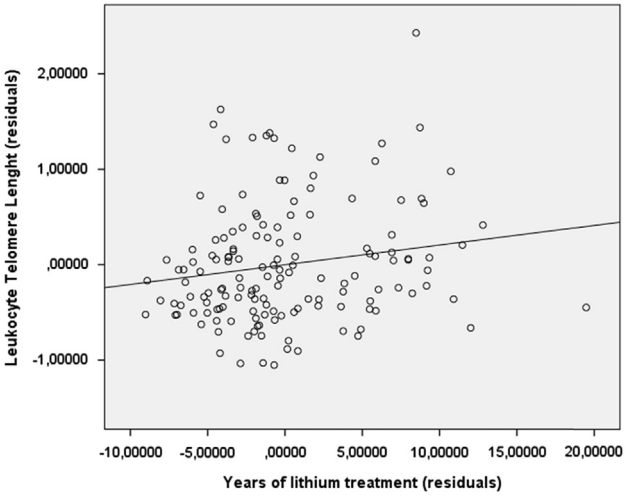


Figure 1 Leukocyte telomere length (LTL) positively depends on duration of lithium treatment in patients exposed for more than 24 months (correlation coefficient=0.17, $p=0.037$).

LTL may involve insulin-like growth factor 1 (IGF-1), a growth factor that plays an important role in regulating apoptosis, neuronal development, neuronal differentiation and stress resistance (Fernandez et al., 2007). Recent studies suggest that high levels of *IGF-1* may be involved in clinical response to lithium treatment (Squassina et al., 2013; Milanesi et al., 2015). Interestingly, high circulating levels of IGF-1 have been associated with longer telomeres (Barbieri et al., 2009), whereas low levels with age-related disorders (Aulinas et al., 2013). However, the hypothesis that IGF-1 may be involved in regulation of LTL remains speculative and should be further investigated in future studies.

Our data did not confirm the finding reported by Martinsson and coworkers for longer LTL in LiRs compared to non-LiRs (Martinsson et al., 2013). Several factors could have contributed to the different findings. In our sample, the group of non-LiR comprised patients with an Alda score of 6 or lower, as defined by the Alda scale, while in the study by Martinsson and colleagues, patients with a score of 6 were excluded from the analyses. On the other hand, we were not able to report significant group differences in LTL when removing patients with a score of 6. In contrast with Martinsson et al., in our study LiRs and non-LiR patients were not matched for age and sex. Nevertheless, including these variables in our models did not significantly affect findings. Indeed, lithium may be able to counteract LTL shortening independently from the clinical response. Interestingly, the neuroprotective effect of lithium treatment has been suggested to be independent of long-term treatment response (Hajek et al., 2014). Specifically, BD patients with limited lifetime exposure to lithium showed smaller hippocampal volumes compared to lithium treated patients, despite a higher activity of illness in the lithium group (Hajek et al., 2014).

While the meta-analysis by Colpo et al. (2015) suggested that telomeres are not shorter in BD patients compared to controls, this finding should be interpreted in light of the significant differences among the studies included in the analysis. Most of the studies used whole peripheral blood samples, which include all leukocytes, while two studies (Elvsåshagen et al., 2011; Rizzo et al., 2013) used peripheral blood mononuclear cell (PBMC). PBMCs do not include granulocytes and therefore findings are difficult to compare with those measuring LTL. Moreover, different techniques were used in different studies (qPCR, Southern Bot, Q-FISH). While qPCR based telomere measurement has been suggested to be a good measure of average TL (Aviv et al., 2011), this has been called into question (Gutierrez-Rodrigues et al., 2014). For this reason, in our study we measured TL with qPCR and Q-FISH in a small subset of patients, reporting significant and strong correlation.

Our results must be interpreted in light of their limitations. Our study implemented a retrospective design for the analysis on the 200 BD patients using one single DNA sample for each patient, therefore not allowing us to test longitudinal differences in LTL. The effect size of the effect of lithium on LTL was small (Cohen's $d=0.37$), suggesting that larger samples are needed to better explore this hypothesis. Main strengths of this study are the relatively large sample size compared to previous similar investigations, the concordance between qPCR and Q-FISH data in a subgroup of patients, the Sardinian ancestry of all subjects under study, and the inclusion of a large number of confounders in the analysis.

Please cite this article as: Squassina, A., et al., Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.03.020

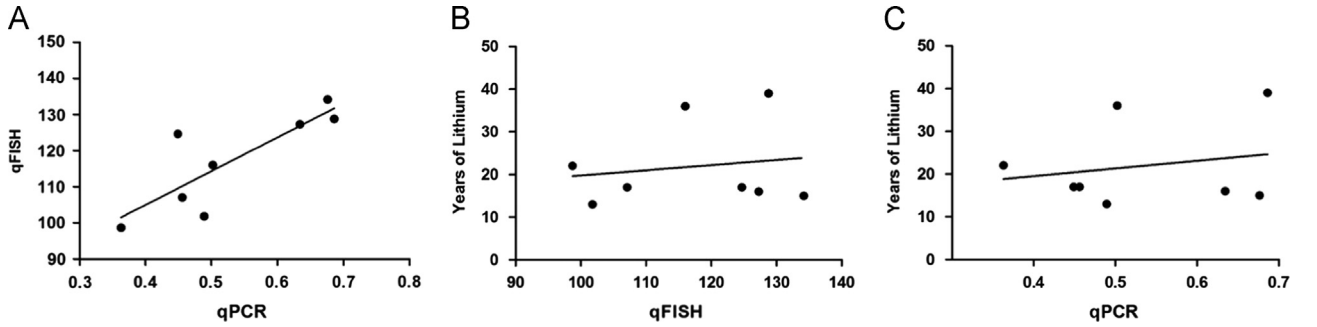


Figure 2 (A) Shows the correlation between Q-FISH and qPCR data (Correlation coefficient=0.826, p=0.012), (B) shows the lack of correlation between years of lithium treatment and telomere length measured with Q-FISH in the subsample of eight subjects, and (C) shows the lack of correlation between years of lithium treatment and telomere length measured with qPCR in the subsample of eight subjects. Q-FISH=quantitative fluorescence in situ hybridization; qPCR=quantitative PCR.

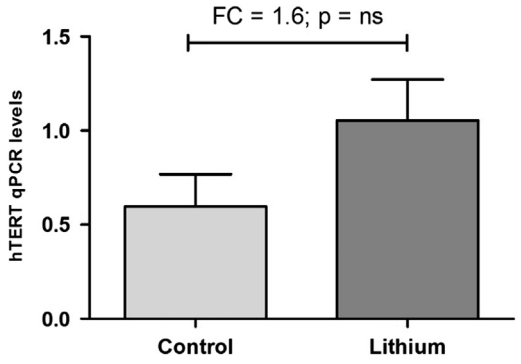


Figure 3 Effect of treatment with lithium 1 mM for one week on the expression of *hTERT* in human neuronal progenitor cells (NPCs). NPCs were derived from induced pluripotent stem cell line GM08330 obtained from a healthy male and divided into three replicates. qPCR=quantitative PCR; FC=fold change; ns=not significant.

In conclusion, we found a correlation between LTL and years of lithium therapy in BD patients with long-term lithium treatment and suggest, for the first time, that lithium increases the expression of hTERT in human-derived neural progenitor cells, supporting the hypothesis that modulation of hTERT might be one of the mechanisms through which lithium may counteract telomeres shortening.

Role of funding source

Funding for this study was provided by Fondazione Banco di Sardegna (Italy). These funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Authors AS, CP, DC, RV and MDZ designed the study and wrote the protocol. Authors C. Chillotti, AB, GS and GB phenotyped the sample included in the study. Authors DC and CP managed the literature searches and analyses. Authors CP, PN, CM, PC and DF performed the laboratory experiments. Authors JPL and C. Cruceanu performed the experiments on neural precursors cells. Author GT coordinated the laboratory work at McGill Group for Suicide Studies. Authors CP and AS undertook the statistical analysis, and authors CP and DC wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgments

Authors wish to thank Dr. Martin Alda and Nathan Corbett for helpful comments on this study.

References

Angelucci, F., Aloe, L., Jimenez-Vasquez, P., Mathe, A.A., 2000. Lithium treatment alters brain concentrations of nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in a rat model of depression. Int. J. Neuropsychopharmacol. 6, 225–231.

Atzmon, G., Cho, M., Cawthon, R.M., Budagov, T., Katz, M., Yang, X., Siegel, G., Bergman, A., Huffman, D.M., Schechter, C.B., Wright, W.E., Shay, J.W., Barzilai, N., Govindaraju, D.R., Suh, Y., 2010. Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. Proc. Natl. Acad. Sci. U S A. 107, 1710–1717.

Aulinas, A., Ramírez, M.J., Barahona, M.J., Mato, E., Bell, O., Surrallés, J., Webb, S.M., 2013. Telomeres and endocrine dysfunction of the adrenal and GH/IGF-1 axes. Clin. Endocrinol. (Oxf.) 79, 751–759.

Aviv, A., Hunt, S.C., Lin, J., Cao, X., Kimura, M., Blackburn, E., 2011. Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. Nucleic Acids Res. 39 e134.

Bakaysa, S.L., Mucci, L.A., Slagboom, P.E., Boomsma, D.I., McClearn, G.E., Johansson, B., Pedersen, N.L., 2007. Telomere length predicts survival independent of genetic influences. Aging Cell. 6, 769e74.

Barbieri, M., Paolisso, G., Kimura, M., Gardner, J.P., Boccardi, V., Papa, M., Hjelmborg, J.V., Christensen, K., Brimacombe, M., Nawrot, T.S., Staessen, J.A., Pollak, M.N., Aviv, A., 2009. Higher circulating levels of IGF-1 are associated with longer leukocyte telomere length in healthy subjects. Mech. Ageing Dev. 130, 771–776.

Beurel, E., Jope, R.S., 2006. The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways. Prog. Neurobiol. 79, 173–189.

Blackburn, E.H., Greider, C.W., Szostak, J,W., 2006. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nat. Med. 12, 1133–1138.

Bodelon, C., Savage, S.A., Gadalla, S.M., 2014. Telomeres in molecular epidemiology studies. Prog Mol Biol Transl Sci. 125, 113–131.

Cai, J., Wu, Y., Mirua, T., Pierce, J.L., Lucero, M.T., Albertine, K.H., Spangrude, G.J., Rao, M.S., 2002. Properties of a fetal multipotent neural stem cell (NEP cell). Dev. Biol. 251, 221–240.

Calado, R.T., 2009. Telomeres and marrow failure, Hematology. Am. Soc. Hematol. Educ. Program., 338–343.

Cantara, S., Pisu, M., Frau, D.V., Caria, P., Dettori, T., Capezzone, M., Capuano, S., Vanni, R., Pacini, F., 2012. Telomere abnormalities and chromosome fragility in patients affected by familial papillary thyroid cancer. J. Clin. Endocrinol. Metab. 97, E1327-E1331.

Caporaso, G.L., Lim, D.A., Alvarez-Buylla, Chao, M.V., 2003. Telomerase activity in the subventricular zone of adult mice. Mol. Cell. Neurosci. 23, 693–702.

Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 30 (10), e47.

Chen, G., Zeng, W.Z., Yuan, P.X., Huang, L.D., Jiang, Y.M., Zhao, Z. H., Manji, H.K., 1999. The mood stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. J. Neurochem. 72, 879–882.

Colpo, G.D., Leffa, D.D., Köhler, C.A., Kapczinski, F., Quevedo, J., Carvalho, A.F., 2015. Is bipolar disorder associated with accelerating aging? a meta-analysis of telomere length studies. J. Affect. Disord. 186, 241–248.

Cruceanu, C., Tan, P.P., Rogic, S., Lopez, J.P., Torres-Platas, S.G., Gigek, C.O., Alda, M., Rouleau, G.A., Pavlidis, P., Turecki, G., 2015. Transcriptome sequencing of the anterior cingulate in bipolar disorder: dysregulation of G protein-coupled receptors. Am. J. Psychiatry 11, 1131–1140.

Elvsåshagen, T., Vera, E., Bøen, E., Bratlie, J., Andreassen, O.A., Josefsen, D., Malt, U.F., Blasco, M.A., 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.

Endicott, J., Spitzer, R.L., 1978. A diagnostic interview: the schedule for affective disorders and schizophrenia. Arch. Gen. Psychiatry 35, 837–844.

Fernandez, S., Fernandez, A.M., Lopez-Lopez, C., Torres-Aleman, I., 2007. Emerging roles of insulin-like growth factor-I in the adult brain. Growth Horm. IGF Res. 17, 89–95.

Flores, I., Benetti, R., Blasco, M.A., 2006. Telomerase regulation and stem cell behaviour. Curr. Opin. Cell. Biol. 3, 254–260.

Flores, I., Canela, A., Vera, E., Tejera, A., Cotsarelis, G., Blasco, M.A., 2008. The longest telomeres: a general signature of adult stem cell compartments. Genes. Dev. 5, 654–667.

Grof, P., Duffy, A., Cavazzoni, P., Grof, E., Garnham, J., MacDougall, M., O'Donovan, C., Alda, M., 2002. Is response to prophylactic lithium a familial trait? J. Clin. Psychiatry 10, 942–947.

Gutierrez-Rodrigues, F., Santana-Lemos, B.A., Scheucher, P.S., Alves-Paiva, R.M., Calado, R.T., 2014. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. PLoS One 9, e113747.

Hajek, T., Bauer, M., Simhandl, C., Rybakowski, J., O'Donovan, C., Pfennig, A., König, B., Suwalska, A., Yucel, K., Uher, R., Young, L.T., MacQueen, G., Alda, M., 2014. Neuroprotective effect of lithium on hippocampal volumes in bipolar disorder independent of long-term treatment response. Psychol. Med. 44, 507–517.

Klapper, W., Shin, T., Mattson, M.P., 2001. Differential regulation of telomerase activity and TERT expression during brain development in mice. J. Neurosci. Res. 64, 252–260.

Lima, I.M.M., Barros, A., Rosa, D.V., Albuquerque, M., Malloy-Diniz, L., Neves, F.S., Romano-Silva, M.A., de Miranda, D.M., 2014. Analysis of telomere attrition in bipolar disorder. J. Affect. Disord. 172, 43–47.

Lopez, J.P., Lim, R., Cruceanu, C., Crapper, L., Fasano, C., Labonte, B., Maussion, G., Yang, J.P., Yerko, V., Vigneault, E., Mestikawy, S., Mechawar, N., Pavlidis, P., Turecki, G., 2014. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nat. Med. 7, 764–768.

Manchia, M., Adli, M., Akula, N., Ardau, R., Aubry, J.M., Backlund, L., Banzato, C.E., Baune, B.T., Bellivier, F., Bengesser, S., Biernacka, J.M., Brichant-Petitjean, C., Bui, E., Calkin, C.V., Cheng, A.T., Chillotti, C., Cichon, S., Clark, S., Czerski, P.M., Dantas, C., Zompo, M.D., Depaulo, J.R., Detera-Wadleigh, S.D., Etain, B., Falkai, P., Frisén, L., Frye, M.A., Fullerton, J., Gard, S., Garnham, J., Goes, F.S., Grof, P., Gruber, O., Hashimoto, R., Hauser, J., Heilbronner, U., Hoban, R., Hou, L., Jamain, S., Kahn, J.P., Kassem, L., Kato, T., Kelsoe, J.R., Kittel-Schneider, S., Kliwicki, S., Kuo, P.H., Kusumi, I., Laje, G., Lavebratt, C., Leboyer, M., Leckband, S.G., López Jaramillo, C.A., Maj, M., Malafosse, A., Martinsson, L., Masui, T., Mitchell, P.B., Mondimore, F., Monteleone, P., Nallet, A., Neuner, M., Novák, T., O'Donovan, C., Osby, U., Ozaki, N., Perlis, R.H., Pfennig, A., Potash, J.B., Reich-Erkelenz, D., Reif, A., Reininghaus, E., Richardson, S., Rouleau, G.A., Rybakowski, J.K., Schalling, M., Schofield, P.R., Schubert, O.K., Schweizer, B., Seemüller, F., Grigoroiu-Serbanescu, M., Severino, G., Seymour, L.R., Slaney, C., Smoller, J.W., Squassina, A., Stamm, T., Steele, J., Stopkova, P., Tighe, S.K., Tortorella, A., Turecki, G., Wray, N.R., Wright, A., Zandi, P.P., Zilles, D., Bauer, M., Rietschel, M., McMahon, F.J., Schulze, T.G., Alda, M., 2013. Assessment of response to lithium maintenance treatment in bipolar disorder: a consortium on lithium genetics (ConLiGen) report. PLoS One 8 (6), e65636.

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

Merikangas, K.R., Akiskal, H.S., Angst, J., Greenberg, P.E., Hirschfeld, R.M., Petukhova, M., Kessler, R.C., 2007. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. Arch. Gen. Psychiatry 5, 543–552.

Milanesi, E., Hadar, A., Maffioletti, E., Werner, H., Shomron, N., Gennarelli, M., Schulze, T.G., Costa, M., Del Zompo, M., Squassina, A., Gurwitz, D., 2015. Insulin-like Growth Factor 1 differentially affects lithium sensitivity of lymphoblastoid cell lines from lithium responder and non-responder bipolar disorder patients. J. Mol. Neurosci. 56, 681–687.

Njajou, O.T., Hsueh, W.C., Blackburn, E.H., Newman, A.B., Wu, S. H., Li, R., Simonsick, E.M., Harris, T.M., Cummings, S.R., Cawthon, R.M., 2009. Health ABC study, Association between telomere length, specific causes of death, and years of healthy life in health, aging, and body composition, a population-based cohort study. J. Gerontol. A. Biol. Sci. Med. Sci. 64, 860–864.

O'Sullivan, R.J., Karlseder, J., 2010. Telomeres: protecting chromosomes against genome instability. Nat. Rev. Mol. Cell. Biol. 11, 171–181.

Osterhage, J.L., Friedman, K.L., 2009. Chromosome end maintenance by telomerase. J. Biol. Chem. 284, 16061–16065.

Please cite this article as: Squassina, A., et al., Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.03.020

Rizzo, L.B., Do Prado, C.H., Grassi-Oliveira, R., Wieck, A., Correa, B.L., Teixeira, A.L., Bauer, M.E., 2013. Immunosenescence is associated with human cytomegalovirus and shortened telomeres in type I bipolar disorder. Bipolar Disord. 15, 832–838.

Rizzo, L.B., Costa, L.G., Mansur, R.B., Swardfager, W., Belangero, S.I., Grassi-Oliveira, R., McIntyre, R.S., Bauer, M.E., 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.

Rowe, M.K., Chuang, D.M., 2004. Lithium neuroprotection: molecular mechanisms and clinical implications. Expert. Rev. Mol. Med. 21, 1–18.

Rybakowski, J.K., 2011. Lithium in neuropsychiatry: a 2010 update. World J Biol. Psychiatry 12, 340–348.

Saliques, S., Zeller, M., Lorin, J., Lorgis, L., Teyssier, J.R., Cottin, Y., Rochette, L., Vergely, C., 2010. Telomere length and cardiovascular disease. Arch. Cardiovasc. Dis. 8–9, 454–459.

Salpea, K.D., Talmud, P.J., Cooper, J.A., Maubaret, C.G., Stephens, J.W., Abelak, K., Humphries, S.E., 2010. Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. Atherosclerosis 1, 42–50.

Simon, N.M., Smoller, J.W., McNamara, K.L., Maser, R.S., Zalta, A.K., Pollack, M.H., Nierenberg, A.A., 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.

Squassina, A., Pisanu, C., Corbett, N., Alda, M., 2015. Telomere length in bipolar disorder and lithium response. Eur. Neuropsychopharmacol. http://dx.doi.org/10.1016/j.euroneuro.2015.10.008.

Squassina, A., Costa, M., Congiu, D., Manchia, M., Angius, A., Deiana, V., Ardau, R., Chillotti, C., Severino, G., Calza, S., Del Zompo, M., 2013. Insulin-like growth factor 1 (IGF-1) expression is up-regulated in lymphoblastoid cell lines of lithium responsive bipolar disorder patients. Pharmacol. Res. 73, 1–7.

Wei, Y.B., Backlund, L., Wegener, G., Mathé, A.A., Lavebratt, C., 2015. Telomerase dysregulation in the hippocampus of a rat model of depression: normalization by lithium. Int. J Neuropsychopharmacol. 7 pyv002.

Zhang, F., Phiel, C.J., Spece, L., Gurvich, N., Klein, P.S., 2003. Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium. Evidence for autoregulation of GSK-3. J. Biol. Chem. 35, 33067–33077.

Please cite this article as: Squassina, A., et al., Leukocyte telomere length positively correlates with duration of lithium treatment in bipolar disorder patients. European Neuropsychopharmacology (2016), http://dx.doi.org/10.1016/j.euroneuro.2016.03.020