

Molecular Evidence for Mitochondrial Dysfunction in Bipolar Disorder

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Background: The disease mechanism of bipolar disorder remains unknown. Recent studies have provided evidence for abnormal gene expression in bipolar disorder.

Objective: To determine the expression of 12558 nuclear genes in the human hippocampus in healthy control subjects and those with bipolar disorder or schizophrenia.

Design: We used gene arrays to study messenger RNA expression. Data were verified with a real-time quantitative polymerase chain reaction assay.

Subjects: We studied 10 healthy control subjects, 9 subjects with bipolar disorder, and 8 subjects with schizophrenia.

Results: The expression of nuclear messenger RNA coding for mitochondrial proteins was significantly decreased in the hippocampus in subjects with bipolar disorder but not in those with schizophrenia. Subjects with bipolar disorder were characterized by a pronounced and extensive decrease in the expression of genes regulating oxidative phosphorylation and the adenosine triphosphate-dependent process of proteasome degradation.

Conclusions: These findings point toward a widespread dysregulation of mitochondrial energy metabolism and downstream deficits of adenosine triphosphate-dependent processes in bipolar disorder.

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BIPOLAR DISORDER AFFECTS approximately 0.5% of the world population, often leading to recurrent illness and a marked decline in social function.¹ The clinical features of bipolar disorder (ie, recurrent episodes of depression and either full-blown mania with frank psychosis or milder bouts of hypomania) have long been recognized.² However, the etiologic and disease mechanisms remain unknown. For example, bipolar disorder shows a high degree of heritability (approximately 0.8%), and several studies have reported linkage of bipolar disorder to chromosomal loci, but not a single locus has repeatedly been linked to bipolar disorder.³

Recent spectroscopic studies have provided evidence for bipolar disorder as a disease of mitochondrial energy metabolism,⁴ including decreased pH⁵ and decreased high-energy phosphates^{6,7} in the frontal and temporal lobes of these patients. Such mitochondrial dysfunction in bipolar disorder could be due to an abnormal expression of nuclear or mito-

chondrial genes coding for mitochondrial proteins.⁸ In this article, we report that the expression of nuclear messenger RNA (mRNA) coding for mitochondrial proteins is significantly decreased in the hippocampus in bipolar disorder but not in schizophrenia.

METHODS

SUBJECTS

We analyzed the expression of 12558 nuclear genes in 3 study groups: healthy controls, subjects with bipolar disorder, and subjects with schizophrenia. Brain specimens were obtained from the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, Mass) and initially consisted of 10 subjects in each diagnostic group. Each control subject was matched with 1 subject who had schizophrenia and 1 who had bipolar disorder for age and postmortem interval to ensure homogeneity of the groups. One subject with bipolar disorder and 1 subject with schizophrenia were excluded from the study because they did not provide sufficient RNA quality, as assessed by the 3'/5' ratio of glyceraldehyde-3-phosphate dehydrogenase (>4), 3'/5' ratio of β -actin (>4),

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and percentage (<37%) of “gene-present calls” (the percentage of genes on the array that were above the detection limit in a sample).

All diagnoses were established by 2 psychiatrists at the Harvard Brain Tissue Resource Center via retrospective review of all available medical records and extensive questionnaires about social and medical history completed by family members of the donors. We applied the criteria of Feighner et al⁹ for the diagnosis of schizophrenia and that of the *DSM-III*¹⁰ for the diagnosis of schizoaffective disorder and bipolar disorder. Probands with a documented history of substance dependence or neurological illness were excluded from the study. During our study it became evident that the documentation of 1 subject with schizophrenia was not sufficient to verify the diagnosis, and that case had to be excluded.

One hemisphere of each brain underwent a comprehensive neuropathologic examination, which revealed no evidence of stroke, tumor, infection, or neurodegenerative changes. After exclusion of 3 cases (insufficient RNA quality in 2 cases and insufficient documentation of the psychiatric history in 1 case), the final sample sizes were 10 control subjects, 9 subjects with bipolar disorder, and 8 subjects with schizophrenia (**Table 1**).

TISSUE PREPARATION AND RNA EXTRACTION

All brains were transported on wet ice and dissected immediately on arrival by specially trained staff at the Harvard Brain Tissue Resource Center using a standard protocol (see <http://www.brainbank.mclean.org/> for details). A coronal block of the hippocampus was obtained at the level of the lateral geniculate nucleus, frozen in liquid nitrogen vapor, and stored at -80°C . Mean \pm SD storage time was 31 ± 14 months with no significant difference between groups. Blocks were trimmed to include only the dentate gyrus and cornu ammonis sectors 1 through 4 without adjacent white matter of the parahippocampal gyrus. Twenty-five slices (10 μm thick) were cut from each hippocampal block in a cryostat and used for RNA extraction.

Human hippocampal RNA was prepared according to the protocol provided by Affymetrix (Santa Clara, Calif). The RNA was extracted from 50 to 100 mg of tissue with an extraction kit (RNAagents kit; Promega, Madison, Wis). The total yield of RNA was the same in all 3 groups. The RNA quality was assessed using an analytical gel and a bioanalyzer (Agilent Technologies, Palo Alto, Calif). We used 8 μg of total RNA for complementary DNA synthesis with a double-stranded complementary DNA synthesis kit (SuperScript; Invitrogen Corp, Carlsbad, Calif), and in vitro transcription was performed with an RNA transcript labeling kit (Enzo IVT kit; Enzo Biochem, Farmingdale, NY). Both the schizophrenia group and the bipolar disorder group had a 20% lower yield of biotinylated RNA. Whereas the difference was not significant in the bipolar disorder group, it reached significance in the schizophrenia group. Biotinylated RNA was fragmented and hybridized to the HG-U95Av2 array (Affymetrix) overnight at 45°C and stained on the washing station with streptavidin-phycoerythrin (Molecular Probes, Eugene, Ore) followed with a biotinylated antistreptavidin antibody (Vector Laboratories, Burlingame, Calif) and a second round of streptavidin-phycoerythrin.

Tissue preparation and RNA extraction were performed in a single batch by the same investigator to limit experimental variability. The order of samples was randomized, investigators were blinded to diagnoses, and the sample code was broken before the arrays were loaded onto the washing station to enable the investigator to randomize samples on the washing station modules.

Samples were analyzed in diagnostic groups using the dChip program (<http://www.dchip.org>).¹¹ Model-based expression was performed on perfect match-only data. A control sample with average intensity was chosen for normalization. We found no significant differences in the quality control criteria provided by the Data Mining Tool (Affymetrix) and dChip analyses (3'/5' ratios for glyceraldehyde-3-phosphate dehydrogenase and β -actin as well as scaling factor and background) or in the ratio of 28S/18S ribosomal RNA obtained with the bioanalyzer. A significant difference was found in gene-present calls, which were lower ($P = .04$) in the bipolar disorder group (Table 1).

We explored expression profiles revealed by the dChip analysis further with the GenMAPP and MAPPfinder (<http://www.genmapp.org>) programs. GenMAPP was used to draw maps of genes in functionally related groups.¹² The MAPPfinder program was used to find regulation trends in groups of genes organized according to biological process, molecular function, or cellular component, as defined by the Gene Ontology Consortium (<http://www.geneontology.org>). The following criteria were chosen for the MAPPfinder analysis: $P < .02$, with gene-present calls in more than 50% of the samples and at a fold induction higher than 1.1. For result verification, data were also computed with Affymetrix Data Mining Tool software version 3.0.

REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION

For real-time quantitative polymerase chain reaction (PCR), complementary DNA was synthesized from 1 μg of total RNA with a synthesis system (SuperScript First-Strand Synthesis System for real-time quantitative PCR; Invitrogen Corp) using oligonucleotide deoxythymidine as the primer. A primer set for each gene was designed with the help of Primer3 software (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>). Amplicons were designed to be between 100 and 150 base pairs in length. Melt curve analysis and polyacrylamide gel electrophoresis were used to confirm the specificity of each primer pair. The real-time quantitative PCR reaction was performed in accordance with described procedures¹³ (DNA Engine Opticon; Opticon Monitor Data Analysis Software version 1.4; MJ Research, Waltham, Mass) with a PCR kit (DyNAmo SYBR Green real-time quantitative PCR kit; Finnzymes, Espoo, Finland) in a volume of 25 μL , with 2.5 μL of 1:5 diluted complementary DNA samples and 0.3- μM primers. The PCR cycling conditions were initially 95°C for 10 minutes followed by 49 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. Data were collected between 72°C and 79°C depending on amplicon melting temperature. A melt curve analysis was performed at the end of each real-time quantitative PCR experiment. Dilution curves were generated for each primer in every experiment by diluting complementary DNA twice from a control sample with a ratio of 1:3, yielding a dilution series of 1.00, 0.33, and 0.11. The logarithm of the dilution value was plotted against the cycle threshold value. Blanks were run with each dilution curve to control for cross-contamination. Dilution curves, blanks, and samples were run in duplicate. Reported values were normalized to the internal control human filamin A α (accession number NM_001456), an actin-binding protein. Human filamin A α was not regulated in the gene array or quantitative PCR analysis. Seven control samples and 6 bipolar disorder samples available from the original group were used for real-time quantitative PCR.

The identical real-time quantitative PCR parameters were used for an analysis of 16 frontal lobe specimens (8 control subjects and 8 with bipolar disorder from the original study sample). Cortical tissue was removed from Brodmann area 9, and RNA was extracted as detailed previously.

Table 1. Demographics and Microarray Quality Control Data

Case No./ Sex/Age, y	Diagnosis	Hemi- sphere	Postmortem Interval, d	3'/5' GAPDH	3'/5' β-Actin	Present Calls, %	Scaling Factor	Back-ground Level	28S/18S	Cause of Death	Chlorpromazine Equivalents, mg	Psychoactive Medication Use
B3806/F/70	NA (Control)	R	15	2.2	3.2	45.1	2.27	49	1	Cardiac arrest	NA	NA
B3898/F/78	NA (Control)	R	14.1	3.1	3.9	45	2.16	50	0.85	Myocardial infarction	NA	NA
B4605/M/29	NA (Control)	L	18.2	2	2.5	43.4	2.5	51	1.44	Renal failure	NA	NA
B4737/F/74	NA (Control)	L	12.5	3.4	3.6	37.3	3.82	49	0.34	Renal failure	NA	NA
B4751/M/54	NA (Control)	L	24.2	2.3	2.7	47.1	1.85	56	1.44	Cardiac arrest	NA	NA
B4810/F/62	NA (Control)	L	16.4	1.6	2.1	45.9	2.39	46	1.11	Lung cancer	NA	NA
B4853/F/70	NA (Control)	L	22.5	2	2.6	43.3	3.1	45	1.03	Liver cancer	NA	NA
B4932/M/67	NA (Control)	R	22.3	1.5	2	47.4	1.74	47	1.3	Cardiac arrest	NA	NA
B5074/M/79	NA (Control)	R	20.9	1.8	2.6	48.3	2	46	0.92	Pancreatic cancer	NA	NA
B5082/F/78	NA (Control)	R	23.9	1.9	2.1	45.4	2.18	65	1.25	Breast cancer	NA	NA
B3817/F/64	Bipolar disorder	R	11	2.11	2.53	46.3	2.25	46	0.56	Respiratory failure	800	Trifluoperazine
B4069/F/80	Bipolar disorder	L	11.6	2	2.6	44.9	1.98	49	1.21	Cerebrovascular accident	67	Perphenazine, benzotropine, valproate
B4403/F/76	Bipolar disorder	L	22.8	2.46	3	41.3	3.49	46	0.54	Cardiopulmonary failure	0	Lithium carbonate
B4462/M/50	Bipolar disorder	R	30.5	3.4	3.8	42	2.7	50	0.77	Cardiac arrest	NA	?
B4464/M/74	Bipolar disorder	L	24.8	2.83	3.3	39.1	5.61	39	0.67	Pneumonia	285	Divalproex sodium, quetiapine
B4661/M/25	Bipolar disorder	L	12.6	2.6	2.7	39	3.01	52	1	Pulmonary edema	0	Sertraline, trazodone, gabapentin, lithium
B4914/F/73	Bipolar disorder	R	20.8	2.2	2.5	42.9	2.85	48	1.03	Sepsis	33	Risperidone, carbamazepine
B4961/M/74	Bipolar disorder	R	14.3	2.6	3.3	42.2	2.55	44	1.04	Pneumonia	0	Lithium, divalproex sodium
B5044/F/73	Bipolar disorder	R	17	2.7	3.5	38.6	3.43	52	0.33	Renal failure	133	Lithium divalproex sodium, risperidone
B4190/F/78	Schizoaffective disorder	L	13.4	2.7	3.6	44.1	2.02	46	1.33	Sinus node disease	1066	Lithium, haloperidol
B4238/M/26	Schizophrenia	R	16	1.6	1.6	47.4	1.36	51	1.23	Suicide by hanging	357	Prolixin decanoate
B4469/M/80	Schizophrenia	L	11	2.5	3.4	47.1	1.96	49	1.44	Cardiopulmonary failure	10	Thioridazine
B4875/F/55	Schizoaffective disorder	R	18	1.7	1.7	47.7	2.22	46	1.17	Cancer	200	Divalproex sodium, intramuscular fluphenazine
B4907/F/73	Schizoaffective disorder	R	24	3	2.8	38	3.82	47	0.67	Lung cancer	600	Prolixin
B5047/M/63	Schizophrenia	R	22.3	2.36	3.84	40	3.12	52	0.91	Cardiac arrest	532	Clozapine, clonazepam
B5100/F/72	Schizophrenia	R	21.7	1.5	2	47.4	2.1	49	1.09	Cancer	267	Risperidone, benzotropine, paroxetine
B5115/M/49	Schizophrenia	L	24.5	1.5	1.6	47.1	1.8	48	1.22	Acute respiratory failure	1066	Haloperidol decanoate
Overall												
Control/6F/4M/66.1		5L/5R	19.0	2.2	2.7	44.8	2.4	50.4	1.1	NA	NA	NA
Bipolar disorder/5F/4M/65.4		4L/5R	18.4	2.5	3.0	41.8*	3.1	47.3	0.8	NA	165	NA
Schizophrenia/4F/4M/62.0		3L/5R	18.9	2.1	2.6	44.9	2.3	48.5	1.1	NA	512	NA

Abbreviations: 3'/5' GAPDH, 3'/5' ratio of glyceraldehyde-3-phosphate dehydrogenase; 3'/5' β-actin, 3'/5' ratio of β-actin; 28S/18S, 28S/18S ratio; L, left; NA, not applicable; R, right.

*P = .04.

Table 2. Decreased Gene Expression in Bipolar Disorder

Gene	GenBank Accession No.	Map Location	Fold	P Value	Present Calls, %
Mitochondrial					
1. ATP synthase, mitochondrial F0 complex, subunit c, isoform 3	U09813	2q31.1	-1.63	.001	100
2. VDAC1 pseudogene, porin protein, isoform 1	AJ002428	X	-1.41	.001	94
3. Ubiquinone-binding protein	D50369	5q31.1	-1.37	.001	100
4. ATP synthase, mitochondrial F0 complex, subunit d	AF087135	17q25	-1.67	.001	100
5. Mitochondrial ribosomal protein L3	X06323	3q21-q23	-1.46	.001	100
6. Cytochrome-c oxidase, subunit VIIb	Z14244	Xq13.2	-1.58	.001	100
7. ATP synthase, mitochondrial F0 complex, subunit f, isoform 2	AF047436	7q11.21	-1.48	.002	100
8. Dynamin 1-like	AF000430	12p12.1	-1.66	.002	68
9. Voltage-dependent anion channel 2; porin	L08666	10q22	-1.4	.002	100
10. Cytochrome c oxidase, subunit VIIa, polypeptide 2 (liver)	X15822	6q12	-1.42	.002	100
11. ATP synthase, mitochondrial F1 complex, O subunit	X83218	21q22.11	-1.53	.002	100
12. Voltage-dependent anion channel 1; porin	L06132	5q31	-1.49	.003	100
13. Single-stranded DNA-binding protein	M94556	7q34	-1.44	.003	94
14. Fumarate hydratase	U59309	1q42.1	-1.47	.004	100
15. Solute carrier family 25, member 4	J02966	4q35	-1.53	.004	100
16. ATP synthase, mitochondrial F1 complex, γ polypeptide 1	D16562	10q22-q23	-1.46	.004	100
17. NADH dehydrogenase (ubiquinone) 1, α/β subcomplex, 1, 8kd	AC002400	16p11.2	-1.45	.005	100
18. 3-Oxoacid CoA transferase	U62961	5p13	-1.62	.009	100
Energy metabolism					
19. UDP-glucose pyrophosphorylase 2	U27460	2p14-p13	-1.44	.002	100
20. ATPase, lysosomal 70kd, V1 subunit A, isoform 1	L09235	3q13.31	-1.54	.004	89
21. ATPase, lysosomal 34kd, V1 subunit D	BC031002	14	-1.47	.006	100
Protein degradation					
22. Sec61 4	AF054184	7p14.1	-1.39	.001	100
23. Proteasome (prosome, macropain) 26S subunit, ATPase 6	D78275	14q22.1	-1.49	.002	100
24. Protein-L-isoaspartate (D-aspartate) O-methyltransferase	D25547	6q24-q25	-1.75	.006	100
25. F-box only protein 9*	AL031178	6p12.3-p11.2	-1.68	.008	100
Neurotransmission					
26. Somatostatin	J00306	3q28	-2.78	.006	84
27. Glutamic acid decarboxylase 67	M81883	2q31	-1.8	.009	100
Structural proteins					
28. Actin-related protein 2/3 complex, subunit 3, 21kd	AF006086	12q24	-1.49	.001	100
29. β -Tubulin, β -2	X02344	Not placed	-1.47	.002	100
30. Actin-related protein 2 homolog (yeast)	AF006082	2p14	-1.5	.002	100
Other					
31. Macrophage migration inhibitory factor	L19686	22q11	-1.35	.001	100
32. ρ Guanine nucleotide exchange factor 4	AB029035	2q22	-1.39	.001	100
33. FSHD region gene 1	L76159	4q35	-1.42	.001	100
34. Eukaryotic translation initiation factor 3, subunit 11	AB019392	19q13.2	-1.53	.002	100
35. Ataxin-10 (spinocerebellar ataxia type 10 protein)	AL050282	22q13.31	-1.67	.003	100
36. UDP-GlcNAc: β -1, 3-N-acetylglucosaminyltransferase 6	AF029893	11q12.1	-1.5	.004	100
37. Contactin 1; glycoprotein gp135	Z1488	12q11-q12	-1.77	.005	63
38. Endosulfine α	X99906	1q21.1	-1.5	.005	100
39. Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	M86400	8q23.1	-1.54	.007	100
40. Chromosome 1 open reading frame 15; KIAA0479 protein	AB007948	1q25	-1.67	.007	94
41. Arg protein tyrosine kinase-binding protein	X95677	2q33	-1.51	.008	73
42. Fk506-binding protein, alternative splice 2	HG1139-HT4910†	Not placed	-1.47	.008	84
43. Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	M37400	10q24.1-q25.1	-1.61	.008	100

Abbreviations: Arg, arginine; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; CoA, coenzyme A; FSHD, facioscapulohumeral muscular dystrophy; NADH, nicotinamide adenine dinucleotide; UDP, uridine 5'-diphosphate; VDAC, voltage-dependent anion channel.

*Encodes a member of the F-box protein family.

†Indicates TIGR sequence number.

RESULTS

GENE ARRAY RESULTS

We initially limited our analysis to genes expressed in at least 60% of all cases, with at least a 1.2-fold differential expression at a 90% confidence limit and significance level of $P < .01$. These statistical thresholds were exceptionally stringent (false discovery rate, 2.9%) and were not

met by a single gene in the schizophrenia group. In contrast, the expression of 43 genes was decreased in bipolar disorder (**Table 2**). Using more liberal statistical thresholds, we found evidence for increased and decreased gene expression in both groups. However, for the purpose of this article we will focus on these 43 genes, in which we discovered a striking pattern: 18 genes (42%) coded for mitochondrial proteins. These included subunits of complexes I (nicotinamide adenine dinucleo-

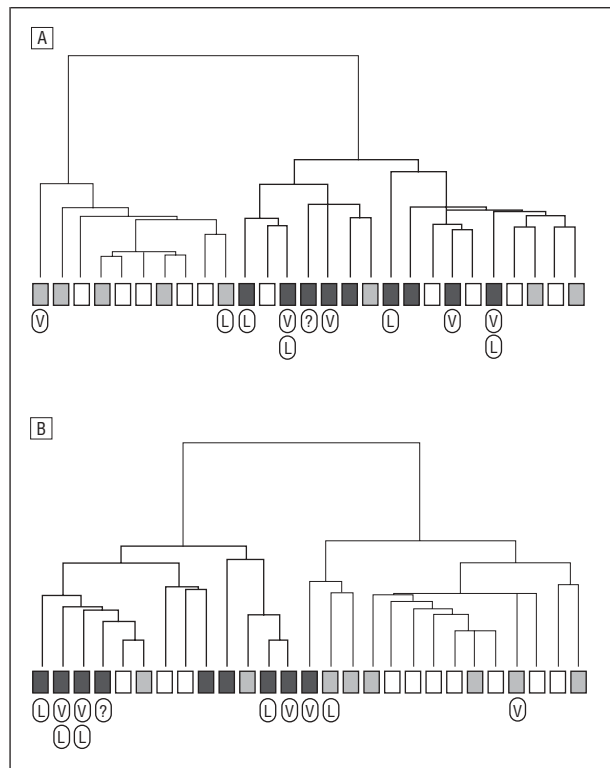


Figure 1. Hierarchical clustering of samples. A, All genes with a standard deviation higher than 4% of the mean of their expression value and present calls in at least 20% of samples were used for clustering (n=216). Significant clustering of bipolar disorder samples was observed ($P=.005$). B, Genes known to be involved in complexes I through V of the mitochondrial respiratory chain and present in at least 20% of samples were used for clustering (n=72). Significant clustering of bipolar disorder samples ($P=.004$) and control samples ($P=.02$) was observed. Redundant probe sets were excluded from clustering analysis. Dark-shaded rectangles indicate bipolar disorder; light-shaded rectangles, schizophrenia; open rectangles, controls; L, lithium carbonate; V, valproic acid; and ?, treatment not known.

tide dehydrogenase in 1 gene), IV (cytochrome-*c* oxidase in 1 gene), and V (adenosine triphosphate [ATP] synthase in 5 genes), which carry out oxidative phosphorylation in the mitochondrial inner membrane.

In addition to the novel evidence for the abnormal regulation of nuclear genes coding for mitochondrial proteins, we also confirmed previous evidence¹⁴ of decreased expression of the 67-kd isoform of glutamic acid decarboxylase (GAD₆₇), the enzyme synthesizing the inhibitory neurotransmitter γ -aminobutyric acid (GABA) in bipolar disorder (Table 2). Furthermore, the mRNA coding for the neuropeptide somatostatin, expressed in the oriens-lacunosum/moleculare subtype of hippocampal interneurons,¹⁵ was most significantly decreased in all 43 differentially affected genes.

We performed hierarchical clustering using the dChip program to identify samples with similar expression profiles.^{16,17} To limit noise and increase the strength of our findings, only genes with ample variability and present calls were used for clustering (Figure 1A). Variability was set at a standard deviation greater than 4% of the mean of the expression value, and genes had to be deemed present in at least 20% of samples. A total of 216 genes met the criteria. These genes showed that bipolar

disorder samples had similar genetic profiles and clustered together ($P=.005$).

To further explore regulation trends in functionally related genes, we used MAPPfinder.¹² Of 365 genes that were down-regulated in bipolar disorder with at least a 1.1-fold difference, $P<.02$, and more than 50% present calls (false discovery rate, 7.2%), 326 linked to terms defined by the Gene Ontology Consortium. Of the 6 groups that achieved a *z* score higher than 10, three were associated with mitochondria and 3 with the ATP-dependent process of proteasome degradation (Table 3). MAPPfinder identified 50 mRNA molecules coding for proteins located in the mitochondrial inner membrane that were in the Gene Ontology Consortium database and HG-U95Av2 array and found 17 (34%) to be decreased in bipolar disorder. Furthermore, the expression of 7 (78%) of 9 genes associated with the proton-transporting ATP synthase complex in the inner mitochondrial membrane was decreased in bipolar disorder. The dChip results were verified with Data Mining Tool software, and down-regulation of the same 2 gene families was confirmed with MAPPfinder.

NUCLEAR GENES RELATED TO MITOCHONDRIAL FUNCTION

To provide an unbiased review of the regulation of genes involved in energy metabolism and proteasome degradation, we used GenMAPP to draw maps of all relevant genes represented in the HG-U95Av2 array.¹² These maps revealed that the decreased expression of genes related to mitochondrial function was not only pronounced but also widespread (Figure 2 and Figure 3). Similar maps for the schizophrenia group revealed that not a single one of the genes listed for oxidative phosphorylation and proteasome degradation, 62 and 28 genes, respectively, reached a probability level of $P\leq.05$.

Hierarchical clustering was performed with 72 genes known to be involved in complexes I through V of the mitochondrial respiratory chain. Significant clustering of bipolar disorder samples ($P=.004$) and control samples ($P=.02$) was observed (Figure 1B), demonstrating that these subjects had a similar profile for genes in the mitochondrial respiratory chain. No significant clustering was observed with valproic acid or lithium carbonate treatment. In a direct comparison of expression levels of mitochondrial genes, no difference was observed in patients treated with valproic acid or lithium compared with those who did not receive the drug.

Because a lower percentage of genes were deemed present in the bipolar disorder group (Table 1), we examined whether a lower percentage of gene-present calls was associated with down-regulation of genes in the mitochondrial respiratory pathway, independent of clinical diagnosis. We combined control and schizophrenia samples, sorted them according to percentage of gene-present calls, compared the 9 samples with the lower percentage of gene-present calls (mean \pm SD, 42.4% \pm 3.1%) with the 9 samples with a higher percentage of calls (mean \pm SD, 47.3% \pm 0.6%; $P<.001$), and performed MAPPfinder analysis. Mitochondrial membrane, mitochondrial inner membrane, and proton-transporting ATP syn-

Table 3. Gene Ontology Groups

Gene Ontology Name	Gene Ontology Identification No.	Gene Ontology Type	No. Changed/No. Measured in Array/ No. of Genes in Gene Ontology Identification	z Score
Mitochondrion				
Mitochondrial membrane	5740	Cellular component	20/75/189	11.4
Mitochondrial inner membrane	5743/19 866	Cellular component	17/50/154	12.2
Proton-transporting adenosine triphosphate synthase complex	5753	Cellular component	7/9/2013	12.5
Proteasome				
26S Proteasome	5837	Cellular component	14/32/43	12.8
20S Proteasome	5839	Cellular component	8/16/2023	10.4
Threonine endopeptidase	4299	Molecular function	8/13/2020	11.7

these complex had z scores of 2.7 (ranked 236), 0.72 (ranked 608), and 1.6 (ranked 387), respectively. These low z scores (compared with the high z scores reported in Table 3 for the bipolar disorder group) indicate that the decreased expression of nuclear genes related to mitochondrial function cannot be explained solely by the lower percentage of gene-present calls in the bipolar disorder group.

REAL-TIME QUANTITATIVE PCR RESULTS

To confirm our finding of decreased expression of nuclear genes involved in energy metabolism and proteasome degradation in bipolar disorder, we selected 4 genes for verification in a real-time quantitative PCR assay: 2 from the mitochondrial respiratory chain and 2 proteasome subunits (Figure 4). All 4 mRNA molecules were corrected for an internal control gene (human filamin A α) and were significantly down-regulated, confirming the gene array data.

To explore whether the decreased expression of these 4 genes was specific for the hippocampus, we performed the same real-time quantitative PCR analysis in frontal cortex specimens from the same subjects (Figure 4). We found a similar pattern of decreased expression in the frontal cortex tissue in subjects with bipolar disorder.

COMMENT

Our results provide evidence for the abnormal regulation of nuclear genes coding for mitochondrial proteins in bipolar disorder. In addition, we confirm and extend previous evidence¹⁴ of abnormal gene expression in hippocampal interneurons in bipolar disorder. The decreased expression of GAD₆₇ and somatostatin points to a specific deficit of the oriens-lacunosum/moleculare subtype of hippocampal interneurons in bipolar disorder.¹⁵ This supports the notion that a subset of hippocampal interneurons, located in the stratum oriens and terminating with apical dendrites of principal cells in conjunction with perforant pathway afferent fibers, is abnormal in bipolar disorder.

In this article, we focus primarily on the novel evidence for abnormal mitochondrial energy metabolism in bipolar disorder. First, the expression of genes coding for the enzymatic complexes governing oxidative phosphory-

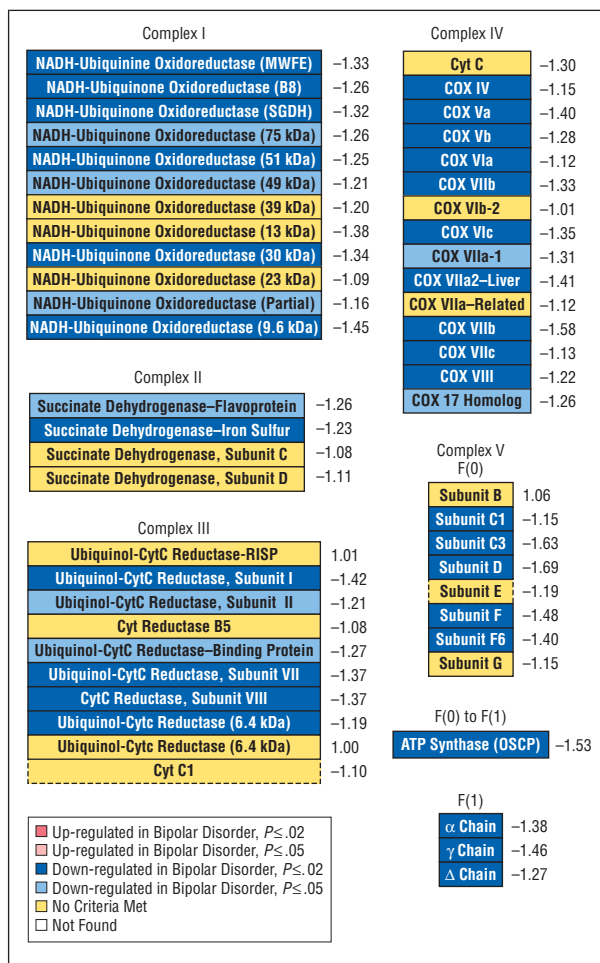


Figure 2. Genes coding for mitochondrial proteins involved in oxidative phosphorylation. The figure includes all genes represented on the HG-U95Av2 array (Affymetrix, Santa Clara, Calif) that code for mitochondrial proteins involved in oxidative phosphorylation. Comparison of the bipolar disorder group with the control group revealed that most genes were down-regulated (indicated in blue), some were not significantly changed or had a presence call lower than 60% (indicated in yellow), and none were significantly increased. ATP indicates adenosine triphosphate; COX, cytochrome-c oxidase; Cyt, cytochrome; NADH, nicotinamide adenine dinucleotide; OSCP, oligomycin sensitivity-conferring protein; and RISP, Rieske iron-sulfur protein. A dashed line around a box indicates that the gene was represented on the chip more than once (eg, Cyt C1); if a box has more than 1 color, the various representations of the gene on the array met different criteria as indicated by the colors (eg, subunit E). Fold difference is shown to the right of the box (positive values calculated as bipolar disorder/control; negative values calculated as control/bipolar disorder).

lation is decreased in bipolar disorder. Second, the ATP-dependent process of proteasome degradation is down-regulated at the level of gene expression. This molecular evidence strengthens the hypothesis that decreased pH and high-energy phosphate levels in bipolar disorder are the result of mitochondrial dysfunction.⁴

It is unclear how a widespread decrease in the expression of nuclear genes coding for mitochondrial pro-

teins could have been produced in the cases of bipolar disorder reported in this study. Although mutations in both mitochondrial¹⁸ and nuclear DNA may contribute to mitochondrial dysfunction in bipolar disorder, it is unlikely that such mutations could induce the pattern of decreased mRNA expression observed in our sample. Possible explanations of the findings are that the number of mitochondria per neuron is reduced in bipolar disorder or that a subset of neurons with high mitochondrial numbers (eg, GABAergic interneurons) is lost. That mRNA coding for the neuropeptide somatostatin, expressed in hippocampal interneurons, is also reduced might support this notion of selective neuron loss. Because glial fibrillary acidic protein mRNA, a marker of gliosis, was not altered in bipolar disorder, neuronal death would probably not be a recent event. Alternatively, mechanisms that control transcription, including the ATP-dependent process of nucleosome remodeling¹⁹ or histone acetylation and methylation,²⁰ could be involved in widespread changes of gene expression. In this context, it is of interest that lithium and valproic acid, 2 therapeutic agents in the treatment of bipolar disorder, affect chromatin remodeling. Inositol polyphosphates, targets of lithium can modulate the activities of chromatin-remodeling complexes in vitro.²¹ The mood-stabilizing drug valproic acid is an inhibitor of histone deacetylase.²² The inhibition of this enzyme results in a widespread increase in gene expression, including the gene *GAD67*,²³ which has been found to be decreased in the hippocampus in bipolar disorder in this and previous studies.^{14,24} It is therefore conceivable that mechanisms of chromatin structuring are affected in bipolar disorder and are targeted by pharmaceutical compounds effective in the treatment of this disease.

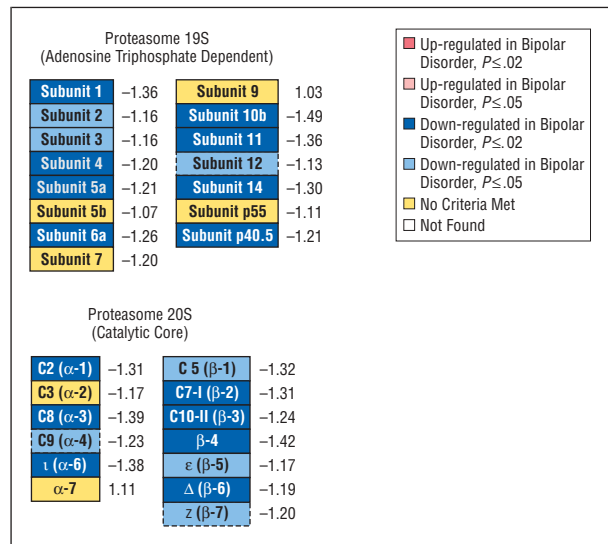


Figure 3. Genes coding for proteins involved in proteasome degradation. The figure includes all genes represented on the HG-U95Av2 array (Affymetrix, Santa Clara, Calif) that code for proteins involved in proteasome degradation. Comparison of the bipolar disorder group with the control group revealed that most genes were down-regulated (indicated in blue), some were not significantly changed (indicated in yellow), and none were significantly increased. See Figure 2 legend for details.

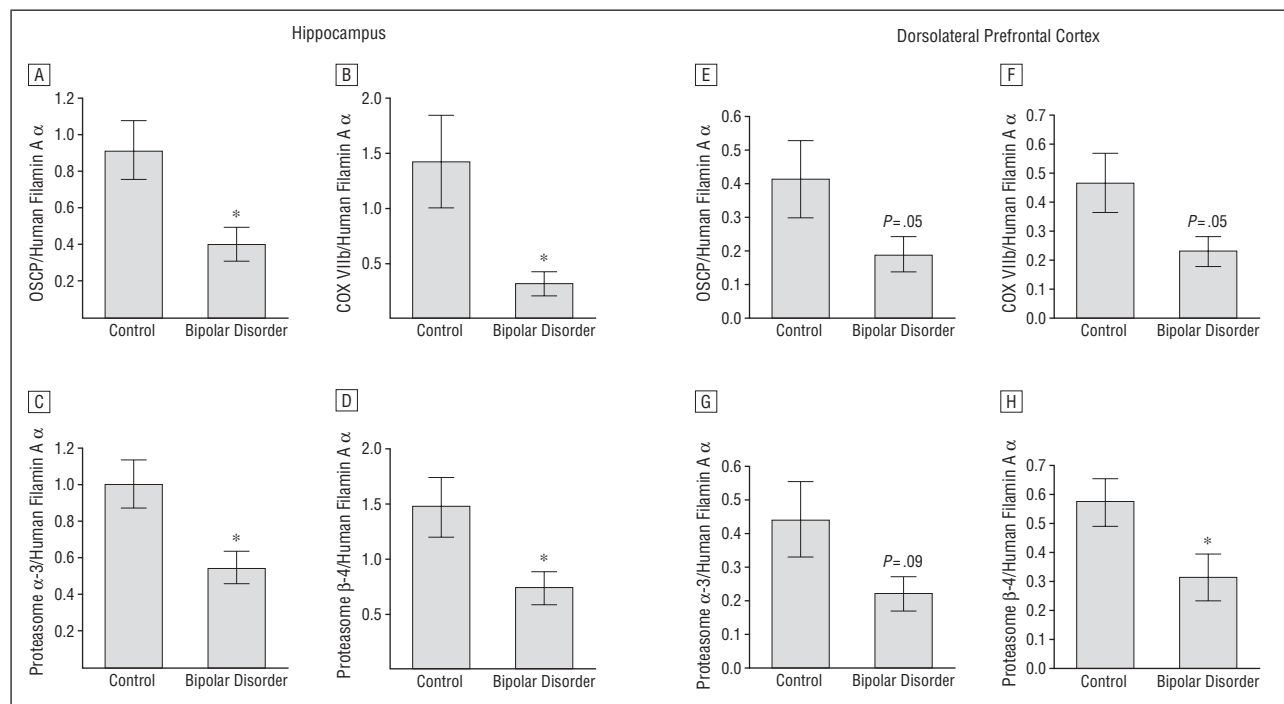


Figure 4. Real-time quantitative polymerase chain reaction of mitochondrial and protosomal genes in subjects with bipolar disorder and controls. Four genes were selected for verification. A and E, The oligomycin sensitivity-conferring protein (OSCP), a subunit of mitochondrial adenosine triphosphate synthase. B and F, The mitochondrial cytochrome-c oxidase subunit, COX VIIb. C and G, The proteasome α-3 subunit. D and H, The proteasome β-4 subunit. The expression of each gene was normalized to human filamin A α. Mean ± SEM is shown. Asterisk indicates significance at $P < .05$.

Despite using all possible measures to avoid the introduction of experimental bias and although equal amounts of biotinylated RNA were used in all arrays, the percentage of gene-present calls was lower in bipolar disorder. Although not significant, data from the 28S/18S ribosomal RNA ratios and 3'/5' glyceraldehyde-3-phosphate dehydrogenase and β -actin ratios might suggest reduced mRNA quality in the hippocampus in bipolar disorder. Compromised energy metabolism could account for this observation, but we cannot entirely exclude the possibility that factors inherent in postmortem studies and beyond the investigators' control might have contributed to reduced RNA quality.

Most subjects with bipolar disorder and all subjects with schizophrenia were treated with neuroleptic medication, which has supportive as well as inhibitory effects on mitochondrial function.²⁵⁻²⁷ If the genes for mitochondrial respiration were down-regulated as a result of antipsychotic drug treatment, this effect should have been more pronounced in the subjects with schizophrenia, who were treated with higher doses of antipsychotic drugs. Conversely, if antipsychotic drug treatment up-regulates genes for mitochondrial respiration, it could explain why the schizophrenia group had levels more comparable with controls. Lithium and valproic acid did not seem to be responsible for the down-regulation of genes because they did not cluster together (Figure 1B), and lithium had no such effect in an animal study.²⁸ We consider this as evidence that the decreased gene expression in our bipolar disorder sample was not due to neuroleptic medication, lithium, or valproic acid.

Recent postmortem studies of schizophrenia have reported that the activity of oxidative enzymes associated with mitochondria, such as the malate-aspartate shuttle system²⁹ and complex IV,³⁰ is also decreased in the frontal cortex in subjects with schizophrenia. This suggests that disturbances in mitochondrial oxidation (at the level of gene expression, as in our study of subjects with bipolar disorder, or at the level of enzyme activity, as previously reported in those with schizophrenia) may play a broader role in psychotic disorders.

We do not know whether our finding of abnormal gene expression in bipolar disorder is specific to the hippocampus. The results of real-time quantitative PCR analysis of the frontal cortex specimens indicate that the changes reported in our article are not limited to the hippocampus. Previous studies have demonstrated abnormal gene expression in the hippocampus and cerebral cortex in bipolar disorder,³¹⁻³⁴ but we are aware of no studies that have systematically examined the expression of genes coding for mitochondrial proteins in this disease.

It is likely that decreased nuclear gene expression governing oxidative phosphorylation has functional implications. Mitochondrial dysregulation associated with decreased oxidative phosphorylation shifts metabolism toward anaerobic energy production via glycolysis, increasing lactate levels and pH and leading to reactive oxygen species, glutamate excitotoxicity, and apoptosis.³⁵ Similarly, decreased expression of genes coding for proteins of the ubiquitin-proteasome system has functional implications, among them an impairment of synapse remodeling.³⁶ Further studies should test the hypothesis

that the pronounced and widespread decrease of mRNA coding for mitochondrial and proteasome proteins leads to abnormal protein concentration and function. It appears that our finding of a decreased expression of genes involved in mitochondrial function and proteasome degradation provides potential targets for the development of novel drug compounds in the treatment of bipolar disorder.

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Weickert et al (p 544) mapped the expression of dysbindin, a schizophrenia susceptibility gene, in postmortem brains from individuals with schizophrenia and suitable controls. They observed reduced levels of dysbindin messenger RNA in the frontal cortex and midbrain, 2 regions in which dopaminergic processes are prominent.

Rosa-Neto et al (p 556) measured an index of serotonin synthesis, brain regional α -[^{11}C] methyl-L-tryptophan trapping (K^* , milliliters per gram per minute), in the areas involved in the regulation of mood in medication-free patients with a current episode of major depression. Compared with healthy men and women, normalized K^* values were significantly decreased in the anterior cingulate and mesial temporal lobe. The results suggest that reduced serotonin synthesis in parts of the limbic and paralimbic cortices may contribute to the development and expression of major depression.

Mufson et al (p 577) conducted an effectiveness study of interpersonal psychotherapy modified for depressed adolescents (IPT-A) as compared with treatment as usual delivered in school-based health clinics serving impoverished urban communities. Adolescents treated with IPT-A as compared with treatment as usual demonstrated greater reduction in depression symptoms and greater improvement in overall functioning. School-based clinicians were able to implement a brief evidence-based treatment intervention in a real-world setting, thereby narrowing the gap between treatment conducted in the laboratory and in the community.

Butters et al (p 587) studied patients with late-life depression and healthy comparison subjects to characterize neuropsychological functioning in the disorder and to examine its association with putative risk factors. They found that more than half of the patients with depression exhibited significant impairment, most often in information-processing speed and visuospatial

and executive abilities. Moreover, the broad-based impairments were mediated almost entirely by the slowed information-processing speed and not by factors more commonly thought to cause cognitive deficits.

Xu et al (p 597) found that a specific haplotype cluster of the D_2 dopamine receptor gene (*DRD2*) was associated with high risk of heroin dependence in a Chinese case-control population. A recombination “hotspot” generated 2 daughter haplotypes associated with lower risk of heroin dependence in a German population. The results of this study helped to resolve previous contradictory findings with substance abuse and provide evidence for a functional role for the *DRD2* gene in heroin dependence.

Hudziak et al (p 608) investigated the genetic and environmental contributions to the Obsessive-Compulsive Scale in large samples of twins aged 7, 10, and 12 years from the Netherlands Twin Registry and a sample of mixed-age twins from the Missouri Twin Study. The analyses revealed that the Obsessive-Compulsive Scale is influenced by significant additive genetic influences (approximately 45%). These findings are consistent across age, sex, and cultures.

Glasson et al (p 618) found an increase in obstetric complications among a population-based cohort of people with autism spectrum disorders compared with controls. Case mothers had greater frequencies of threatened abortion during pregnancy and were more likely to receive epidural caudal anesthesia and have labor induced. Case infants were more likely to experience fetal distress and be delivered by either an elective or emergency cesarean section.

Correction

Error in Table Heading. In the article titled “Molecular Evidence for Mitochondrial Dysfunction in Bipolar Disorder,” published in the March issue of the ARCHIVES (2004;61:300-308), the fourth column heading in Table 1 read “Postmortem Interval, d.” It should have read “Postmortem Interval, h.” The ARCHIVES regrets the error.