Lithium Regulates Glycogen Synthase Kinase-3β in Human Peripheral Blood Mononuclear Cells: Implication in the Treatment of Bipolar Disorder

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Methods: The PBMCs were obtained from 23 healthy control subjects, 9 bipolar subjects currently treated with lithium, and 13 lithium-free bipolar subjects. Immunoblot analyses were used to measure the inhibited, serine9-phosphorylated GSK3β.

Results: The level of phospho-Ser9-GSK3β in PBMCs was regulated by agents that modified kinases and phosphatases acting on GSK3β and was increased by in vitro lithium treatment. More important, phospho-Ser9-GSK3β levels were eightfold higher in PBMCs from lithium-treated bipolar than healthy control subjects.

Conclusions: Signaling pathways regulating serine9-phosphorylation of GSK3β can be studied in human PBMCs. Both in vitro and in vivo therapeutic lithium treatment is associated with a large increase in phospho-Ser9-GSK3β in PBMCs. Therefore, the inhibitory serine9-phosphorylation of GSK3β in human PBMCs may provide a biochemical marker to evaluate the association between GSK3β inhibition and therapeutic responses to lithium treatment.

Keywords
Bipolar disorder, GSK3, lithium, PBMC

Author(s)
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Xiaohua Li, Ari B. Friedman, Wawa Zhu, Li Wang, Sherer Boswell, Roberta S. May, Lori L. Davis, and Richard S. Jope
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Abstract
Background—Bipolar disorder has been linked to alterations in the multifunctional enzyme glycogen synthase kinase-3β (GSK3β). The mood stabilizer lithium inhibits GSK3β in vitro and in mouse brain, and this is currently the strongest known potential therapeutic target of lithium. We tested whether lithium modified GSK3β in vivo or in vitro in peripheral blood mononuclear cells (PBMCs) from healthy control and bipolar disorder subjects.

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Keywords
Bipolar disorder; GSK3; lithium; PBMC
may be a key process in susceptibility, onset, therapeutic responses, or a combination of these factors in mood disorders and schizophrenia (Duman et al 2000; Nestler et al 2002).

Lithium has dual effects that work in combination to inhibit GSK3β in vivo. First, lithium binds directly to GSK3β to inhibit its activity, with 2 mmol/L lithium providing approximately 50% inhibition of GSK3β activity (Klein and Melton 1996; Stambolic et al 1996). This relatively high concentration of lithium compared with its therapeutically effective concentration of approximately 1 mmol/L initially suggested that in vivo concentrations of lithium achieved in treated patients may only moderately inhibit the activity of GSK3β; however, the direct inhibitory effect of lithium is amplified in vivo by a second mechanism involving phosphorylation of GSK3β. The activity of GSK3β is principally regulated by phosphorylation of a regulatory serine-9 in its N-terminal region. Phosphorylation of this serine is the major physiologic mechanism that inhibits the activity of GSK3β in cells (Stambolic and Woodgett 1994; Sutherland et al 1993). Thus, the likelihood that lithium significantly inhibits GSK3β in vivo was bolstered by the finding that a therapeutically relevant level of lithium greatly increases the serine9-phosphorylation of GSK3β in mouse brain in vivo (De Sarno et al 2002). This finding revealed that the direct modest inhibition of GSK3β by lithium was amplified in vivo by the serine9-phosphorylation of GSK3β, allowing lithium at therapeutic concentrations to provide a more robust inhibition of GSK3β than could be achieved solely by its direct inhibition of the enzyme (Jope 2003).

Because lithium-induced serine9-phosphorylation of GSK3β may be a key factor in allowing a therapeutically relevant concentration of lithium to substantially inhibit the activity of GSK3β, it is important to determine whether lithium causes this modification in cells from healthy and bipolar human subjects. Unfortunately, this cannot be addressed using postmortem human brain samples because GSK3β is rapidly dephosphorylated following death (Li et al 2005). Therefore, in this study we examined whether in vitro and therapeutic in vivo lithium treatment increased the serine9-phosphorylation of GSK3β in human peripheral blood mononuclear cells (PBMCs). The results show that lithium robustly increases this inhibitory modification of GSK3β in human PBMCs, further supporting the likelihood that lithium substantially inhibits GSK3β in human brain and that this may be an important therapeutic target of lithium.

Methods and Materials

Subjects and Procedures

The study was conducted in the Department of Psychiatry and Behavioral Neurobiology at the University of Alabama at Birmingham Center for Psychiatric Medicine. The study was approved by the University of Alabama at Birmingham Institutional Review Board, and written informed consent was obtained from all subjects after receiving a complete description of the study.

Men and women aged 19–65 years with a primary diagnosis of bipolar disorder (BD) according to DSM-IV criteria were recruited from the psychiatric outpatient clinic or the Center for Psychiatric Medicine in-patient hospital at the University of Alabama at Birmingham. Subjects were excluded if they concomitantly met criteria for any other major DSM-IV Axis I diagnosis, such as psychotic disorder, major depressive disorder, anxiety disorder, or alcohol or other substance dependence. At the initial screening, eligible subjects may have been taking any psychotropics for bipolar disorder, such as lithium, anticonvulsants, antipsychotics, antidepressants, or anxiolytics. If they were taking lithium, the length of lithium treatment must have been at least 4 weeks. The pharmacologic treatment was the decision of the primary physician and was not influenced in any way by
this study. A total of 22 bipolar subjects were enrolled in the study, among which 9 were taking lithium with an average serum level of 0.87 ± 0.18 mEq/L at the time of blood collection; the other 13 were either medication-free (n = 3) or taking any psychotropic except lithium for bipolar disorder. In addition, 23 healthy control subjects who had no history of psychiatric illnesses or any major medical illness were enrolled in the study. These control subjects were medication-free at the time of blood collection. Demographic characteristics of the subjects are shown in Table 1.

Psychiatric and medical histories along with vital signs, a physical examination, and a neurologic examination, were obtained before blood collection. Serum levels of lithium, valproate, or carbamazepine were measured if subjects were taking one of these mood stabilizers. The most recent (within 1 week) laboratory data on fluid balance, blood glucose, CBC with differential, thyroid function, and liver function were reviewed to ensure that these parameters were within the normal range. Clinical symptoms of BD were assessed using the Young Mania Rating Scale (YMRS), the Hamilton Depression Rating Scale (HAM-D), and the Clinical Global Impression of Severity (CGI-S) scale. All the assessments were completed within 3 days of blood collection.

**PBMC Preparation, In Vitro Treatments, and GSK3β Measurements**

Approximately 50 mL of blood from each subject was collected by venipuncture into heparin-containing vacutainers. Whole blood from each subject was diluted with RPMI-1640 cell culture media (Cellgro, Herndon, Virginia). The PBMCs were extracted from whole blood by addition of 0.25 volume of IsoPrep solution (Robbins Scientific, Sunnyvale, California) into the bottom of the blood-containing tube followed by centrifugation (2000 rpm for 20 min at room temperature). The cell layer containing PBMCs was transferred to a clean tube and was washed with RPMI-1640 media twice. Approximately 5 × 10^7 PBMCs were obtained from 50 mL of whole blood. Aliquots of PBMCs (3 × 10^6 cells) were placed into individual microfuge tubes or 24-well cell culture dishes. One aliquot of PBMCs was immediately lysed in lysis buffer (10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L ethylenediamine tetraacetic, 1 mmol/L ethylene glycol bis-2-aminoethyl ether-N,N′,N″,N‴-tetraacetic acid, .5% NP-40, 10 μg/L leupeptin, 10 μg/L aprotinin, 5 μg/L pepstatin, .1 mmol/L β-glycerophosphate, 1 mmol/L phenylmethanesulfonyl fluoride, 1 mmol/L sodium vanadate, and 100 nmol/L okadaic acid) and centrifuged at 14,000 rpm for 10 min. The supernatant was incubated on ice for 2 hours and then processed along with the in vitro–treated PBMC samples. In one group of experiments, the PBMC samples were incubated for 2 hours in RPMI-1640 media at 37°C in the absence or the presence of the indicated agents for the indicated times up to 1 hour. In the second group of experiments, PBMC samples were preincubated for 1 hour in RPMI-1640 media containing 10% Fetal Clone II (HyClone, Logan, Utah) at 37°C, followed by the addition of lithium and a further incubation for 20 hours. Agents tested in these experiments include lithium chloride, phenylarsine oxide (Sigma), phorbol 12-myristate 13-acetate (PMA; Alexis, California), and calyculin A (Alamore Labs, Jerusalem, Israel). After incubation, PBMCs were lysed with lysis buffer and centrifuged, and the supernatants were collected. Protein concentrations were measured using the Bradford protein assay (Bradford 1976).

For immunoblotting, proteins from PBMC lysates were mixed with Laemmli sample buffer (2% sodium dodecyl sulfate [SDS]) and placed in a boiling water bath for 5 min. Proteins (15 μg) were resolved in 10% SDS-polyacrylamide gels and transferred to nitrocellulose. Blots were probed with antibodies to phospho-Ser9-GSK3β (Cell Signaling Technology, Beverly, Massachusetts) and total GSK3β (Southern Biotech, Birmingham, Alabama). Immunoblots were developed using horseradish peroxidase–conjugated goat antimouse or
goat antirabbit immunoglobulin G, followed by detection with enhanced chemiluminescence. Protein bands were quantitated with a densitometer.

**Data Analysis**

Data analyses were performed using Microsoft Excel (2003) and Sigma Stat (3.0). The analyzed data set consisted of three groups of subjects: healthy control subjects, BD subjects currently treated with lithium, and lithium-free BD subjects. The unpaired rank sum test for two-group comparison was used to analyze differences between in vitro treatments and between healthy control and BD subjects. Data from lithium-concentration response experiments within each group of subjects were analyzed using one-way repeated measure ANOVA on rank.

**Results**

We first tested whether the serine9-phosphorylation of GSK3β could be modulated in human PBMCs. To do this, freshly isolated PBMCs from healthy control subjects were incubated in vitro and then immunoblot analyses were carried out using a phosphorylation-dependent antibody that specifically identifies GSK3β only when it is phosphorylated on its regulatory residue, serine-9 (phospho-Ser9-GSK3β). This was compared with the total amount of GSK3β, measured using an antibody that identifies all GSK3β to assess the proportion of all the GSK3β in PBMCs that contained the inhibitory serine9-phosphorylation modification. Cells were treated with several agents known to increase phospho-Ser9-GSK3β in other types of cells to test if phospho-Ser9-GSK3β levels dynamically changed in PBMCs. PBMCs were incubated with a phorbol ester (PMA) to activate protein kinase C, which is known to phosphorylate GSK3β in other cells (Goode et al 1992; Tsuji et al 2000). Treatment with PMA caused a rapid increase in the serine9-phosphorylation of GSK3β that was evident within 15 min of treatment, reached a peak after 60 min, and was maintained above the initial basal level for at least 2 hours during exposure to PMA (Figure 1A and 1B). Furthermore, the PMA-induced increase in phospho-Ser9-GSK3β was concentration-dependent with an EC₅₀ of approximately 10 nmol/L PMA (Figure 1C). Cells also were treated with two inhibitors of protein phosphatases; PAO was used because it inhibits protein phosphotyrosine phosphatases. Phosphotyrosine residues are commonly associated with activated growth factor receptors, which can stimulate intracellular signaling cascades leading to serine9-phosphorylation of GSK3β, and thus PAO may potentiate this signal to phospho-Ser9-GSK3β. Treatment with PAO (1 μmol/L) caused time-dependent increases in the phosphorylation of GSK3β on serine-9 in human PBMCs, with the increase lasting more than 90 min (Figure 1A and 1B). The effect of PAO was concentration-dependent with an EC₅₀ of approximately 0.2 μmol/L and a maximal effect at 1 μmol/L (Figure 1C). Additionally, calyculin A was used because it inhibits protein phosphatases 1 and 2A (Millward et al 1999). In other types of cells protein phosphatases 1 and 2A are known to remove the phosphate from the serine-9 of GSK3β (Grimes and Jope 2001). If these protein phosphatases dynamically regulate phospho-Ser9-GSK3β in human PBMCs, phosphatase inhibition by calyculin A should elevate phosphorylation levels. This was found to be the case because treatment with calyculin A robustly increased the level of phospho-Ser9-GSK3β time- and concentration-dependently (Figure 1). With all treatments only minor changes in the total level of GSK3β were detected (Figure 1A), indicating that the effects were primarily on the serine-9 phosphorylation, not expression, of GSK3β. These results show that the serine9-phosphorylation of GSK3β is a dynamic process in human PBMCs and is regulated by changing the activities of kinases and phosphatases in these cells.

We next compared the basal levels of phospho-Ser9-GSK3β in human PBMCs from three groups of subjects: healthy control subjects, lithium-treated BD subjects, and lithium-free
BD subjects. The basal levels of phospho-Ser9-GSK3β from the three groups of subjects clearly stratified into three distinct categories (Figure 2). The basal level of phospho-Ser9-GSK3β was lowest in healthy control subjects, it was elevated about threefold in lithium-free BD subjects, and it was highest (eightfold the level in healthy control subjects; $p = .038$ compared with healthy controls) in lithium-treated BD subjects. These findings are consistent with the conclusion that therapeutic in vivo treatment with lithium increased the serine9-phosphorylation of GSK3β in PBMCs as it did in mouse brain in vivo and in cultured cells (reviewed by Jope 2003). The elevated serine9-phosphorylation of GSK3β in PBMCs from both lithium-treated and lithium-free BD subjects was found to be reversible because incubation of PBMCs in serum-free media for 2 hours caused the levels of phospho-Ser9-GSK3β to revert to a low level that was similar to the basal levels in healthy control subjects.

We also tested whether in vitro treatment with lithium was capable of increasing the serine9-phosphorylation of GSK3β in PBMCs from each group of subjects. To test this, PBMCs were incubated in serum-free media for 1 hour and then treated with a maximal concentration of lithium (20 mmol/L) for a short period of time (1 hour) followed by immunoblot analyses of phospho-Ser9-GSK3β (Figure 2). This high concentration of lithium was used to attain maximal inhibition of GSK3β within a short treatment time. In PBMCs from healthy control subjects, this in vitro lithium treatment caused a significant fivefold increase ($p = .002$ compared with serum-free no treatment samples) in the level of phospho-Ser9-GSK3β. In vitro lithium treatment of PBMCs from lithium-treated BD subjects raised the level of phospho-Ser9-GSK3β to the high basal level found in these subjects. The effect of in vitro lithium treatment of PBMCs from lithium-free BD subjects also raised phospho-Ser9-GSK3β to a high level. The effect of in vitro lithium treatment in BD subjects was statistically significant when compared with serum-free no treatment samples, with $p = .004$ in lithium-treated BD subjects and $p = .016$ in lithium-free BD subjects. Neither in vivo therapeutic lithium treatment nor in vitro lithium treatment changed the level of total GSK3β in PBMCs (Figure 2A). Thus, these results show that in vivo therapeutic lithium treatment was associated with elevated phospho-Ser9-GSK3β and that in vitro lithium treatment also raised phospho-Ser9-GSK3β levels in human PBMCs.

Finally, we examined the concentration-dependence of the in vitro lithium-induced increases in phospho-Ser9-GSK3β by incubating human PBMCs with lithium in a concentration range of 1.25 to 10 mmol/L (Figure 3). In these experiments, the incubation time with lithium was increased to 20 hours to allow detection of the effects of lower concentrations of lithium. The levels of phospho-Ser9-GSK3β following in vitro lithium treatment were evaluated as a percent of the basal level to take into account the different initial levels in each group of subjects. These experiments showed that the phospho-Ser9-GSK3β in PBMCs from all three groups of subjects significantly increased ($p < .01$) in a lithium concentration–dependent manner within each group of subjects. The concentration-dependence of PBMCs was similar among all three groups of subjects at the lower lithium concentration range (1.25–2.5 mmol/L), with PBMCs from BD subjects tending to have greater responses than those from healthy control subjects at the higher concentration range (5–10 mmol/L). This prolonged in vitro lithium treatment (20 hours) did not change the total level of GSK3β in PBMCs (Figure 3A). Thus, even lower concentrations of lithium caused increases in phospho-Ser9-GSK3β after human PBMCs were treated with lithium in vitro for a prolonged period of time.

**Discussion**

Lithium inhibits GSK3β directly, and this inhibitory effect is amplified in mouse brain and in cultured cells by an increase in the inhibitory serine9-phosphorylation of GSK3β (Jope.
The lithium-induced increase in serine9-phosphorylation of GSK3β may be critical because it provides a mechanism whereby a moderate direct inhibitory effect of lithium achieved by therapeutically relevant lithium concentrations is amplified to achieve more pronounced inhibition of GSK3β by the therapeutic level of lithium. Therefore, it was important to determine whether this amplification mechanism is active in human cells. To assess this, we tested whether serine9-phosphorylation of GSK3β was dynamically regulated in human PBMCs and if in vivo therapeutic lithium administration as well as in vitro lithium treatment of PBMCs was associated with increased levels of phospho-Ser9-GSK3β. The results provide, for the first time, evidence that increases in phospho-Ser9-GSK3β are associated with both in vivo and in vitro lithium treatment in PBMCs from BD subjects.

Our first goal was to assess the utility of using human PBMCs to study the regulation of phospho-Ser9-GSK3β. Several signaling molecules or pathways were found to regulate the serine9-phosphorylation of GSK3β in PBMCs. Treatment with PMA greatly increased phospho-Ser9-GSK3β, showing that activation of protein kinase C can phosphorylate GSK3β in PBMCs. Inhibition of protein phosphatases 1/2A with calyculin A also increased phospho-Ser9-GSK3β, implicating these phosphatases in controlling the dephosphorylation of GSK3β in PBMCs. Perhaps most interesting, the phosphotyrosine phosphatase inhibitor PAO increased phospho-Ser9-GSK3β in PBMCs. This demonstrates the likelihood that growth factor receptors activated by tyrosine phosphorylation stimulate signaling cascades, leading to serine9-phosphorylation of GSK3β in PBMCs. Further studies are needed to identify the specific growth factor receptors that are involved, such as neurotrophic factor receptors (Besser and Wank 1999), because dysregulation of neurotrophic receptor signaling pathways are known to be linked to mood disorders (Duman et al 2000). Overall, these in vitro results demonstrate that the serine9-phosphorylation of GSK3β in human PBMCs is a dynamic process and can be used to identify modulators of this inhibitory regulation of GSK3β.

We found that in vitro and in vivo lithium treatment was associated with increased serine9-phosphorylation of GSK3β, an effect matching changes previously reported in mouse brain and continuous cultured cell lines (reviewed by Jope 2003). The mechanism through which lithium increases phospho-Ser9-GSK3β in cells is not completely understood. Some data indicate that lithium treatment causes activation of Akt, which leads to increased phospho-Ser9-GSK3β (Chalecka-Franaszek and Chuang 1999), but this appears to be a cell-type selective action. Other data indicate that as a result of lithium’s direct inhibition of GSK3β, there is a secondary inhibition of the protein phosphatase responsible for dephosphorylating GSK3β, which leads to greater serine9-phosphorylation of GSK3β (Zhang et al 2003). This regulation allows for a phosphorylation-dependent amplification of the direct inhibitory effect of lithium on GSK3β. Regardless of the mechanism, lithium clearly increased the level of phospho-Ser9-GSK3β in human PBMCs. The most interesting finding was that the level of phospho-Ser9-GSK3β was highly elevated in PBMCs from BD subjects currently undergoing lithium therapy. This suggests that therapeutic lithium treatment causes increased serine9-phosphorylation of GSK3β in human PBMCs. Taken in conjunction with findings of the same effect of lithium in mouse brain (De Sarno et al 2002), these results suggest that therapeutic levels of lithium are likely to increase the serine9-phosphorylation of GSK3β in human brain. Unfortunately, this modification cannot be studied in postmortem brain because there is a rapid loss of the phosphorylation during short postmortem intervals (Li et al 2005). Nevertheless, these results suggest that the direct inhibitory effect of lithium is amplified in humans by the increased inhibitory serine9-phosphorylation of GSK3β.

Recently increases in serotonergic activity and administration of the antidepressants imipramine or fluoxetine were found to increase the serine9-phosphorylation of GSK3β in mouse brain, suggesting that the deficient serotonergic activity that is associated with depression may involve impaired inhibitory control of GSK3β (Li et al 2004).
findings suggest that if the increased phospho-Ser9-GSK3β caused by lithium treatment in human PBMCs also occurs in human brain then lithium may be able to counteract this deficiency associated with impaired serotonergic activity.

This study assessed the feasibility of examining the regulation of serine9-phosphorylation of GSK3β in human PBMCs. This investigation is limited by the small sample size used in this initial examination, as well as the mixed demographic characteristics of the subjects and the presence of other psychotropic agents in many of the BD subjects. Additionally, PBMC preparations contain a heterogeneous population of cells, which vary among individuals, a factor that likely contributes to interindividual variations in the basal level of phospho-Ser9-GSK3β. Our results indicate, however, that PBMCs are useful for examining both the in vitro actions of drugs on GSK3β in human subjects and, more important, the in vivo therapeutic effects of psychotropic agents. The increased phospho-Ser9-GSK3β levels caused by lithium add support to the concept that GSK3β may be an important therapeutic target of lithium, as well as other therapeutic agents. GSK3β is an interesting therapeutic target because it is known to regulate neural plasticity in many ways through its phosphorylation of numerous proteins involved in gene expression, neuronal remodeling, and neuronal survival (Jope and Johnson 2004). Thus further studies of GSK3β phosphorylation in human PBMCs should be useful for increasing our understanding of the in vitro and in vivo actions of psychotropic agents in human subjects and to assess whether the in vivo level of phospho-Ser9-GSK3β in PBMCs may serve as a biomarker related to specific clinical symptoms or therapeutic response.

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Figure 1.
Increased phospho-Ser9-GSK3β in human peripheral blood mononuclear cells (PBMCs) induced by activation of protein kinase C and phosphatase inhibitors. (A) Immunoblots of phospho-Ser9-GSK3β (left panel) and total GSK3β (right panel) after PBMCs from healthy control subjects were incubated at 37°C in serum-free media for 2 hours in the absence or the presence of phorbol 12-myristate 13-acetate (PMA [1 μmol/L]), phenylarsine oxide [PAO; 1 μmol/L], or Calyculin A [0.1 μmol/L] for the indicated times. (B) Quantitative analysis of immunoblots showing the time-dependent increases of phospho-Ser9-GSK3β in PBMCs after treatment with PMA, PAO, and calyculin A. (C) Quantitative analysis of immunoblots showing the concentration-dependent increases of phospho-Ser9-GSK3β in PBMCs after treatment with PMA, PAO, and calyculin A. The graphs in (B) and (C) show...
data from individual samples of PBMCs that demonstrate representative rates of change or concentration-dependent responses typical of changes in PBMCs in response to these agents.
Figure 2.
Phospho-Ser9-GSK3β in peripheral blood mononuclear cells (PBMCs) from healthy control subjects and subjects with bipolar disorder (BD) before and after in vitro treatments. Freshly isolated PBMCs from healthy control subjects \( (n = 13) \), BD subjects treated with lithium (Lithium-Tx BD, \( n = 9 \)), or BD subjects without lithium treatment (lithium-free BD, \( n = 13 \)) were (i) immediately lysed and incubated on ice for 2 hours (basal), (ii) incubated in serum-free media for 2 hours (SF), or (iii) incubated in serum-free media for 1 hour followed by incubation with 20 mmol/L lithium for 1 hour (LiCl). Phospho-Ser9-GSK3β and total GSK3β in protein lysates (15 \( \mu \)g) from each subject were detected on the same immunoblot.

(A) Representative immunoblots from a healthy control subject, a lithium-treated BD subject, and a lithium-free BD subject. (B) Immunoblots were analyzed by densitometry, and values are calculated as the absolute optical density (OD) volume. Data shown are average ± SEM. Statistical significance was calculated using an unpaired rank sum test for two-group comparison. *\( p < .05 \) between basal phospho-Ser9-GSK3β of healthy control subjects and lithium-treated BD subjects; **\( p < .05 \) between serum-free samples and in vitro lithium-treated samples within each group of subjects.
Figure 3.
Lithium concentration–dependent increase of phospho-Ser9-GSK3β in human peripheral blood mononuclear cells (PBMCs). Freshly isolated cells were incubated in serum-containing media for 1 hour followed by addition of lithium (0, 1.25, 2.5, 5, or 10 mmol/L) and incubation for 20 hours. Phospho-Ser9-GSK3β and total GSK3β levels in PBMCs from each subject were detected on the same immunoblot. (A) Representative immunoblots from a healthy control subject. (B) Immunoblots were analyzed by densitometry, and values were calculated as the percent of value obtained in PBMCs incubated in parallel without lithium (0 mmol/L). Data shown are average values (n = 7–9) from healthy control subjects and each bipolar group. In this set of experiments, only those samples that had treatments with all five lithium concentrations are included for data analysis. Statistical significance was calculated using one-way repeated-measures analysis of variance on rank for each lithium concentration response within the same group of subjects. There is a significant (p < .01) difference in the mean values among the lithium concentrations within each group of subjects, and *p < .05 when the individual lithium concentration is compared with the corresponding basal value (0 mmol/L).
### Table 1

**Demographic and Clinical Summary**

<table>
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<th>Lithium-Treated BD</th>
<th>Lithium-Free BD</th>
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AA, African American; BD, bipolar disorder; BDI, bipolar disorder type I; BDII, bipolar disorder type II; BD NOS, bipolar disorder not otherwise specified; CA, Caucasian; F, female; M, male; Others, any race other than CA or AA.