Mitochondrial Complex I Activity and Oxidative Damage to Mitochondrial Proteins in the Prefrontal Cortex of Patients With Bipolar Disorder

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Context: Accumulating evidence suggests that mitochondrial dysfunction and oxidative stress contribute to the pathogenesis of bipolar disorder and schizophrenia. It remains unclear whether mitochondrial dysfunction, specifically complex I impairment, is associated with increased oxidative damage and, if so, whether this relationship is specific to bipolar disorder.

Objective: To evaluate whether decreased levels of the electron transport chain complex I subunit NDUFS7 are associated with complex I activity and increased oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder, schizophrenia, or major depressive disorder.

Design: Postmortem prefrontal cortex from patients and controls were assessed using immunoblotting, spectrophotometric, competitive enzyme immunoassay to identify group differences in expression and activity of complex I, and in oxidative damage in mitochondria.

Setting: University of British Columbia, Vancouver, Canada.

Patients: Forty-five patients with a psychiatric disorder (15 each with bipolar disorder, schizophrenia, and major depressive disorder) and 15 nonpsychiatric control subjects were studied.

Main Outcome Measures: Oxidative damage to proteins and mitochondrial complex I activity.

Results: Levels of NDUFS7 and complex I activity were decreased significantly in patients with bipolar disorder but were unchanged in those with depression and schizophrenia compared with controls. Protein oxidation, as measured by protein carbonylation, was increased significantly in the bipolar group but not in the depressed or schizophrenic groups compared with controls. We observed increased levels of 3-nitrotyrosine in the bipolar disorder and schizophrenia groups.

Conclusions: Impairment of complex I may be associated with increased protein oxidation and nitration in the prefrontal cortex of patients with bipolar disorder. Therefore, complex I activity and mitochondrial dysfunction may be potential therapeutic targets for bipolar disorder.

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Bipolar disorder (BD) is a chronic psychiatric illness characterized by recurrent episodes of mania, hypomania, mixed states, and depression. It has been increasingly recognized that individuals with BD are at a higher risk for chronic general medical conditions, such as obesity, diabetes mellitus, and cardiovascular disease, which are directly associated with the increased morbidity and mortality observed in BD. Despite decades of extensive investigation, the etiology and pathogenesis of this disorder remain unclear. Several postmortem studies have reported reduced neuronal and glial density in discrete regions of the prefrontal cortex of patients with BD. In addition, Davis et al found reductions in cortical gray matter and cerebral white matter volumes in male patients with familial BD type I. Mitochondrial dysfunction and the consequent oxidative damage to lipids, proteins, and DNA might be one of the possible mechanisms contributing to neuronal or glial impairment in BD.

Mitochondria are intracellular organelles that have a crucial role in adenosine triphosphate (ATP) production through oxidative phosphorylation, a process performed by the electron transport chain (ETC) complexes I through V. Mitochondria also serve as calcium buffers, regulators of apoptosis, and generators of reactive oxygen species (ROS). Mitochondrial ATP production occurs through the flow of electrons that are passed along ETC complexes in the inner mitochondrial membrane. Energy lost by protons...
reduced levels of nitric oxide, and increased DNA damage. 

Mitochondrial O$_2^-$ reacts with superoxide dismutase, which converts the O$_2^-$ into hydrogen peroxide (H$_2$O$_2$), which can, in the presence of ferrous iron (Fe$^{2+}$) via the Fenton reaction (H$_2$O$_2$ + Fe$^{2+}$ → Fe$^{3+}$ + OH$^-$ + OH$^-$), result in the production of highly reactive hydroxyl radicals (OH$^-$). Another relevant event is the reaction of O$_2^-$ with nitric oxide (NO$^-$) to form peroxynitrite (ONOO$^-$). When mitochondrial and cytoplasm enzymatic and nonenzymatic antioxidant systems are overwhelmed by elevated levels of ROS and reactive nitrogen species, oxidative damage can occur to DNA, lipids (cell and organelle membranes), and proteins (receptors, transcription factors, and enzymes). Oxidative damage to proteins may be caused by reactions of amino acid residues with (1) ROS, especially OH$, catalyzed by Fe$^{2+}$ and cupric (Cu$^{2+}$), which introduce carboxyl groups in lysine, proline, arginine, and threonine residues, or (2) ONOO$^-$, which nitrates sulphydryl and hydroxyl residues in cysteine, methionine, phenylalanine, and tyrosine; these modifications could inactivate the membrane signaling pathways and key enzymes.

Several lines of evidence suggest that mitochondrial dysfunction has a role in the pathogenesis of BD because these individuals have been noted, for example, to have altered cerebral energy metabolism and an increased ratio of mitochondrial DNA deletion. Recent DNA microarray analyses in postmortem prefrontal cortex and hippocampus revealed that the expression of many messenger RNAs (mRNAs) coding for ETC complexes I to V subunits was decreased in patients with BD. In addition, evidence from at least some genotyping studies suggest that polymorphisms of the complex I subunit NDUFV2 may be associated with BD. Several studies have also suggested altered activity and expression of mitochondrial ETC components in postmortem brain samples from patients with schizophrenia. Furthermore, mitochondrial ATP production measured in a muscle biopsy specimen was reported to be decreased in patients with major depressive disorder (MDD). Complex I is one of the main sites in which electrons are released and react with oxygen, resulting in ROS production, thus causing oxidative stress. Indeed, recent studies have demonstrated alterations in a diverse set of oxidative stress parameters in patients with BD. For example, studies conducted with peripheral blood cells have demonstrated that BD is associated with alterations in antioxidant enzymes, increased lipid peroxidation, increased levels of nitric oxide, and increased DNA fragmentation. Moreover, we reported increased levels of lipid peroxidation in the cingulate cortex of patients with BD.

Sun et al. using high-density complementary DNA spot microarrays, reported downregulation of 8 mitochondrial ETC-related genes: NDUFS7 and NDUFS8 (complex I), UQRC2 (complex III), COX5A and COX6C (complex IV), and ATP5C1, ATP5J, and ATP5G3 (complex V). Using real-time quantitative polymerase chain reaction, we further verified that mRNA levels of NDUFS7 were decreased. Because NDUFS7 levels were decreased and may contribute to decreased complex I activity, in the present study we examined NDUFS7 protein levels and complex I activity as an indication of mitochondrial complex I impairment. Complex I is a major source of ROS production, which can cause oxidative damage to proteins. We, therefore, also analyzed protein oxidation (carbonyl content) and tyrosine nitration (3-nitrotyrosine levels) as markers of oxidative damage to mitochondrial proteins to improve understanding of the pathogenesis of BD.

**METHODS**

Prefrontal cortex tissue samples were from Brodmann area 10 (1.0-g blocks). Participants were divided into 4 groups: BD, MDD, schizophrenia, and nonpsychiatric control subjects (n=15 per group) matched for age, sex, and postmortem interval (PMI). Diagnoses were retrospectively established by 2 senior psychiatrists using DSM-IV criteria. Detailed clinical information, diagnostic procedures, and demographic information on these individuals have been previously published. The investigators were blinded to the group identity, diagnosis, and demographic variables of the participants during all experiments and measurements. Samples were randomly coded numerically, and the code was lifted only during data analyses, after all of the experiments were completed.

**MITOCHOENDIAL EXTRACTION**

Mitochondria-enriched extracts were prepared as described by Smith, with minor modifications. Briefly, prefrontal cortex tissues were homogenized in buffer 1 (0.25M sucrose, 2mM EDTA, and 10mM Tris hydrochloride; pH 7.2) at a ratio of 20 µL/mg of tissue. Homogenized samples were centrifuged at 5600g for 3 minutes). The supernatant was kept on ice, and the pellet was resuspended in buffer 1, homogenized, and recentrifuged (at 5600g for 3 minutes). The combined supernatants were centrifuged at 37 500g for 20 minutes). Mitochondria-enriched pellets were dissolved in buffer 2 (2M aminoacaproic acid, 150mM Bis-Tris hydrochloride, and 300mM EDTA; pH 7.0) at a ratio of 4 µL/mg of tissue. These homogenates were centrifuged (at 12 000g for 15 minutes). The supernatants were collected and used for complex I enzymatic assay and immunoblotting analysis. Protein concentrations in the supernatants were determined using the Bradford assay. To verify that this method is reliable with frozen samples, we compared the protein concentrations and the complex I activity in fresh and frozen rat brain. The cerebral cortex was removed and submersed in ice-cold calcium (Ca$^{2+}$) and magnesium (Mg$^{2+}$)-free Hanks balanced saline solution. Mean (SD) protein concentrations in fresh (2.15 [0.30] µg/µL) and frozen (1.91 [0.21] µg/µL) mitochondrial fractions were not significantly different (t=1.99; P=.13). No significant difference was found in...
Complex I activity was performed as described by Estornell et al. Briefly, mitochondria-enriched pellets were diluted to 10 to 15 µg/mL in the assay buffer (50mM potassium chloride, 10mM Tris hydrochloride, 1mM EDTA, and 2mM potassium cyanide; pH 7.4). The NADH, 75µM, was added. The reaction was started by the addition of 50µM coenzyme Q1 and was read at 340 nm against the blank containing all the components except the coenzyme Q1 for 5 minutes. The rate after inhibition was determined with the same reagents plus the inhibitor rotenone for 5 minutes. Complex I activity was calculated by subtracting the rate after the addition of rotenone (10µM) from the overall rate.

Table. Demographic and Clinical Data for Postmortem Brain Tissue in Patients and Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>BD</th>
<th>MDD</th>
<th>Schizophrenia</th>
<th>F</th>
<th>P Value</th>
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<tr>
<td>Age, mean (SEM) [range], y</td>
<td>48 (2.7) [29-68]</td>
<td>42 (2.9) [25-61]</td>
<td>47 (2.3) [30-65]</td>
<td>45 (3.3) [25-62]</td>
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<td>.54</td>
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<td>9/6</td>
<td>9/6</td>
<td>9/6</td>
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<td>NA</td>
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<tr>
<td>PMI, mean (SEM) [range], h</td>
<td>23.7 (2.4) [8-42]</td>
<td>32.5 (4.02) [13-62]</td>
<td>27.5 (2.68) [7-47]</td>
<td>33.7 (3.65) [12-61]</td>
<td>1.857</td>
<td>.15</td>
</tr>
<tr>
<td>pH, mean (SEM) [range]</td>
<td>6.3 (0.1) [5.8-6.6]</td>
<td>6.2 (0.1) [5.8-6.5]</td>
<td>6.2 (0.1) [5.8-6.5]</td>
<td>6.2 (0.1) [5.8-6.6]</td>
<td>0.611</td>
<td>.61</td>
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<td>Cardiopulmonary</td>
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<td>5</td>
<td>7</td>
<td>8</td>
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<tr>
<td></td>
<td>Accident</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
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<tr>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
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<td>0</td>
<td>12</td>
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**PROTEIN OXIDATION**

Protein oxidation was assessed by measuring the levels of carbonyl groups using the OxyBlot Protein Oxidation Detection Kit (catalog No. ST750; Chemicon, Kankakee, Illinois). The carbonyl groups in the protein side chains are derivatized from 2,4-dinitrophenylhydrazine by reaction with 2,4-dinitrophenylhydrazine. The 2,4-dinitrophenylhydrazone–derivatized protein samples were separated by means of polyacrylamide gel electrophoresis and Western blotting following the OxyBlot kit instructions. Protein bands were analyzed densitometrically and were normalized against the intensity of porin using VersaDoc.

**TYROSINE NITRATION–INDUCED DAMAGE**

Tyrosine nitration–induced damage was assessed by measuring 3-nitrotyrosine levels using the Oxiselect Nitrotyrosine ELISA [enzyme-linked immunosorbent assay] Kit (catalog No. STA-305; Cell Biolabs Inc, San Diego, California). The unknown protein nitrotyrosine sample or nitrated bovine serum albumin standards were first added to a nitrated bovine serum albumin–preabsorbed enzyme immunoassay plate. After a brief incubation, the anti–nitrotyrosine antibody was added, followed by the horseradish peroxidase–conjugated secondary antibody. The protein nitrotyrosine content in the unknown samples was determined by comparison with a standard curve prepared using predetermined nitrated bovine serum albumin standards.

**COMPLEX I ACTIVITY**

mean (SD) complex I activity comparing fresh (2.37 [0.22] µmol/min) and frozen (2.15 [0.21] µmol/min) rat brain samples (t = 1.82; P = .16).

**NDUFS7 LEVELS**

Protein levels of NDUFS7 were measured by means of immunoblotting. Thirty micrograms of mitochondrial extract was loaded on 12% acrylamide sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel and were subsequently transferred onto polyvinylidene difluoride membranes. Membranes were blocked with 0.05% polysorbate 20 in 0.01M phosphate-buffered solution containing 5% nonfat milk (PBS-T) for 2 hours at room temperature. The blots were independently probed using either mouse anti–human NDUFS7 antibody (Novus Biologicals, Littleton, Colorado), 1:1000, or antiporin antibody (Abcam Inc, Cambridge, Massachusetts), 1:3000, as mitochondria-loading control in PBS-T at 4°C overnight with gentle shaking. Membranes were washed with PBS-T 4 times (10 minutes each time) before applying electrochemiluminescence reagents (GE Co, Piscataway, New Jersey). Protein bands were probed using either mouse anti–human NDUFS7 antibody (Novus Biologicals, Littleton, Colorado), 1:1000, or antiporin antibody (Abcam Inc, Cambridge, Massachusetts), 1:3000, as mitochondria-loading control in PBS-T at 4°C overnight with gentle shaking. Membranes were washed with PBS-T 4 times (10 minutes each time) before applying electrochemiluminescence reagents (GE Co, Piscataway, New Jersey). Protein bands were analyzed densitometrically and were normalized against the intensity of porin using VersaDoc.

**STATISTICAL METHODS**

Statistical analyses were performed using a computer software program (SPSS for Windows version 16.0; SPSS Inc, Chicago, Illinois) software. Normal distribution of data was determined using the Kolmogorov-Smirnov test. For further analysis, parametric tests were used because most of the data (90%) had a normal distribution. Data were analyzed by means of 2-way analysis of variance, followed by the least significant difference post hoc test. Age, sex, PMI, and brain pH were added as covariates, and persistence of the significant difference in main effect between diagnostic groups was assessed by means of analysis of covariance. Correlations were analyzed using the Pearson correlation test. Data are given as mean (SD).
RESULTS

DEMOGRAPHIC VARIABLES

Demographic and clinical characteristics of patients and controls are given in the Table. Controls (n=15) were matched with patients with BD (n=15), MDD (n=15), and schizophrenia (n=15) for age and sex; therefore, as expected, no significant differences were noted among groups on these measures. The PMI ($F = 1.857, P = .15$) and pH ($F = 0.611, P = .61$) were not significantly different among groups. The PMI was not correlated with NDUFS7 expression ($r^2 = 0.021, P = .10$), complex I activity ($r^2 = 0.016, P = .11$), carbonyl levels ($r^2 = 0.001, P = .22$), or 3-nitrotyrosine levels ($r^2 = 0.005, P = .33$). The pH did not correlate with complex I activity ($r^2 = 0.023, P = .72$), carbonyl levels ($r^2 = 0.008, P = .29$), or 3-nitrotyrosine levels ($r^2 = 0.022, P = .11$) but correlated positively with NDUFS7 levels ($r^2 = 0.105, P = .01$) (Figure 1).

To assess whether patients who committed suicide had more mitochondrial dysfunction or protein damage, we divided the patients into 2 subgroups: death by suicide (n=20) and death by other causes (n=25). No significant difference between these groups was observed in complex I activity, NDUFS7 expression, protein carbonylation, or 3-nitrotyrosine levels.

MITOCHONDRIAL COMPLEX I DYSFUNCTION AND PROTEIN DAMAGE

We found that NDUFS7 levels were significantly different comparing the 4 groups. The group difference in NDUFS7 levels ($F_{3,56} = 5.691, P = .002$) was due to a significant decrease in NDUFS7 levels in the BD group (62.38%, $P = .003$) compared with controls (Figure 2A). Expression of NDUFS7 was not different in patients with MDD ($P = .16$) or in those with schizophrenia ($P = .41$) compared with controls. To control for potential confounding variables, age, sex, PMI, and brain pH were added as covariates and were assessed by means of analysis of covariance. The differences between the groups remained significant even after these variables were added to the analysis ($F_{3} = 4.798, P = .01$). Adding pH to the analysis had a significant effect ($F_{3} = 6.271, P = .02$) but did not alter the significant decrease in NDUFS7 levels.

Complex I activity was markedly decreased (53.12%) in patients with BD ($F_{3,56} = 12.85, P < .001$) and was decreased to a lesser extent in those with MDD ($P = .16$) or in those with schizophrenia ($P = .41$) compared with controls. To control for potential confounding variables, age, sex, PMI, and brain pH were added as covariates and were assessed by means of analysis of covariance. The differences between the groups remained significant even after these variables were added to the analysis ($F_{3} = 4.798, P = .01$). Adding pH to the analysis had a significant effect ($F_{3} = 6.271, P = .02$) but did not alter the significant decrease in NDUFS7 levels.
To assess oxidative and nitrosative damage to mitochondrial proteins, we analyzed protein carbonylation and 3-nitrotyrosine levels in the postmortem samples. There were significant differences between groups in carbonylation levels ($F_{3,36}=3.01, P=.04$) (Figure 3A) and nitration levels ($F_{3,36}=4.56, P=.007$) (Figure 3B). Patients with BD had increased carbonyl content ($P=.01$) and 3-nitrotyrosine levels ($P=.001$) compared with controls. 3-Nitrotyrosine levels were also significantly increased in patients with schizophrenia compared with the control group ($P=.03$). Patients with MDD did not differ from the control and other patient groups on either of these protein oxidation markers. Analysis of covariance did not demonstrate any effect from the potential confounding factors.

Next, we analyzed the relationship between complex I activity and NDUFS7 levels, protein oxidation, and tyrosine nitration. As expected, complex I activity was positively correlated with NDUFS7 levels ($n=60; r^2=0.185, P=.001$). The present results show that complex I activity was correlated negatively with carbonyl levels ($n=57; r^2=0.299, P=.02$) and 3-nitrotyrosine levels ($n=57; r^2=0.113, P=.01$) (Figure 4).

**EFFECT OF MEDICATION**

Antipsychotic drugs have been reported to be potential inhibitors of complex I activity. To assess the potential effect of antipsychotics on the present results, we divided the patients into 2 subgroups: patients with schizophrenia or BD treated with antipsychotics (conventional and second-generation drugs) at the time of death ($n=20$ [12 with schizophrenia and 8 with BD]) (Table) and those who were not ($n=10$ [3 with schizophrenia and 7 with BD]). Because none of the patients in the other 2 groups were treated with this class of drugs at the time of death, they were excluded from the analysis. No significant difference was observed between the 2 subgroups in complex I activity, NDUFS7 levels, protein carbonyl content, or 3-nitrotyrosine levels. There was a nonsignificant trend for decreased mean (SD) complex I activity in patients treated with antipsychotics ($0.81 [0.34] \mu\text{mol/min}$) compared with those who had not been treated with antipsychotics ($1.08 [0.30] \mu\text{mol/min}$) ($t=-0.456, P=.08$). Next, to assess the possible effects of antidepressant medications on the results, we divided the patients into 2 subgroups: those treated with antidepressants ($n=23$ [10 with MDD, 5 with schizophrenia, and 8 with BD]) and those not treated with antidepressants ($n=22$ [5 with MDD, 10 with schizophrenia, and 7 with BD]). No significant differences were observed between the groups for any of these measures. Finally, recent studies, including ours, have suggested that lithium carbonate has potential antioxidant capacity. We divided the patients into groups treated with lithium ($n=14$ [2 with MDD, 2 with schizophrenia, and 10 with BD]) and those who had not received lithium ($n=31$ [13 with MDD, 13 with schizophrenia, and 5 with BD]). No significant differences were observed between these 2 subgroups in complex I activity, NDUFS7 expression, protein carbonyl content, or 3-nitrotyrosine levels.

**COMMENT**

We report that NDUFS7 levels and complex I activity are decreased and levels of mitochondrial protein oxidation and tyrosine nitration are increased in postmortem prefrontal cortex of patients with BD compared with age and sex-matched nonpsychiatric controls. There were no differences in NDUFS7 levels in patients with either MDD or schizophrenia, but a small decrease in complex I activity was found in the MDD group compared with controls. Increased 3-nitrotyrosine levels were also found in patients with schizophrenia compared with the control group.
group. We found a negative correlation between complex I activity and protein carbonylation or 3-nitrotyrosine levels. These results, together with evidence from other neurologic diseases, provide evidence that decreased NDUFS7 levels contribute to complex I impairment, in this case, in BD. Decreased complex I activity is widely reported to increase superoxide production, and this free radical, in turn, induces oxidative and nitrosative damage to proteins. Nevertheless, multiple factors regulate complex I activity, including alterations in other complex I subunits, increased neurotoxin levels, glutathione depletion, decreased ATP production, and increased ONOO- levels. The present study identifies NDUFS7 as a factor that potentially contributes to complex I impairment. There is growing evidence that mitochondrial impairment, particularly in mitochondrial complex I, contributes to the pathogenesis of BD.

Microarray data have suggested that decreased expression of many mRNAs coding for complexes I through V subunits are associated with BD. More specifically, Iwamoto et al demonstrated decreased mRNA levels of the complex I subunit NDUFS1, complex III subunit UQCRCC2, and complex IV subunit COX15 in the prefrontal cortex of patients with BD. Sun et al showed that 8 genes coding for subunits of ETC complexes I, III, IV, and V were downregulated in postmortem prefrontal cortex of patients with BD: NDUFS7 and NDUFS8 (complex I), UQRC2 (complex III), COX5A and COX6C (complex IV), and ATP5C1, ATP5J, and ATP5G3 (complex V). To confirm these data, Sun et al evaluated mRNA levels of NDUFS7, UQRC2, COX6C, and ATP5G3 using real-time quantitative polymerase chain reaction and found decreased mRNA levels of NDUFS7 and COX6C. Mitochondrial dysfunction has also been reported in schizophrenia. Using transcriptomic, proteomic, and metabolomic analysis, Prabakaran et al showed that half of the altered protein expression in patients with schizophrenia was related to mitochondrial dysfunction and oxidative stress and that these were mirrored by transcriptional and metabolite perturbations. NDUFS1 was shown to be decreased in white and gray matter of the prefrontal cortex from patients with schizophrenia. In addition, decreased activity of complex IV was found in the caudate nucleus and frontal and temporal cortices, and succinate dehydrogenase (complex II) activity was increased in the putamen and nucleus accumbens of patients with schizophrenia. Complex I activity was also found to be reduced in the temporal cortex but not in the frontal cortex or caudate nucleus in patients with schizophrenia. More recently, Karry et al observed reduced expression of 2 catalytic subunits of mitochondrial complex I, 24 kDa (NDUFV2) and 51 kDa (NDUFV1), in the prefrontal cortex of patients with schizophrenia compared with controls. The present results did not find differences in NDUFS7 levels and complex I activity in the schizophrenia group. The differences in the results may be explained, in part, by the fact that Karry et al evaluated the RNA expression levels of subunits different than those analyzed herein and used brain tissue from Brodmann area 46/9, whereas the present samples were from Brodmann area 10.

The findings highlighted herein illustrate that downregulation of several complex I subunits occurs in BD, which may be associated with the susceptibility of BD to damage through oxidative stress. Human complex I is composed of 45 to 46 different subunits and is divided into 3 functional modules. First, the dehydrogenase module is responsible for the oxidation of NADH via flavin mononucleotide onto a chain of iron-sulfur clusters. Second, the hydrogenase module guides the released electrons to electron acceptors. Third, the transporter module is responsible for translocation of protons across the membrane. In BD, decreased expression of NDUFV2 and NDUFS1 in the dehydrogenase module and decreased expression of NDUFS7 and NDUFS8 in the hydrogenase module have been reported. These results suggest that patients with BD may have a reduced ability to oxidize NADH and to transfer electrons to ubiquinone. In that respect, electrons may persist for sufficient time to react with molecular oxygen and, thus, produce O2. In addition, Benes et al found that superoxide dismutase, catalase, several isoforms of glutathione peroxidase, and glutathione S-transferase, and other genes associated with antioxidant reactions were downregulated in the hippocampus of patients with BD but

Figure 4. Correlations among complex I activity and NDUFS7 levels (A), protein oxidation (carbonyl levels) (B), and tyrosine nitration-induced damage (3-nitrotyrosine) (C). Results were assessed using the Pearson correlation test. AU indicates arbitrary units.
not schizophrenia. Together, the organization of complex I, the downregulation of complex I subunits, and the decreased levels of the antioxidant system strongly support the susceptibility of mitochondrial proteins to oxidative damage in BD.12-18

In this study, patients with BD demonstrated increased levels of carbonylated proteins, which were shown to be negatively correlated with complex I activity. One possible mechanism through which impaired complex I activity could be associated with increased protein carbonylation in BD is the overproduction of OH·, by reaction of O₂⁻ with superoxide dismutase.15,16 OH· reacts with lysine, proline, arginine, and threonine residues of proteins by creating carbonyl groups.15 Carbonylation can alter protein function or can lead to deleterious intermolecular aggregates that preclude their degradation by the proteasomal system.15,16 Konradi et al14 demonstrated decreased expression of genes involved in the proteasome degradation process in prefrontal cortex from patients with BD, suggesting that in addition to potential mechanisms leading to increased protein carbonylation, the normal process of degradation may also be impaired, leading to further accumulation. Indeed, accumulation of carbonylated proteins has been implicated in the etiology or progression of several chronic central nervous system disorders, including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and multiple sclerosis.35,36 Therefore, future studies using redox proteomic techniques will be critical to identify whether specific mitochondrial proteins are targets of protein oxidation and, if so, to define the relationship between oxidative protein modification (ie, carbonylation) and cellular function in BD.

These results also demonstrate increased levels of 3-nitrotyrosine in the prefrontal cortex of patients with BD and those with schizophrenia compared with nonschizophrenic controls. 3-Nitrotyrosine is a posttranslational modification in protein tyrosine residue nitrated by ONOO⁻ (peroxynitrite).18,46 In the brain, NO⁻ is produced by microglia and astrocytes and is subsequently transported to neurons, where it may react with superoxide to yield ONOO⁻.19 Neuronal nitric oxide synthase 1, the enzyme that generates NO⁻, was found to be upregulated in the hippocampus of patients with BD.20 In addition to increased serum levels of NO⁻ in patients with the same disorder.35-37 Our group35 previously reported increased serum levels of 3-nitrotyrosine in patients with BD early (0-3 years) and late (10-20 years) in the course of the illness. In addition, Murray et al19 showed that ONOO⁻ reactions with mitochondrial membranes from bovine heart occur predominantly in complex I subunits, resulting in significant inhibition of complex I activity, suggesting a functional relation between complex I activity and nitrination. This is further supported by the report of Naou et al18 of increased 3-nitrotyrosine levels in the mitochondrial complex I subunits but not in other mitochondrial proteins of SH-SY5Y cells incubated with ONOO⁻. These findings, along with those of the present study, may suggest that mitochondrial complex I proteins are susceptible to the nitrination process and that this modification contributes importantly to the mitochondrial dysfunction observed in BD.

These results must be interpreted in light of the limitations of the samples and methods. First, some of these reported changes could be related to lower brain pH, which is commonly associated with antemortem agonal states, postmortem delay, and storage of tissue.58 We suggest that such changes in pH may, indeed, be related to the diagnosis and treatment of BD, as suggested by Kato et al19 and Hamakawa et al.60 We did not find differences in pH or PMI between groups (Table). However, pH correlated positively with NDUF5L levels but not with complex I activity, 3-nitrotyrosine levels, and carbonyl levels. The covariates pH, PMI, age, and sex did not contribute significantly to the main findings, as demonstrated by the subsequent data analysis. Second, drug treatment is another important consideration; however, we did not find significant effects of treatment with lithium, antidepressants, or antipsychotics on any of these measures. Third, considering that we studied postmortem brain, the results also might be limited by a small sample and analysis of a single region, the prefrontal cortex. Results of a recent study60 also indicate oxidative stress in the anterior cingulate cortex of patients with BD. The authors acknowledge the efforts of many groups to collect postmortem brain samples from larger groups of patients with better control over these important variables as a future strategy to control for these limitations. Therefore, additional brain regions require further investigation to understand the specificity of oxidative stress in psychiatric diseases.

In conclusion, these results provide evidence of the involvement of mitochondrial complex I dysfunction and consequent oxidative damage to proteins in BD but not in schizophrenia. Accumulation of oxidative damage to mitochondrial proteins is thought to lead to neuronal cell death by apoptosis or as a consequence of aggregation of oxidized protein that may result in neurodegeneration.86 Complex I deficiency may sensitize neurons to mitochondria-dependent apoptosis in response to the proapoptotic protein Bax, which releases the soluble pool of cytochrome c in the mitochondrial intermembrane space, activating the programmed cell death by caspase 3 and 9.61,62 In addition, Benes et al33 showed that several apoptotic genes, including FAS, BAK, APAF-1, NFκB56, TRAF1, BID, c-Myc, c-jun, and MDM2, are upregulated in the hippocampus of individuals with BD but not in patients with schizophrenia, suggesting an important role of the apoptotic process in BD. Future studies are needed to identify which specific mitochondrial proteins are targets of carbonylation and nitrination in patients with BD and possibly to identify new targets of neuroprotective strategies and to help elucidate a better understanding of the pathogenesis of BD.

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**Correction**

**Figure Parts Mislabeled.** In the article titled “Mitochondrial Complex I Activity and Oxidative Damage to Mitochondrial Proteins in the Prefrontal Cortex of Patients With Bipolar Disorder,” by Andreazza et al, published in the April issue of the Archives (2010;67[4]:360-368), the first (A) and second (B) graphs of Figure 4 on page 365 should be switched. The legend is correct.