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REGULAR RESEARCH ARTICLE

Telomere Length, Oxidative Stress, Inflammation and BDNF Levels in Siblings of Patients with Bipolar Disorder: Implications for Accelerated Cellular Aging

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Abstract

Background: Growing evidence supports the existence of neurobiological trait abnormalities in individuals at genetic risk for bipolar disorder. The aim of this study was to examine potential differences in brain-derived neurotrophic factor, cytokines, oxidative stress, and telomere length markers between patients with bipolar disorder, their siblings, and healthy controls.
Methods: Thirty-six patients with bipolar disorder type I, 39 siblings, and 44 healthy controls were assessed. Serum levels of brain-derived neurotrophic factor, interleukin-6, interleukin-10, tumor necrosis factor-α, C-C motif chemokine 11, C-C motif chemokine 24, and 3-nitrotyrosine were measured, as were the activities of glutathione peroxidase, glutathione reductase, and glutathione S-transferase. Telomere length (T/S ratio) was measured using quantitative polymerase chain reaction.

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Significance Statement

Bipolar disorder (BD) presents with progressive clinical and neurobiological changes probably associated with poor metabolic stress compensation. Growing evidence supports the existence of neurobiological trait abnormalities in individuals at genetic risk for BD. Aging and senescence processes could contribute to the search for new and promising molecular targets to help explain and ameliorate neuroprogression in BD. This study reinforces that aging is probably associated with the neuropathological processes seen in BD and shows that unaffected siblings may present senescence features. Prospective studies of the neurobiology and clinical features of those with a high familiar risk for BD may help researchers identifying heritable subphenotypes of the disorder and assess how this familial risk is clinically expressed. Furthermore, research with siblings of patients with BD may shed light on the pathophysiology of the condition.

Results: Telomere length was different between the 3 groups ($P = .041$) with both patients and siblings showing a shorter T/S ratio compared with healthy controls. Patients showed increased levels of interleukin-6 ($P = .005$) and interleukin-10 ($P = .002$) compared with controls as well as increased levels of interleukin-6 ($p = 0.014$) and CCL24 ($P = .016$) compared with their siblings. C-C motif chemokine 11 levels were increased in siblings compared with controls ($P = .015$), and a similar tendency was found in patients compared with controls ($P = .045$). Glutathione peroxidase activity was decreased in patients compared with controls ($P = .006$) and siblings ($P = .025$). No differences were found for the other markers.
Conclusions: The present results suggest that unaffected siblings may present accelerated aging features. These neurobiological findings may be considered as endophenotypic traits. Further prospective studies are warranted.

Keywords: bipolar disorder, siblings, biomarkers, telomere, aging.

Introduction

The deleterious neuropathological processes seen in bipolar disorder (BD), including its accelerating, progressive course, have led investigators to believe that the pathophysiology of the condition is probably associated with poor metabolic stress compensation (Berk et al., 2011). Neurobiological underpinnings that underlie these associations remain little known, but a growing body of evidence suggests that changes in oxidative stress, inflammatory cytokines, neurotrophins, neurogenesis, mitochondrial energy generation, and stress-related hormones may play a role in BD neuropathology (Berk et al., 2011) involved in complex and reciprocal interactions.

Brain-derived neurotrophic factor (BDNF) plays a role in neurogenesis, neuronal survival, synaptic plasticity, dendritic growth, and long-term memory (Post, 2007). Studies have shown reduced BDNF levels in depressive and manic episodes in patients with BD (Goldstein and Young, 2013) and a negative correlation with the severity of symptoms (Shimizu et al., 2003; Cunha et al., 2006; Machado-Vieira et al., 2007). In addition, BDNF levels are decreased even in euthimic patients in late stages of BD and are negatively correlated with length of illness (Kauer-Sant´Anna et al., 2009). Inflammation is a mechanism that plays a part in several neuropsychiatric disorders, and in BD there is substantial evidence for this involvement (Brietzke and Kapczinski, 2008); BD mood episodes are proinflammatory states. Kauer-Sant´Anna et al. (2009) showed that both early and late stages of the disease are also associated with inflammatory activity, like alterations on interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α. Regarding oxidative stress, brain cells are more vulnerable to its damage as they present fewer antioxidant enzymes and a high level of oxidant metabolism (Olmez and Ozyurt, 2012). Research has showed an imbalance between oxidative damage and antioxidant defenses in BD (Magalhães et al., 2012; Pfaffenseller et al., 2013); depressive and manic episodes are associated with elevated oxidative stress activity (Andreazza et al., 2007; Machado-Vieira et al., 2007). In a review, Bauer et al. (2014) found results suggesting that elevated levels of peripheral inflammatory cytokines and oxidative stress and reduced levels of neurotrophic factors were associated with poorer cognitive performance in patients with BD.

In addition, recent studies have suggested that telomeres are shorter in patients with BD compared with controls (Simon et al., 2006; Elvsåshagen et al., 2011; Rizzo et al., 2013; Lima et al., 2015). Telomeres are structures found at the ends of chromosomes. They consist of protein complexes and noncoding DNA comprising hundreds to thousands of tandem repeats of TTAGGG (de Lange, 2005), which are crucial for maintaining chromosomal integrity and protecting them from loss of genetic material and end-to-end recombination (Blackburn, 2005). Telomere length (TL) is determined by the number of divisions that the cell has undergone: the higher the number of divisions, the shorter the length (Bojesen, 2013); at a certain critical short length, the cell loses the ability to divide and enters senescence (Nitta et al., 2011). Therefore, telomere shortening has been used as a biological marker of aging (Blackburn, 2005).

Short telomeres have been associated with increased risk of early death in the general population, but it is unclear whether the former are the cause or only represent a trait of risk for the latter (Bojesen, 2013). Of note, some studies have suggested that telomere shortening may be associated with aging-related diseases, such as cardiovascular disease and diabetes (Salpea et al., 2010; Wong et al., 2010). Moreover, factors such as age, sex, obesity, smoking, and lifestyle are known to influence TL (Hastie et al., 1990; Cassidy et al., 2010; Fitzpatrick et al., 2011; Njajou et al., 2012; Sun et al., 2012; Weischer et al., 2012). Finally, heritability (i.e., the variation in TL explained by hereditary factors) has been found to vary between 60% and 80% (Njajou et al., 2007; Atzmon et al., 2010).

There is evidence suggesting that high levels of proinflammatory cytokines during a depressive episode are associated with telomere shortening (Damjanovic et al., 2007). Simon et al. (2006)

reported a reduction in TL in peripheral leukocytes in individuals with major depression disorder and BD compared with healthy controls, and other authors have confirmed telomere shortening in BD (Elvsåshagen et al., 2011; Martinsson et al., 2013; Lima et al., 2015). Rizzo et al. (2014) published a review focusing on a theory of BD as an illness of accelerated aging. According to these authors, investigation of aging and senescence processes could contribute to the search for new and promising molecular targets to help explain and ameliorate neuroprogression in BD (Rizzo et al., 2014).

In line with the aging approach, Villeda et al. (2011) suggested a mechanism whereby systemic toxicity could have direct effects on the CNS; this study showed that C-C motif chemokine 11 (CCL11) could cause deficits in synaptic plasticity, neurogenesis, and cognition that are associated with aging. Magalhães et al. (2014), in a population-based sample of young adults, confirmed previous findings (Brietzke et al., 2009; Barbosa et al., 2013) that pointed to a role played by CCL11 in BD-related neuropathological processes.

Siblings of patients with BD may represent a vulnerable group, as BD has a strong component of heritability (Antypa and Serretti, 2014). The risk for BD in first-degree relatives is approximately 10-fold higher than in the general population (Kessler et al., 1994), and patients with BD reporting a family history of mood disorder have been shown to present a worse clinical profile upon presentation for treatment and a more symptomatic course of the disorder (Antypa and Serretti, 2014). Recent evidence has suggested the existence of neurobiological trait abnormalities in individuals at genetic risk (siblings or offspring), including neuroimaging findings (Fusar-Poli et al., 2012), cognitive parameters (Arts et al., 2008), and hypothalamic-pituitary-adrenal (HPA) axis functioning (Ellenbogen et al., 2010; Fries et al., 2014). Specifically, Fries et al. (2014) showed that HPA axis activity in siblings of patients with BD lies somewhere between that of controls and patients with BD.

Despite the evidence supporting the existence of neurobiological trait abnormalities in individuals at genetic risk for BD, most individuals with a family history of BD do not develop the disorder (Kessler et al., 1994). Thus, a better understanding of the familial risk for BD in siblings of patients (a population with a high genetic load for BD) could help researchers to identify endophenotypes of the disorder. Assessing how this familial risk is clinically manifested could provide insights into characterizing subphenotypes of the disorder, shed light on the pathophysiology of the condition, and foster further research into the genetic architecture of BD. In an attempt to advance knowledge in this area, in this study, we investigated the role played by telomere shortening and aging pathways in this pathological process.

To characterize the neurobiological profile of a population at genetic risk for BD, the aim of this study was to examine differences in potential biological markers between patients with BD, their unaffected siblings, and healthy subjects. Therefore, we choose a combination of biomarkers to evaluate in this sample that examine changes in neurotrophins (BDNF), TH2 inflammatory response (IL6, IL10, and TNF-α), and oxidative stress (glutathione peroxidase [GPx], glutathione reductase [GR], glutathione S-transferase [GST], and 3-nitrotyrosine [3-NT]) as well as biomarkers associated with accelerating aging process (TL, CCL11, and CCL24). We hypothesized that biomarkers would be altered in patients with BD and in unaffected siblings of BD compared with control subjects.

Methods

Participants

This cross-sectional study recruited subjects from the Bipolar Disorders Program of Hospital de Clínicas de Porto Alegre, Brazil, their siblings, and healthy controls. A total of 119 participants were included. Of these, there were 36 outpatients with a diagnosis of BD type I, their respective unaffected siblings (n = 39), and healthy comparison subjects (n = 44). Patients with bipolar type I diagnosis were invited to this study if they had at least one (or more) unaffected sibling that agreed with the study. Thus, the group of siblings is larger than the group of index patients. Also, the control group was recruited based on frequency math; therefore, a larger number was included to be comparable with patients and siblings regarding age, sex, and years of education. Investigators contacted the siblings by phone to invite them to this study, with the consent of the index patient. Inclusion criteria for patients were: age 18–60 years, BD type I diagnosis according to the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), currently on regular clinical assistance for pharmacological treatment, euthymia for at least 45 days, Hamilton Depression Rating Scale (HDRS) and Young Mania Rating Scale (YMRS) scores ≤7, and having siblings without a diagnosis of BD. Siblings were included only if they did not show any psychiatric diagnosis according to the DSM-IV, scored ≤7 on HDRS and YMRS, age 18–60 years,, and share with index patient the same mother and father. Siblings and patients were evaluated with clinical interview conducted by a well-trained psychiatrist; if either the index patient or their sibling did not met criteria for inclusion or meet exclusion criteria, both were excluded from the study.

Healthy subjects for the study were recruited from volunteer blood donors to the Blood Bank Program of Hospital de Clínicas de Porto Alegre. Inclusion criteria for the control group were: age 18–60 years, not fulfilling criteria for any psychiatric diagnosis according to DSM-IV (checked with SCID-I), HDRS and YMRS scores ≤7, a negative history of psychiatric disorders, and a negative history of psychiatric disorders in first degree-relatives. All controls underwent a comprehensive clinical interview conducted by a well-trained psychiatrist, using a short version of the SCID-I, HDRS, and YMRS.

The exclusion criteria for BD patients, siblings, and controls were history of autoimmune diseases, chronic infection/inflammatory disorders, use of immunosuppressive therapy, any severe systemic or neurological illnesses, and if any inclusion criteria are not confirmed at the end of the interview. So, after the interview, if a psychiatric diagnosis according to DSM-IV was detected in siblings or in healthy subjects, they were excluded.

All study procedures were approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre. Written informed consent was obtained from all participants (patients with BD, siblings, and controls) prior to their inclusion in the study.

BDNF Analysis

Ten milliliters of blood was withdrawn from each subject by venipuncture into a vacuum tube without anticoagulant and one containing EDTA. The blood was centrifuged at 4000 g for 10 minutes, and serum and plasma samples were kept frozen at -80°C until assayed. BDNF serum levels were measured with sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the manufacturer's instructions (Millipore, Billerica, MA). Briefly, 96-well flat-bottom microtiter plates were coated for 24 hours at 4°C with the samples diluted 1:100 in sample diluent and a standard curve ranging from 7.8 to 500 pg of BDNF. Plates were then washed 4 times with buffer followed by the addition of biotinylated mouse anti-human BDNF monoclonal antibody (diluted 1:1000 in sample diluent), which was incubated for 3 hours at room temperature. After washing, a second incubation with streptavidin-horseradish

peroxidase conjugate solution (diluted 1:1000) for 1 hour at room temperature was carried out. After the addition of substrate and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrated a direct relationship between optical density and BDNF concentration. Intra- and inter-assay variation of the kit was 3.7% and 8.5%, respectively.

Oxidative Stress Parameters

Oxidative stress parameters included GPx, GR, GST, and 3-NT. GPx, GR, and GST activities were measured in EDTA-plasma using commercial kits from Cayman Chemical (Ann Arbor, MI), and 3-NT levels were assessed using a commercially available competitive ELISA kit (Millipore) according to the manufacturer's instructions.

Serum Inflammatory Markers

IL-6, IL-10, and TNF-α concentrations were determined by flow cytometry using the B Cytometric Bead Array Human Enhanced Sensitivity Flex Set (BD Biosciences, San Jose, CA). Sample processing and data analysis were performed using a FACScalibur flow cytometer (BD Biosciences) according to the manufacturer's instructions. Samples for the measurement of eotaxin-1/CCL11 and eotaxin-2/CCL24 chemokines were assayed by sandwich ELISA according to the manufacturer's instructions (DuoSet, R&D Systems, Minneapolis, MN).

Measurement of Relative TL (T/S Ratio)

Whole peripheral venous blood was used for genomic DNA (gDNA) extraction using a commercial kit (Illustra blood genomicPrep Mini Spin Kit, GE Healthcare) according to the manufacturer's instructions. Nucleic acid quantification and purity were assessed spectrophotometrically (BioPhotometer Plus, Eppendorf, Hamburg, Germany), and samples were stored at -20°C for subsequent analysis. gDNA (25 ng/reaction) was used as template for the quantification of mean relative TL (T/S) using real-time qPCR, with minor modifications to the method previously described (Cawthon et al., 2002). Briefly, for each sample, 2 separate qPCR assays were performed, in triplicate, in separate 96-well plates, and in the same position. One reaction amplified the telomere (T) repeat sequence, while the other amplified a single copy gene (S), 36B4, which served as quantitative control. For each participant, relative TL was expressed as T/S ratio. Previously published primer sequences (Cawthon et al., 2002) were (5'→3'): tel 1, GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT; tel 2, TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA; 36B4u, CAGCAAGTGGGAAGGTGTAATCC; and 36B4d, CCCATTCTATCATCAACGGGTACAA. T and S master mix reactions were identical in a final volume of 20 µL with 0.1x SYBR Green (Molecular Probes, CA), 2 mM $MgCl_2$, 0.1 mM of each deoxynucleotide, 1% dimethyl sulfoxide, and 0.5 U of Platinum Taq DNA Polymerase (Invitrogen). Primer concentrations for telomere amplification were 270 nM for the forward primer and 1125 nM for the reverse primer. For the amplification of the RPLP0 gene (36B4), we used 300 nM for the forward primer (36B4u) and 500 nM for the reverse primer (36B4d). We arbitrarily chose a gDNA sample from an independent study as a reference sample. This sample was present in all the plates and was used to generate the standard curve present in each plate for telomeres and 36B4 amplification. Standard curves were generated over a 5-fold range serial dilution from 100 to 0.16 ng of gDNA. In previous experiments, we also used synthetic oligos previously published for the generation of the standard curves for the amplification of the telomeres' sequence for telomeres or the RPLPO gene with the same results (O'Callaghan et al., 2008). We have already published 2 studies with these conditions (Barbé-Tuana et al., 2016; Czepielewski et al., 2016).

PCR reactions were performed using the StepOnePlus Real-Time PCR System (Applied Biosystems, Carlsbad, CA) and analyzed using StepOne Software v2.3. (Applied Biosystems). The thermal cycling profile used for telomere amplification consisted of an initial incubation step for 10 minutes at 94°C to activate hot start Platinum Taq DNA Polymerase, followed by 22 cycles of denaturing at 94°C for 15 seconds and annealing and extension for 2 minutes at 54°C. For 36B4, amplification consisted of 30 cycles of denaturing at 94°C for 15 seconds followed by annealing and extension for 2 minutes at 60°C. The specificity of the amplification was confirmed at the end of each run using melting curve analysis. Additionally, PCR products were confirmed using agarose gel electrophoresis. In each run, a reference sample was included as a calibrator to normalize the participant's T/S ratio and calculate the final T/S ratio. Finally, to check for PCR amplification efficiency, standard curves for telomere and 36B4 amplification were generated from the reference sample over a 5-fold range by serial dilution from 100 to 0.16 ng of gDNA.

Statistical Analysis

Demographic data were compared using 1-way ANOVA or chi-square tests, as appropriate. All continuous variables were initially tested for normality using the Kolmogorov-Smirnov test. T/S ratios obtained in the different groups were compared using the Jonckheere-Terpstra trend test for continuous variables. The Jonckheere-Terpstra test for nonparametrical samples can test an ordered alternative hypothesis within independent samples (Jonckheere, 1954). Different from the Kruskal-Wallis test, the Jonckheere-Terpstra allows not only to detect differences between the groups but also to detect a priori ordering from which the samples are drawn and expresses a statistically significant trend.

BDNF levels, as well as GPx and GR activities, showed a normal distribution and were therefore analyzed using 1-way ANOVA followed by Tukey's posthoc test. All other biochemical parameters showed a nonnormal distribution and were analyzed using Kruskal-Wallis's followed by Mann-Whitney's tests. P values <.05 were considered significant, except when Bonferroni correction was applied to control for multiple comparisons (in this case, $P < .016$ was considered significant).

Results

A total of 135 subjects were evaluated with the interview. Of these, 6 families (patient-sibling) were excluded (a total of 12 subjects): 1 patient due to use of immunosuppressive therapy, 2 patients due to a inclusion criteria not confirmed at the end of the interview (HAMD >7), 3 siblings due to a inclusion criteria not confirmed at the end of the interview (2 with psychiatric diagnosis according to DSM-IV, and 1 did not share the same mother and father with patient). Regarding to healthy controls, 4 subjects were excluded: 3 due to inclusion criteria not confirmed at the end of the interview (1 with psychiatric diagnosis according to DSM-IV and 2 with history of psychiatric disorders in first degree-relatives) and 1 due to chronic infection/inflammatory disorders. Results are shown for 119 subjects: 36 patients, 39 siblings, and 44 controls.

Table 1. Characteristics of Controls, Siblings, and Patients with Bipolar Disorder

Characteristic	Controls (n = 44)	Siblings (n = 39)	Patients (n = 36)	*P*
Age (y)[a,d]	45.8 (12.1)	49.7 (14.4)	47.2 (10.1)	.359
Gender (male/female)[c]	20/24	12/27	9/27	.134
HDRS[a,d]	0.7 (1.15)	3.59 (3.5)	4.11 (2.9)	<.001
YMRS[a,d]	0.09 (0.36)	1.1 (1.5)	0.94 (1.53)	.001
Body mass index[a,d]	27.6 (4.8)	26.6 (5.09)	29.22 (5.5)	.124
Smoking[c]	11.36%	21.6%	27.7%	.173
Education (y)[a,d]	12.1 (5.2)	10.5 (4.7)	10.2 (3.9)	.167
Age at illness onset[a]			27.47 (10.5)	
Length of illness (y)[a]			19.7 (11.54)	
Number of manic episodes[a]			5.06 (4.85)	
Number of depressive episodes[a]			7.6 (7.8)	
Number of hospitalizations[a]			4.89 (7.8)	
Comorbidities, %				
Hypothyroidism[c]	2.27	16.2	33.3	.001
Hypertension[c]	18.1	21.6	33.3	.265
Diabetes mellitus[c]	2.27	0	16.6	.005
Dyslipidemia[c]	4.5	27.02	19.44	.02
Obesity[c]	0	0	2.7	.313
Other[c]	22.7	45.9	27.7	.107
Psychiatric medication, n (%)				
Lithium			19 (52.78)	
Valproic acid			14 (38.89)	
Typical antipsychotic			6 (16.67)	
Atypical antipsychotic			15 (41.66)	
Antidepressants			3 (8.33)	
Benzodiazepines			6 (16.67)	

Abbreviations: HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.
[a] Mean (standard deviation).
[b] Median (interquartile range).
[c] Chi-square test.
[d] 1-way ANOVA.

The 3 groups were similar with regard to demographic data, for example, age, gender, body mass index, smoking status, and years of education (Table 1). With regard to clinical variables, patients and siblings showed higher HDRS and YMRS scores compared with controls, even though all of the participants were euthymic at enrollment. Groups also differed in regard to prevalence of hypothyroidism, diabetes mellitus, and dyslipidemia (Table 1).

Subjects with BD presented shorter telomeres (T/S ratio) compared with their siblings and controls (patients' mean = 1.007; siblings' mean = 1.017; healthy controls' mean = 1.300) (Figure 1). The Jonckheere-Terpstra test revealed a significant negative trend for T/S ratio (P = .041) between the 3 groups. Further comparisons revealed that patients had significantly shorter telomeres than healthy controls (U = 339, P = .033) and that siblings also had shorter telomeres than controls (U = 550, P = .05). However, no differences were found between patients and siblings (U = 292, P = .837). Differences in T/S ratios remained significant after adjustment for age (β = -0.141, P = .171) and body mass index (β = 0.057, P = .586) in a multivariate model ($t_{(2,129)}$, P = .036).

There were no differences in BDNF levels between the 3 groups ($F_{(2,105)}$ = 0.844, P = .433) (Figure 2). Similarly, oxidative stress markers such as GR activity ($F_{(2,100)}$ = 0.313, P = .732; Figure 3a), GST activity (χ^2 = 5.182, P = .072; Figure 3b), and 3-NT levels (χ^2 = 0.99, P = .607; Figure 3c) also did not differ between the 3 groups. However, GPx activity was significantly lower in patients with BD compared with both controls ($F_{(2,101)}$ = 5.548, P = .006; Figure 3d) and siblings (P = .025), but similar between siblings and controls (P = .864).

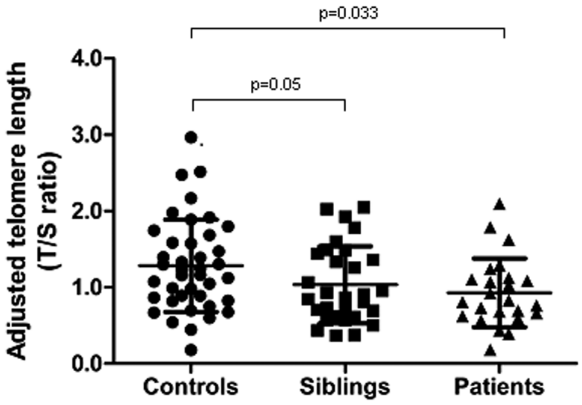

Figure 1. Telomere length in controls, siblings, and patients with bipolar disorder (BD).

Regarding inflammatory markers, IL-6 levels were undetectable in 21% of the samples, while IL-10 was undetectable in 11.7%. Either way we proceeded with the comparison between groups, in which we found that IL-6 levels were significantly higher in patients than in controls (U = 405, P = .005; Figure 4a), but no significant differences were found between patients and siblings (U = 486, P = .150) or between siblings and controls (U = 609, P = .092). The median and interquartile ranges are the following: patients, 103.68 (315.51); siblings, 66.54 (187.84); controls, 11.65 (125.83). Also, patients presented significantly higher levels of IL-10 compared with controls (U = 270, P = .002;

Figure 4b), whereas no differences were found between patients and siblings (U = 334.5, P = .018) or between siblings and controls (U = 493.5, P = .397). The median and interquartile ranges are the following: patients, 88.05 (90.34); siblings, 52.68 (62.66); controls, 49.84 (73.7). CCL11 levels were significantly increased in siblings compared with controls (U = 458.5, P = .015; Figure 4c), and a similar tendency was found in the comparison between patients and controls (U = 367, P = .045). Patients and siblings showed similar CCL11 levels (U = 506.5, P = .879). Finally, CCL24 levels were increased in patients compared with siblings (U = 339, P = .016; Figure 4d), whereas no differences were found for the other comparisons (patients vs controls, U = 404, P = .087; siblings vs controls, U = 588.5, P = .392). TNF-α concentrations were also assessed by flow cytometry using Cytometric Bead Array, but the results could not be included because TNF-α levels were undetectable in 85.7% of the samples. This can be explained by the extremely low stability of TNF-α in the periphery. Unfortunately, there was not enough biological sample to repeat or fix this, regarding TNF-α. This may be a limitation, and future studies should focus on the measurement of its soluble receptors sTNFR1 and sTNFR.

Of note, even though the sample storage time at -80ºC was higher than 1 month for most of the samples (after which some biochemical markers can be altered, particularly oxidative stress markers), we did not see any significant correlations between the assessed markers and storage time (BDNF – r = -.064, P = .509; IL10 – rho = .015, P = .883; IL6 – rho = .173, P = .069; CCL11 – rho = -.016, P = .871; CCL24 – rho = .036, P = .717; GPx – r = -.004, P = .966; GR – r = .154, P = .121; GST – rho = .130, P = .189; 3-NT – rho = -.099, P = .313).

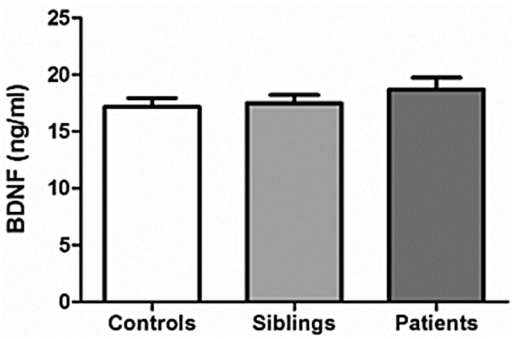

Figure 2. Brain-derived neurotrophic factor (BDNF) levels in patients with bipolar disorder (BD), siblings, and controls.

Discussion

To our knowledge, this is the first study to show a significant negative trend in telomere shortening in patients with BD, siblings, and healthy controls. We found evidence to support the hypothesis of progressive development of a senescent phenotype supported by a prooxidative and proinflammatory milieu that may contribute to the maintenance of the aging phenotype in patients with BD.

The findings of this research can be summarized as follows: (1) TL was reduced in patients compared with healthy controls but did not differ between patients and siblings; in addition, siblings showed a tendency to have a shorter TL than healthy controls; (2) CCL11, also an aging marker, was significantly increased in siblings compared with controls, and a similar tendency was also found between patients and controls; (3) IL-6 and IL-10 levels were significantly higher in patients than in controls, but no significant differences were found between patients and siblings; (4) GPx activity was significantly reduced in patients compared with controls, whereas there were no differences in GR

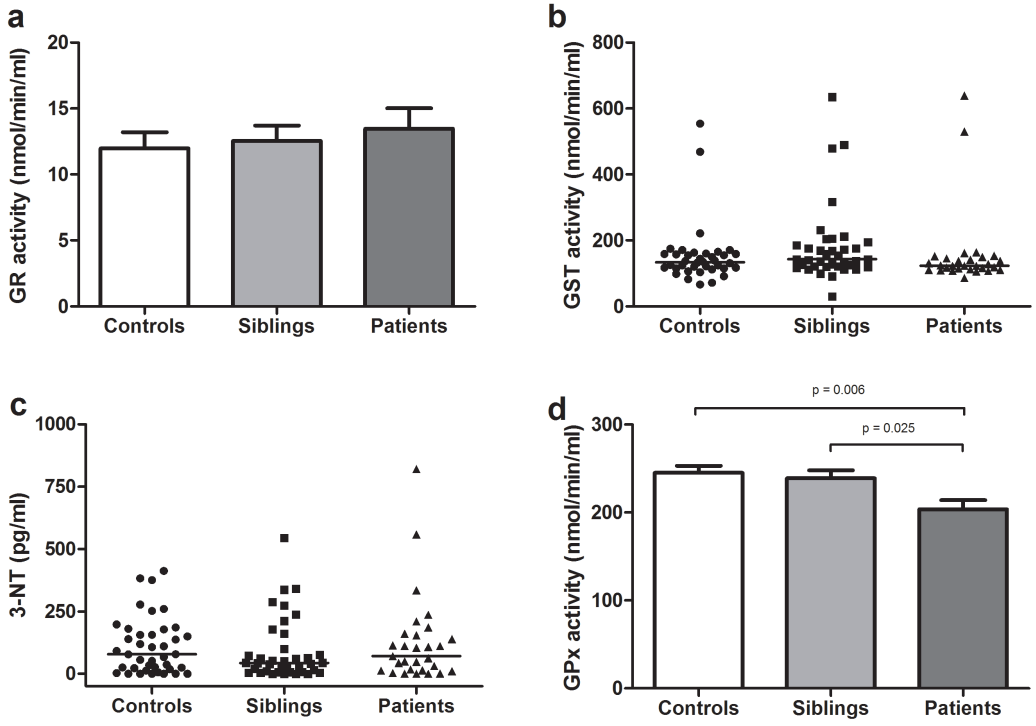

Figure 3. Oxidative stress parameters in patients with bipolar disorder (BD), siblings, and controls. GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; 3-NT, 3 nitrotyrosine.

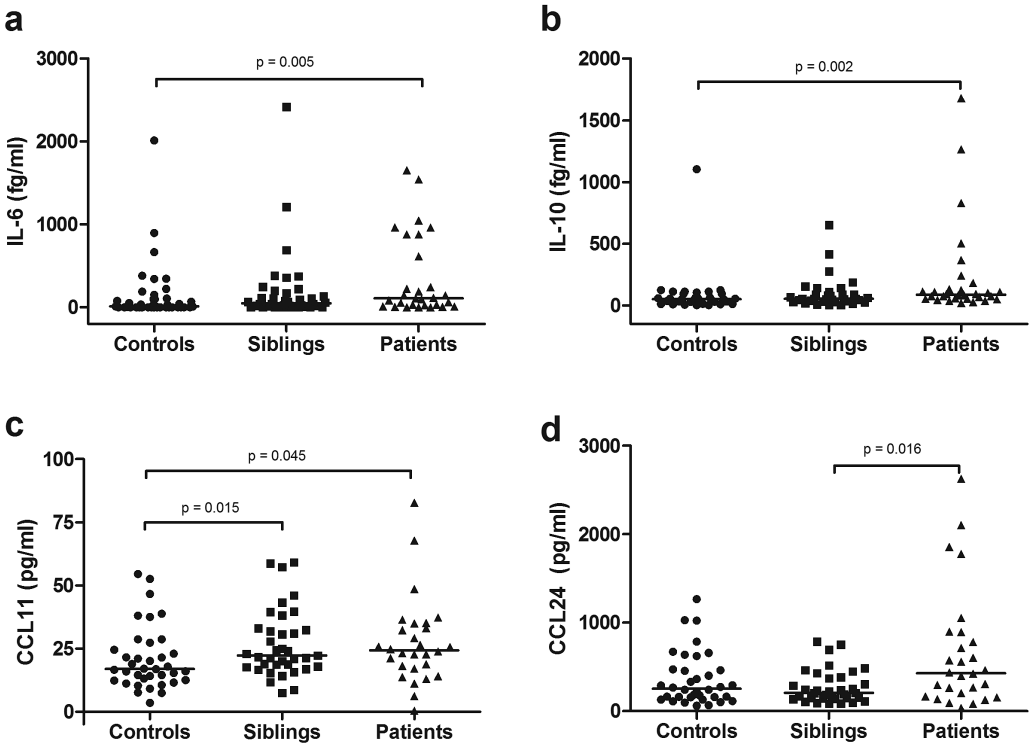

Figure 4. Inflammatory markers in patients with bipolar disorder (BD), siblings, and controls. CCL11, C-C motif chemokine 11; CCL24 = C-C motif chemokine 24; IL, interleukin.

and GST activities or in 3-NT levels between the groups; and (5) BDNF did not differ between the groups.

This study reinforces that aging is probably associated with the neuropathological processes seen in BD and shows that unaffected siblings may present senescence features, which is an original finding. This new finding is supported by the fact that 60% to 80% of TL is determined by heritability (Njajou et al., 2007; Atzmon et al., 2010), as patients and siblings share genetic background. Moreover, the results found in our patients are in line with previous studies suggesting that telomeres play a role in the neurobiological underpinnings of BD (Simon et al., 2006; Elvsåshagen et al., 2011; Lima et al., 2015). In BD type II, an increased load of short telomeres has been associated with lifetime number of depressive episodes, but not with illness duration (Elvsåshagen et al., 2011). Association of telomere shortening with depressive episodes, but not with illness duration, has also been observed in major depression disorder (Hartmann et al., 2010; Wolkowitz et al., 2011).

Previous studies have linked certain BD biological markers to aging. Yatham et al. (2009) have suggested that the pathophysiology of BD could be associated with an accelerated aging process, as suggested by the accelerated decrease observed in BDNF levels with age in patients with BD. Also, older euthymic patients with BD have shown greater levels of oxidative lipid damage than older controls, supporting the hypothesis of a persistent effect, into late life, of reactive oxygen species in patients with BD (Andreazza et al., 2015). In fact, oxidative stress has been related and intrinsically connected to aging and life span (Finkel and Holbrook, 2000; Harman, 2009). For instance, oxidative stress causes DNA damage and stimulates inflammatory activation (Rizzo et al., 2014). Moreover, Epel et al. (2004) have pointed out that greater perceived stress was associated with higher oxidative stress and shorter TL, mediated in part by stress-related hormones (Aschbacher et al., 2013). In our sample, patients with BD showed higher levels of inflammatory and oxidative markers. Our findings are in agreement with several literature data that indicate that patients with BD are in a proinflammatory state (Brietzke and Kapczinski, 2008; Kauer-Sant´Anna et al., 2009). Siblings showed intermediate values between those of patients and healthy controls despite the absence of statistically significant differences in relation to patients. A tendency in siblings to present intermediate values was also found for HPA axis functioning in previous analyses of our group (Fries et al., 2014). The meaning of these intermediate values is not clear; it would be worthy of further studies to attempt to address if there really are no statistically significant differences in siblings, perhaps using other more sensitive techniques, such as soluble receptors.

Our results are in accordance with previous studies showing high levels of CCL11 in patients with BD (Brietzke et al., 2009; Barbosa et al., 2013; Magalhães et al., 2014). Interestingly, CCL11 levels were higher in siblings compared with patients. It is important to note the fact that patients are usually medicated (all our patients were) with lithium or other medications with neuroprotective properties (Berk et al., 2011). Martinsson et al. (2013) reported a positive effect of long-term lithium treatment on TL, and longer telomeres were associated with improved lithium response in patients with BD; these findings suggest that lithium exerts a protective effect against telomere shortening and thus against aging, especially when therapeutically effective. For future research, it would be interesting to evaluate how to control potential interferences of medications to make the biochemical findings cleaner. Nevertheless, the differences between unaffected siblings and control groups help us to speculate that accelerated aging may be a neurobiological change without

influence of medications in this research, regarding these groups, as they were medication free. Findings reported by Villeda et al. (2011), which showed direct effects of systemic toxicity on the CNS, particularly of CCL11 on synaptic plasticity, neurogenesis, and cognition, may shed light on the possible neurobiological vulnerability of siblings, underscoring the need to devote more attention and research to this population. From a different perspective, it could be speculated that the siblings included in the present study were resilient, as they remained without a psychiatric diagnosis despite the higher levels of CCL11 and the genetic and environmental factors shared with the patients. Of note, mean age in the group of siblings was 45.6 years, which is beyond the first peak age range of BD onset (Kroon et al., 2013).

Our findings need to be considered in light of some limitations. First, our sample size was small, and this was a cross-sectional study. It would be interesting to see the effects of a larger sample on our results, especially in parameters that showed a tendency toward intermediate, nonsignificant results. Moreover, there are limitations regarding to some comorbid conditions that differed significantly between groups, such as hypothyroidism, diabetes, and dyslipidemia.

In conclusion, our results reinforce the hypothesis of neurobiological trait abnormalities in individuals at genetic risk for BD and suggest that unaffected siblings may present accelerated aging features compared with healthy controls, which is in line with the genetic background shared with patients. These findings may contribute to the development of markers in genetic risk population studies and may be considered as endophenotypic traits of BD. Further prospective studies are needed to advance knowledge in this area and to clarify whether unaffected siblings are a resilient or vulnerable group.

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