ORIGINAL ARTICLE

Differences in Lymphocyte Electron Transport Gene Expression Levels Between Subjects With Bipolar Disorder and Normal Controls in Response to Glucose Deprivation Stress

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Context: Bipolar disorder (BPD) is among the top 10 causes of disability worldwide. Recent findings on the etiology of the disease point to a disturbed mitochondrial energy metabolism in the brain of subjects with BPD.

Objective: To test whether gene transcripts for proteins of the mitochondrial respiratory chain have altered levels in glucose-deprived lymphocytes from patients with BPD.

Design: Microarrays were used to measure gene expression levels in fresh lymphocytes and in lymphocytes cultured for 5 days in regular or low-glucose medium.

Setting: Subjects with BPD were recruited through the Schizophrenia and Bipolar Disorders Program, McLean Hospital, Belmont, Mass. Controls were recruited through advertising.

Patients: A total of 21 patients with BPD (inpatients and outpatients) and 21 control subjects.

Main Outcome Measure: Expression levels for genes of proteins involved in mitochondrial respiration.

Results: We found an opposite molecular response of control and BPD lymphocytes to glucose deprivation. Whereas lymphocytes of normal controls responded to glucose deprivation with an up-regulation of nuclear transcripts for proteins of the electron transfer chain, subjects with BPD had a tendency to down-regulate these transcripts.

Conclusions: The results suggest that the normal molecular adaptation to energy stress is deficient in lymphocytes from patients with BPD.

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BIPOLAR DISORDER (BPD) AFfects between 1% and 3% of the US population,[1] is associated with a high risk of suicide,[2] and places an annual economic burden on the nation in excess of $40 billion (estimated in 1991).[3] Diagnosis of BPD is based on the nature and course of symptoms, but the etiology of the disease remains elusive and no diagnostic test is available. Recent studies showed decreased hippocampal and dorsolateral prefrontal cortex levels of creatine kinase messenger RNA (mRNA)[4] as well as decreased levels of high-energy phosphates[5,6] or increased lactate levels[7] in the frontal and temporal lobes of patients with BPD, introducing the hypothesis that mitochondrial energy metabolism plays an important role in the etiology of the disease.[8] Previously, we described a down-regulation in nuclear mRNA coding for mitochondrial electron transfer proteins in postmortem hippocampal tissue from patients with BPD.[9] To follow up on this study, we examined gene expression levels in lymphocytes of patients with BPD.

Gene expression patterns in lymphocytes have been analyzed recently in BPD and schizophrenia.[10-12] Lymphoblastoid cell lines of patients with BPD have been used to study the expression of nuclear genes coding for mitochondrial pro-

teins.[13] Lymphocytes are easier to collect than brain tissue, and it has been shown that peripheral gene expression can be informative about gene expression in the central nervous system. Whole blood has significant gene expression similarities with multiple central nervous system tissues, and the expression levels of many classes of biologically relevant processes are not significantly different between whole blood and prefrontal cortex.[14] In patients with schizophrenia, a comparison of gene expression profiles from brain tissue with profiles from peripheral blood cells identified disease-associated genes that were common to both tissues, confirming the validity of gene expression profiling of blood for detecting schizophrenia biomarkers.[10]

Lymphocytes not only are easily accessible but, unlike postmortem brain tissue, can also be subjected to experimental manipulations. We decided to go beyond gene expression profiling from freshly drawn blood and to culture lymphocytes of patients with BPD and normal controls (NCs) in medium with normal or low glucose levels for 5 days to examine gene expression levels after low-glucose stress.

METHODS

Twenty-one healthy NCs and 21 patients diagnosed with BPD according to the criteria of *DSM-IV*[15] (**Table 1**) provided informed consent as approved by the institutional review board at McLean Hospital, Belmont, Mass. Samples from patients with BPD and NCs were collected over the course of 6 months, and batches of BPD samples were matched with NC samples. Lymphocytes from 10 to 30 mL of freshly drawn blood were separated by centrifugation using Histopaque columns (Sigma-Aldrich, St Louis, Mo), washed 3 times, and split into 3 batches. One batch, containing two thirds of all cells, was frozen at –80°C and later subjected to gene expression microarray analysis, whereas 1 smaller batch each was cultured in either regular RPMI-1640 medium or low-glucose RPMI-1640 medium (25% normal glucose content; 0.5 g/L) for a period of 5 days, after which cells were frozen at –80°C. After sample collection was concluded, RNA was extracted from each batch (RNagents kit; Promega, Madison, Wis), complementary DNA (cDNA) was synthesized from 4 µg of RNA from fresh lymphocytes (SuperScript double-stranded cDNA synthesis kit; Invitrogen Corp, Carlsbad, Calif) or 1 µg of RNA from cultured lymphocytes (MessageAmp II-96 kit; Ambion, Austin, Tex), and biotinylated RNA was synthesized from cDNA (for fresh lymphocytes, Enzo IVT kit; Enzo Biochem, Farmingdale, NY; for cultured lymphocytes, MessageAmp II-96 kit). Biotinylated RNA was fragmented and hybridized to the HG-U133A 2.0 array (Affymetrix, Santa Clara, Calif) overnight at 45°C and stained on a washing station with 2 rounds of streptavidin-phycoerythrin (Molecular Probes, Eugene, Ore) separated by a round of biotinylated antistreptavidin antibody (Vector Laboratories, Burlingame, Calif) as described previously.[9,16] All of the fresh-frozen lymphocytes were worked up in 1 batch for gene array experiments. All of the cultured lymphocytes were worked up together in a separate batch with an improved protocol developed during the course of this project, for which the amount of input RNA could be lowered from 4 µg to 1 µg. Because of the small sample sizes and the variable amount of lymphocytes yielded from individual probands, a number of samples did not yield enough mRNA for gene array analysis (Table 1). The number of samples per group ranged from 10 to 17.

Gene expression levels were calculated with the robust multichip analysis algorithm (RMAExpress; http://rmaexpress.bmbolstad.com) and compared using the comparison analysis of the dChip program (http://biosun1.harvard.edu/complab/dchip), which computes *P* values based on the *t* distribution, with the degrees of freedom set according to the Welch-modified 2-sample *t* test.[17,18] Only samples that met quality-control criteria provided by the GeneChip Operating Software (Affymetrix) and DNA-Chip Analyzer (dChip 2006)[19] were incorporated into the analysis (Table 1) (mean±SD noise, 0.9±0.1; mean±SD percentage present call, 56.2%±1.7%; mean±SD 3′-5′ glyceraldehyde-3-phosphate dehydrogenase ratio, 1.4±0.4; mean±SD 3′-5′ β-actin ratio, 1.6±0.8; mean±SD percentage of array outliers, 0.16%±0.22%; mean±SD percentage of single outliers, 0.046%±0.043%; no significant differences were observed between groups).

All of the genes differently expressed between 2 groups ($P \leq .05$; ≥50% present call; 4 groups: BPD over NC for low glucose, BPD over NC for normal glucose, low over normal glucose for NC, and low over normal glucose for BPD) were subjected to a classification analysis using the Gene Ontology database gene product attributes (http://www.geneontology.org) calculated with the dChip software. Multiples of same transcripts were masked for classification analyses. Similar results were obtained with $\log_2$-transformed and natural scale data. Analysis of variance filtering was carried out in dChip. Permuted and adjusted *P* values for mitochondrial genes were obtained with the MAPPFinder program (http://www.genmapp.org).[20] We used 271 groupings (MAPPs) of individual genes for this analysis, grouped in a manner that avoided duplication of the same genes in independent groups. MAPPFinder calculates a nonparametric statistic based on 2000 permutations of the data, randomizing the gene associations for each sample to generate a distribution of *z* scores for each MAPP, which are then used to assign permuted *P* values. In addition, the Westfall-Young adjustment, which calculates the familywise error rate for each sample and accounts for multiple testing, is used for multiple testing. This adjustment gives the adjusted *P* value. Fisher exact test was used to examine the statistical difference between the percentage of regulation of mitochondrial transcripts vs the percentage of regulation of all of the transcripts.

Families of genes, such as genes of the mitochondrial respiratory chain or genes specific for B or T cells, were compared between NC and BPD samples with 2-tailed, paired *t* tests using the natural expression values. For example, for the mitochondrial respiratory chain, the expression level of each of the 114 individual transcripts in an experimental group was divided by the average expression level of each transcript in all of the groups. False discovery rates were calculated in the dChip program by estimating the empirical false discovery rate for a group of genes (ie, the 114 mitochondrial transcripts) using 2000 random permutations.

Real-time quantitative polymerase chain reaction (qPCR) was used for data verification and carried out as previously described.[9,16] Briefly, cDNA was synthesized from 1 µg of total RNA (SuperScript First-Strand Synthesis System for real-time qPCR; Invitrogen Corp) and oligonucleotide deoxythymidine primers. Primer sets for each gene were designed with the Primer3 software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) for amplicons of 100 to 200 base pairs. Melt curve analysis and polyacrylamide gel electrophoresis were used to confirm the specificity of each primer pair. The iQ SYBR Green Supermix (Bio-Rad, Hercules, Calif) was used for the experiment carried out with a MyiQ real-time PCR detection system (Bio-Rad) in a volume of 20 µL, with 4 µL of 1:10 diluted cDNA samples and 0.3 µM primers. The PCR cycling conditions were initially 95°C for 5 minutes followed by 39 cycles of 94°C for 10 seconds, 55°C for 15 seconds, and 72°C for 20 seconds. Data were

Table 1. Sample Information for Subjects With Bipolar Disorder and Normal Controls

Patient Information											Tests*				
Subject No.	Diagnosis	Current Episode	Inpatient	Psychosis	Sex	Age, y	Medications	MPE, y	YMRS Score	BDI Score	GL	GN	PL	PN	FL
Patients With Bipolar Disorder															
1	BP-I	Manic	Yes	None	M	53	Lithium carbonate, 900 mg/d; synthroid, 100 µg/d; VPA, 1500 mg/d; ω-3 fatty acids, 1 g/d; quetiapine fumarate, 850 mg/d	16	1	0	X				
2	BP-I	Manic	Yes	AH	F	54	Quetiapine fumarate, 300 mg/d; lithium carbonate 600 mg/d; buspirone hydrochloride, 10 mg/d	Unknown	30	12	X		X		
3	BP-I	Manic	Yes	Delusions + AH	M	22	Risperidone (Risperdal Consta) 25 mg every 2 wk; risperidone, 6 mg/d; haloperidol, 5 mg/d; lithium carbonate, 1500 mg/d; lorazepam, 3 mg/d; benztropine mesylate, 2 mg/d†	Unknown	14	3	X	X	X	X	
4	BP-I	Mixed	Yes	None	M	44	ω-3 Fatty acids, 1 g/d; oxcarbazepine, 600 mg/d; fluoxetine hydrochloride, 20 mg/d; trazodone hydrochloride, 150 mg/d;	15	1	34	X	X	X	X	X
5	BP-I	Manic	Yes	None	M	49	Aripiprazole, 2 mg/d; lithium carbonate, 600 mg/d;	Unknown	18	26	X	X	X	X	X
6	BP-I	None	No	None	M	50	Valproate extended release, 500 mg/d; fluoxetine hydrochloride, 10 mg/d; topiramate, 200 mg/d; ω-3 fatty acids, 1800 mg/d; propoxyphene napsylate–acetaminophen (Darvocet), 5 tablets/d	12	ND	1	X	X	X	X	X
7	BP-II	None	No	None	F	50	Lithium carbonate, 300 mg/d; sertraline hydrochloride, 100 mg/d; topiramate, 25 mg/d	15	1	8	X	X	X	X	
8	BP-I	Manic	Yes	None	F	39	Lithium carbonate, 1000 mg/d; olanzapine, 2.5 mg/d;	15	6	0	X	X		X	X
9	BP-I	None	Yes	Delusions	F	37	Carbamazepine, 500 mg/d; fluoxetine hydrochloride, 80 mg/d; venlafaxine hydrochloride, 37.5 mg/d;	12	2	15.5	X	X		X	X
10	BP-I	Depressed	Yes	AH	M	19	Lorazepam, 3 mg/d	12	10	9.5	X	X			X
11	BP-I	Depressed	Yes	Delusions	F	39	Carbamazepine, 600 mg/d; bupropion hydrochloride, 400 mg/d; lorazepam, 3 mg/d; quetiapine fumarate, 150 mg/d	18	3	33.5		X	X	X	
12	BP-I	None	No	None	F	42	Escitalopram oxalate, 20 mg/d; VPA, 1000 mg/d; levothyroxine sodium (Synthroid), 112 µg/d‡	12	2	33	X	X	X	X	X
13	BP-I	None	No	None	F	47	Aripiprazole, 20 mg/d; clonazepam, 0.5 mg/d; paroxetine hydrochloride, 60 mg/d; clonidine hydrochloride, 0.02 mg/d;	12	1	26	X	X	X	X	X
14	BP-I	None	No	None	M	48	Gabapentin, 1800 mg/d; venlafaxine hydrochloride, 75 mg/d	Unknown	2	30	X	X	X	X	
15	BP-I	None	No	None	F	34	Venlafaxine hydrochloride, 300 mg/d; quetiapine fumarate, 100 mg/d; oxcarbazepine, 300 mg/d	12	0	42	X	X	X	X	X
16	BP-I	None	Yes	Delusions + AH	M	43	VPA, 1000 mg/d; olanzapine, 10 mg/d	12	6	13		X	X	X	X
17	BP-I	None	No	Delusions + AH	F	34	Levatiracetam, 1000 mg/d	16.5	9	8			X	X	X
18	BP-II	None	Yes	None	F	49	Sibutramine hydrochloride, 15 mg/d; bupropion hydrochloride sustained release, 400 mg/d; zonisamide, 400 mg/d; clonazepam, 3 mg/d; vigabatrin, 8 mg/d; lamotrigine, 500 mg/d; pramipexole dihydrochloride, 1.5 mg/d; gabapentin, 1200 mg/d;	5	3	13	X	X			
19	BP-I	None	Yes	None	M	26	Oxcarbazepine, 450 mg/d; gabapentin, 1200 mg/d; escitalopram oxalate, 15 mg/d; lamotrigine, 200 mg/d;	18	1	6			X	X	X
20	BP-I	None	Yes	None	M	23	Lamotrigine, 450 mg/d; perphenazine, 4 mg/d; fluoxetine hydrochloride, 20 mg/d; zolpidem tartrate, 10 mg/d; fexofenadine hydrochloride, 180 mg/d; lorazepam, 1 mg/d;	15	3	22			X	X	X
21	BP-II	None	No	None	F	49	None	19	3	12			X	X	
Entire group with bipolar disorder						Mean, 40.5		Mean, 13.9	Mean, 5.8	Mean, 16.5	n = 15	n = 15	n = 16	n = 17	n = 13

(continued)

Table 1. Sample Information for Subjects With Bipolar Disorder and Normal Controls (cont.)

Patient Information										Tests*				
Subject No.	Diagnosis	Current Episode	Inpatient	Psychosis	Sex	Age, y	Medications	MPE, y	YMRS Score	BDI Score	GL	GN	PL	PN	FL
NC Subjects															
1	NC				F	34		19			X	X	X	X	X
2	NC				F	23		16			X	X	X		X
3	NC				M	28		Unknown			X	X	X	X	X
4	NC				M	35		Unknown			X	X	X	X	
5	NC				M	21		17				X		X	X
6	NC				M	22		16			X	X		X	X
7	NC				M	23		15			X	X	X		
8	NC				M	47		10			X		X	X	X
9	NC				F	23		14				X		X	X
10	NC				M	46		13						X	X
11	NC				F	57		12				X	X	X	X
12	NC				M	59		14			X	X	X	X	X
13	NC				M	30		14			X		X	X	
14	NC				F	43		14			X		X	X	
15	NC				M	53		12					X	X	X
16	NC				M	52		18						X	
17	NC				M	54		12						X	X
18	NC				F	36		12					X		
19	NC				F	46		10.5				X	X		
20	NC				F	57		13					X		
21	NC				F	36		14					X	X	
Entire NC group						Mean, 39.3		Mean, 14.0			n = 10	n = 11	n = 15	n = 16	n = 12

Abbreviations: AD, antidepressants; AH, auditory hallucinations; BDI, Beck Depression Inventory; BP-I, bipolar I disorder; BP-II, bipolar II disorder; FL, fresh lymphocytes; GL, low-glucose gene arrays; GN, normal-glucose gene arrays; NC, normal control; ND, not determined; MPE, medium parental education; PL, low-glucose quantitative polymerase chain reaction; PN, normal-glucose quantitative polymerase chain reaction; VPA, valproic acid; YMRS, Young Mania Rating Scale.
*X denotes that the test was performed for the individual. No entry indicates that the test was not performed for the individual.
†Risperdal Consta is manufactured by Alkermes, Inc, Cambridge, Mass.
‡Synthroid is manufactured by Abbott Laboratories, Abbott Park, Ill.

Table 2. Entrez GeneID Numbers and Primer Sequences of Genes Chosen for Quantitative Polymerase Chain Reaction Experiments*

Genes of Interest	Respiratory Chain Complex	Entrez GeneID No.	Forward Sequence	Reverse Sequence
Cytochrome c oxidase IV-1 (*COX4I1*)	IV	1327	CGAGCAATTTCCACCTCTGT	CAGGAGGCCTTCTCCTTCTC
ATP synthase, F0, c2 (*ATP5G2*)	V	517	TGGGATTGGAACTGTGTTTG	TCACATGGCAAAGAGGATGA
ATP synthase, F0, g (*ATP5L*)	V	10632	TGTTGTTGGACCATGTGTGA	GCGGGCTAAACAGACGTGTA
ATP synthase, F1, O (*OSCP*)	V	539	CTGAAGGAACCCAAAGTGG	GAAAAGGCAGAAACGACTCC
Control genes				
Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	NA	2597	CTCCCATTCTTCCACCTTTG	GTCCACCACCCTGTTGCT
Keratin 10	NA	3858	GGGCGAGTCTTCATCTAAGG	AATGGTCTGTGTGAAGGGAGA
Integral membrane protein 2A (*ITM2A*)	NA	9452	CATTCGTGAGGATGACAACA	CAGCAACAAGTCCAGGTAAGC

Abbreviation: NA, not applicable.
*For array data, see Figure 1.

collected between 72°C and 82°C depending on amplicon melt temperature. A melt curve analysis was performed at the end of each qPCR experiment, from 60°C to 95°C. Dilution curves were generated for each primer pair in every experiment by diluting cDNA from a vehicle sample to a final concentration of 1.00, 0.20, 0.04, and 0.008. The logarithms of the dilution values were plotted against the cycle values for the standard curve. 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 average of 3 internal standards (**Table 2**), which were not regulated in the gene array analysis. Using an average of multiple internal standards for normalization leads to increased accuracy, as the conventional use of a single gene for normalization leads to relatively large errors.[21] Genes for qPCR were chosen based on gene array results (**Figure 1**). Sequences and Entrez GeneID numbers are provided in Table 2.

RESULTS

In the comparison of NC and BPD lymphocytes in low-glucose medium, among the Gene Ontology database categories with the most significant hits for down-regulated genes were mitochondrion ($P \leq .001$), cytochrome c oxidase activity ($P < .001$), mitochondrial electron transport chain ($P = .001$), and ubiquinol–cytochrome c reductase activity ($P < .001$). These groups were second only to ribosomal proteins, a category that

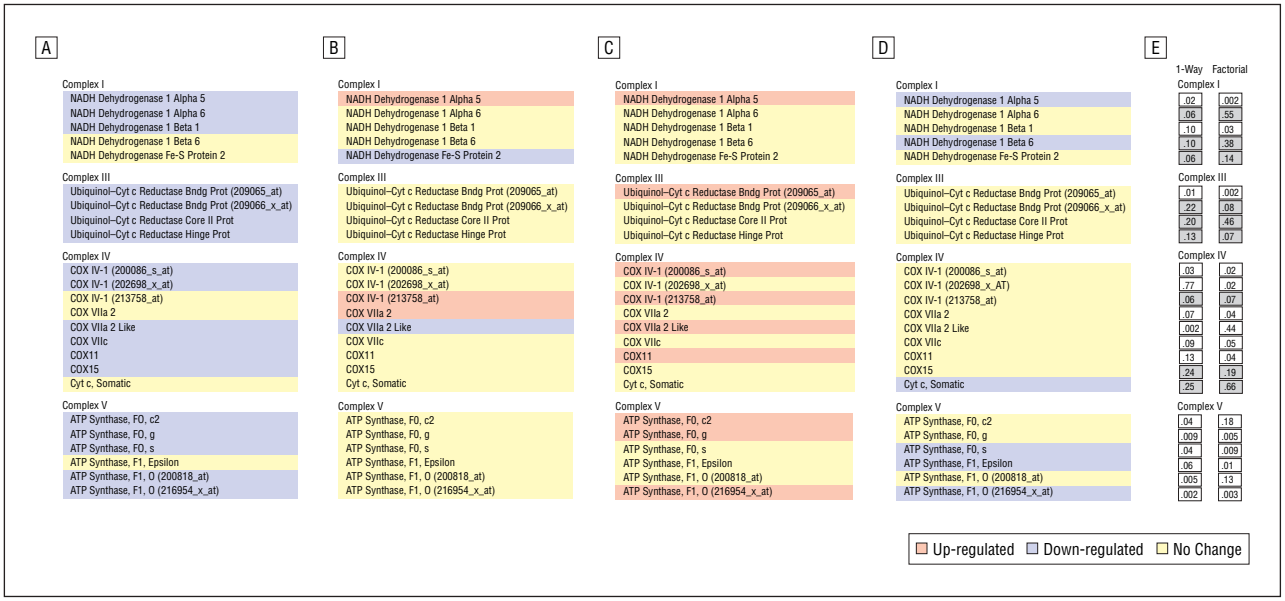

Figure 1. Probe sets of the electron transfer chain with $P<.05$ (*t* test) in low glucose for bipolar disorder lymphocytes over normal control lymphocytes (A), normal glucose for bipolar disorder lymphocytes over normal control lymphocytes (B), low over normal glucose for normal control lymphocytes (C), and low over normal glucose for bipolar disorder lymphocytes (D). NADH indicates reduced nicotinamide adenine dinucleotide; Fe-S, iron-sulfur; cyt c, cytochrome c; bndg, binding; prot, protein; COX, cytochrome c oxidase; and ATP, adenosine triphosphate. E, *P* values of 1-way and factorial analyses of variance (glucose level × treatment); shading indicates that the analysis of variance did not reach significance in both the 1-way and factorial analyses.

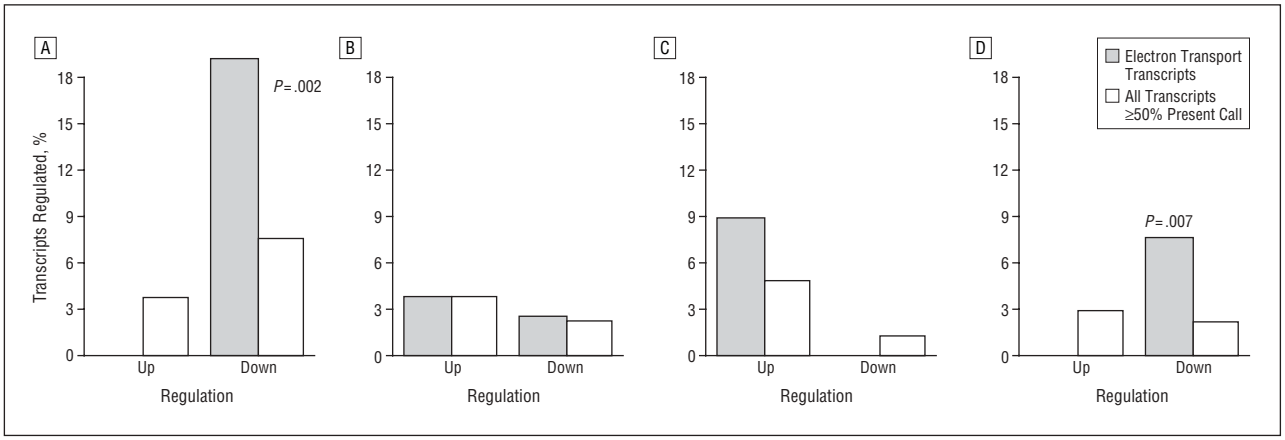

Figure 2. Comparisons of regulated electron transfer transcripts to all regulated transcripts (9399 nonredundant probe sets) in low glucose for bipolar disorder lymphocytes over normal control lymphocytes (A), normal glucose for bipolar disorder lymphocytes over normal control lymphocytes (B), low over normal glucose for normal control lymphocytes (C), and low over normal glucose for bipolar disorder lymphocytes (D). Redundant probe sets were masked; transcripts had to be present in at least 50% of all samples. *P* values were obtained using Fisher exact test.

was also affected in our previous study in the human hippocampus in BPD.[9] In the MAPPFinder program, the permuted *P* value for transcripts of the electron transport chain was less than or equal to .001 and the adjusted *P* value was .01 (*z* score, 6.1). Further analyses revealed that the expression of 18 probe sets of electron transfer transcripts, of 114 on the array (for GenBank and Entrez Gene numbers of all 114 transcripts, see eTable 1 [http://www.archgenpsychiatry.com]), was significantly lower in BPD lymphocytes under glucose deprivation (Figure 1A), whereas no probe sets were expressed at higher levels than in NC lymphocytes. The 18 probe sets represented 15 individual mRNA transcripts, composing 19% of all individual electron transfer mRNAs on the array (35/114 probe sets were duplicate probe sets), whereas on average only 8.2% of the probe sets were lower in BPD lymphocytes under glucose deprivation (**Figure 2**A). This difference was significant in Fisher exact test. Furthermore, the entire group of electron transfer transcripts was shifted significantly toward lower expression levels in BPD (**Figure 3**A and **Table 3**), and these trends were also seen in qPCR (**Figure 4**). No significant shift in expression levels of mitochondrial transcripts was observed between BPD and NC lymphocytes under normal glucose concentrations (Figure 1B, Figure 2B, Figure 3B, and Table 3) or in fresh, uncultured lymphocytes (**Figure 5** and Table 3). The pattern of electron transfer transcript expression in subjects with BPD and NCs suggests a different molecular response to glucose deprivation. Whereas NCs showed

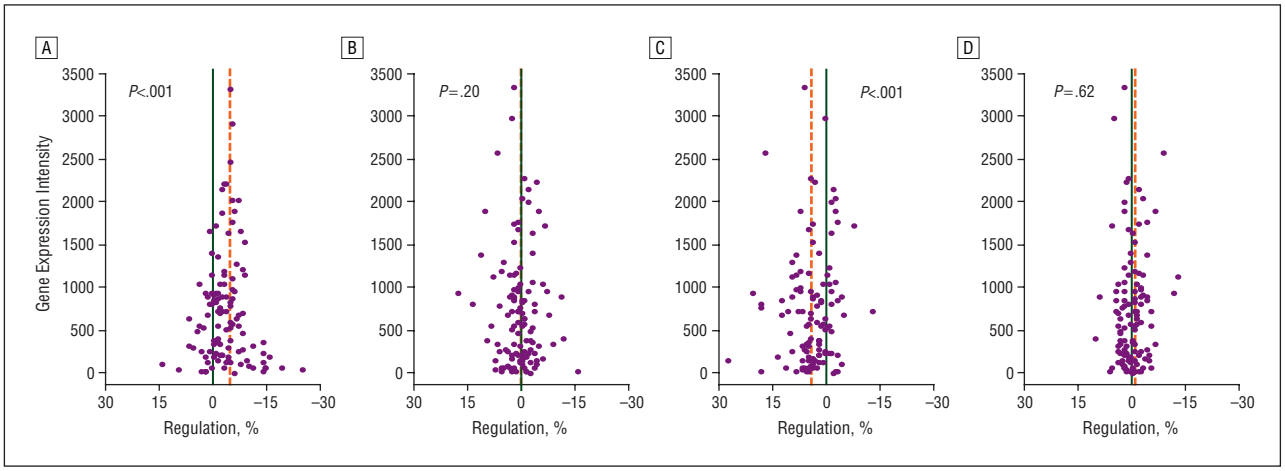

Figure 3. Of all probe sets on the array that were present in at least 50% of all samples (n=14 245), 114 coded for proteins involved in the electron transfer chain. Expression levels of each individual probe set were compared between low glucose for bipolar disorder lymphocytes and low glucose for normal control lymphocytes (A), normal glucose for bipolar disorder lymphocytes and normal glucose for normal control lymphocytes (B), low and normal glucose for normal control lymphocytes (C), and low and normal glucose for bipolar disorder lymphocytes (D). Green line indicates equal regulation; red line, actual average regulation of all transcripts.

Table 3. Statistics for the Entire Group of Mitochondrial Respiratory Chain Transcripts

Comparison	*P* Value for 2-Tailed, Paired *t* Test of Expression % Values*	Up-regulation FDR, %†	Down-regulation FDR, %†
Low glucose, NC‡ vs BPD§	<.001	≤6	Not calculable
Normal glucose, NC‡ vs BPD§	.21	≤33	≤50
BPD, normal‡ vs low§ glucose	.62	Not calculable	≤17
NC, normal‡ vs low§ glucose	<.001	≤12	Not calculable
Fresh lymphocytes, NC‡ vs BPD§	.05	≤100	≤17

Abbreviations: BPD, bipolar disorder; FDR, false discovery rate; NC, normal control.

*Values are for genes of the mitochondrial respiratory chain (for GeneID numbers, see eTable 1 [http://www.archgenpsychiatry.com]). For percentage expression values, the expression level of each of the 114 individual transcripts in an experimental group was divided by the average expression level of this transcript in all of the groups.

†The FDRs were calculated in the dChip program (http://biosun1.harvard.edu/complab/dchip) by estimating the empirical FDR using 2000 random permutations.

‡Baseline group.

§Experimental group.

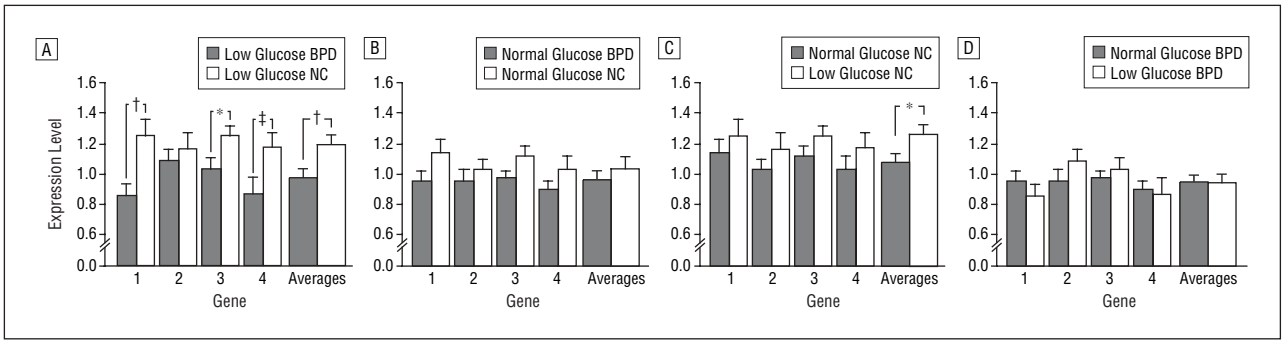

Figure 4. Real-time quantitative polymerase chain reaction analysis for low glucose for bipolar disorder (BPD) lymphocytes (n=16) vs normal control (NC) lymphocytes (n=15) (A), normal glucose for BPD lymphocytes (n=17) vs NC lymphocytes (n=16) (B), normal glucose vs low glucose for NC lymphocytes (C), and normal glucose vs low glucose for BPD lymphocytes (D). Four genes were used in the quantitative polymerase chain reaction verification: (1) the oligomycin sensitivity–conferring protein subunit of adenosine triphosphate synthase (analysis of variance [ANOVA], *P*=.006); (2) adenosine triphosphate synthase subunit c (ANOVA, *P*=.49); (3) adenosine triphosphate synthase subunit g (ANOVA, *P*=.04); and (4) cytochrome c oxidase IV isoform 1 (ANOVA, *P*=.06). For each set, we also show the averages of all 4 genes (ANOVA, *P*<.01). Factorial ANOVAs and Fisher post hoc protected *t* tests were used. *$P \leq .05$. †$P \leq .01$. The *t* test reached significance (*P*=.03) but the ANOVA did not (*P*=.06). Error bars indicate standard error. See eTable 1 (http://www.archgenpsychiatry.com) for all GeneID numbers.

an up-regulation of these transcripts in response to energy stress (Figure 1C, Figure 2C, Figure 3C, and Table 3), subjects with BPD showed no response (Figure 3D and Table 3). Indeed, under low-glucose stress, lymphocytes of subjects with BPD had a number of individual mitochondrial transcripts that were down-regulated

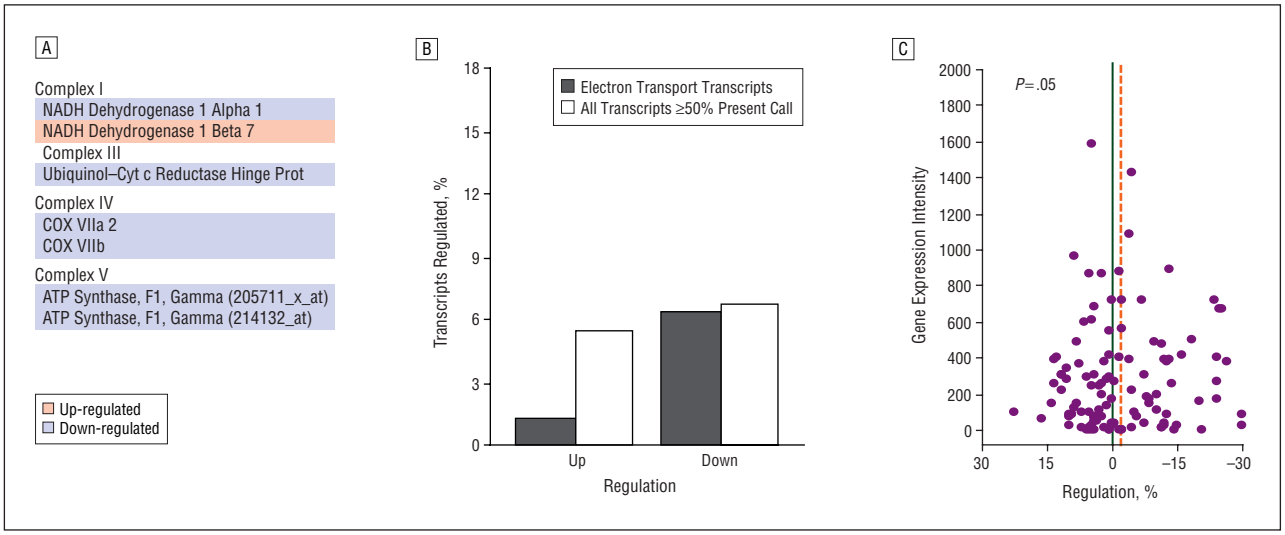

Figure 5. Fresh lymphocytes from bipolar disorder over normal controls. A, Probe sets of the electron transfer chain with $P<.05$ in bipolar disorder lymphocytes over normal control lymphocytes in fresh lymphocytes. NADH indicates reduced nicotinamide adenine dinucleotide; cyt c, cytochrome c; prot, protein; and ATP, adenosine triphosphate. B, Comparisons of regulated electron transfer transcripts to all regulated transcripts (9399 nonredundant probe sets) for bipolar disorder fresh lymphocytes over normal control fresh lymphocytes. Redundant probe sets were masked; transcripts had to be present in at least 50% of all samples. C, Expression levels of the same 114 probe sets shown in Figure 3 are compared between bipolar disorder lymphocytes and normal control lymphocytes in fresh lymphocytes. Green line indicates equal regulation; red line, actual average regulation of all transcripts. The Enzo-IVT kit (Enzo Biochem, Farmingdale, NY) was used for biotinylation. This kit is less efficient than the kits we used for cultured lymphocytes and yielded lower gene expression intensities.

(Figure 1D), although the entire group of genes was not significantly shifted (Figure 3D).

Up-regulated transcripts in NC lymphocytes in low-glucose medium as compared with NC lymphocytes in normal-glucose medium had significant hits in the Gene Ontology database categories of mitochondrion ($P=.002$) and cytochrome c oxidase activity ($P=.002$), whereas down-regulated transcripts in BPD lymphocytes in low-glucose medium as compared with BPD lymphocytes in normal-glucose medium had a significant hit in the Gene Ontology database category of mitochondrion ($P=.01$). However, although the entire group of electron transfer transcripts was significantly shifted toward up-regulation in the NC lymphocytes under glucose deprivation stress (Figure 3C and Table 3), no significant shift was observed in the BPD lymphocytes under energy stress (Figure 3D and Table 3). Regulation trends were verified with qPCR (Figure 4). Four electron transfer transcripts that were used to verify the gene array data replicated the major patterns observed in the gene array analysis (Figure 4), although the levels of difference seen in the gene expression microarray study are at the threshold of detectability for qPCR. When the analysis was limited to paired samples (n=13 for subjects with BPD, n=7 for NCs; see Table 1 for pairs), 15 transcripts showed high between-group variability as determined in a factorial analysis of variance (eTable 2 [http://www.archgenpsychiatry.com]). These 15 transcripts were averaged and plotted (**Figure 6**). In BPD lymphocytes, these transcripts were down-regulated under low-glucose stress ($P\leq.003$, paired t test), whereas in NC lymphocytes, these transcripts were up-regulated ($P\leq.02$, paired t test). In the paired samples, a comparison of NC and BPD lymphocyte mRNA expression levels in low glucose showed that 17 transcripts were expressed significantly lower in

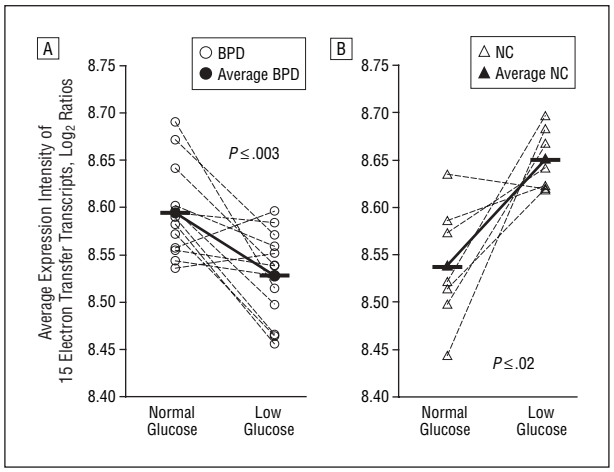

Figure 6. Pairwise comparison of 13 bipolar disorder (BPD) lymphocyte samples in normal and low-glucose medium (A) as well as 7 normal control (NC) lymphocyte samples in normal and low-glucose medium (B). Analysis of variance filtering (factorial analysis of variance, glucose concentration × treatment) was used to select electron transport transcripts with high variations between the groups. Fifteen transcripts survived the filtering and their logarithm-transformed values were averaged for each paired sample (n=13 for BPD; n=7 for NCs). Bipolar disorder lymphocytes showed a down-regulation of these transcripts under low-glucose stress ($P\leq.003$, paired t test), whereas NC lymphocytes showed an up-regulation of these transcripts ($P\leq.02$, paired t test). Dashed line indicates pair; solid line, average of group.

BPD lymphocytes, similar to the larger sample. Finally, no significant relationship between electron transfer transcript expression and medication was found when mitochondrial expression levels obtained in the gene arrays were plotted against drug treatment in a hierarchical cluster analysis or when analyses of variance were calculated (each group of drug compared with absence of that drug in low and normal glucose) using qPCR data (data not shown).

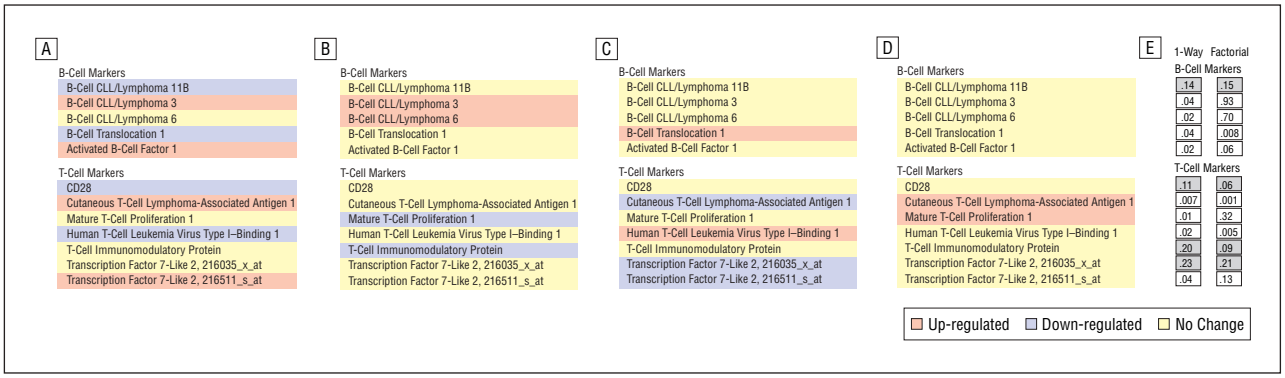

Figure 7. Individual B-cell and T-cell markers that were regulated in the comparison between low glucose for bipolar disorder lymphocytes and low glucose for normal control lymphocytes (A), normal glucose for bipolar disorder lymphocytes and normal glucose for control lymphocytes (B), low and normal glucose for normal control lymphocytes (C), and low and normal glucose for bipolar disorder lymphocytes (D). E, *P* values of 1-way and factorial analyses (glucose level × treatment); shading indicates that the analysis of variance did not reach significance in both the 1-way and factorial analyses.

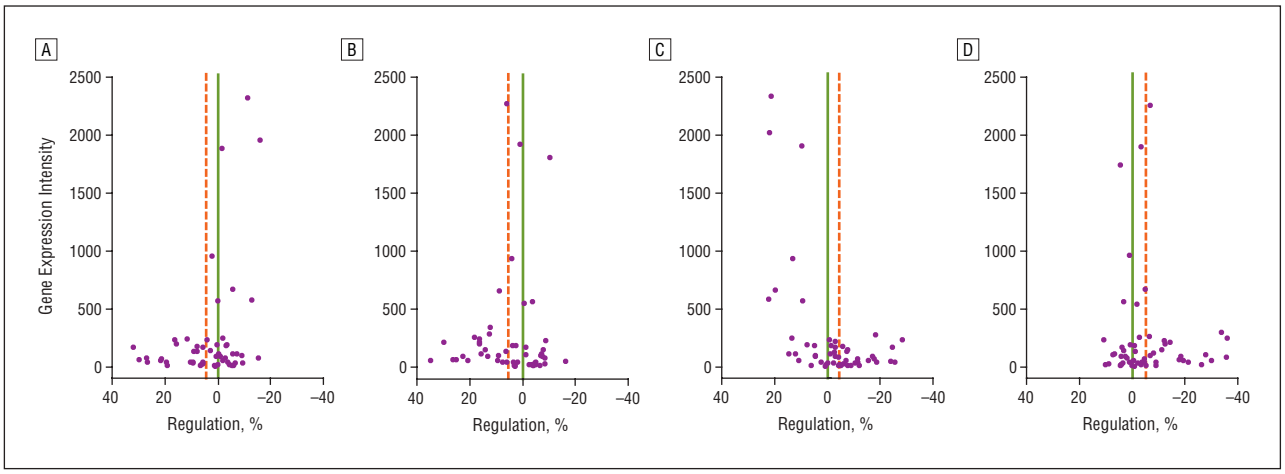

Figure 8. Regulation of the entire group of 54 B-cell markers. Expression levels of each individual probe set were compared between low glucose for bipolar disorder lymphocytes and low glucose for normal control lymphocytes (A), normal glucose for bipolar disorder lymphocytes and normal glucose for normal control lymphocytes (B), low and normal glucose for normal control lymphocytes (C), and low and normal glucose for bipolar disorder lymphocytes (D). Green line indicates equal regulation; red line, actual average regulation of all transcripts. See eTable 3 (http://www.archgenpsychiatry.com) for all GeneID numbers.

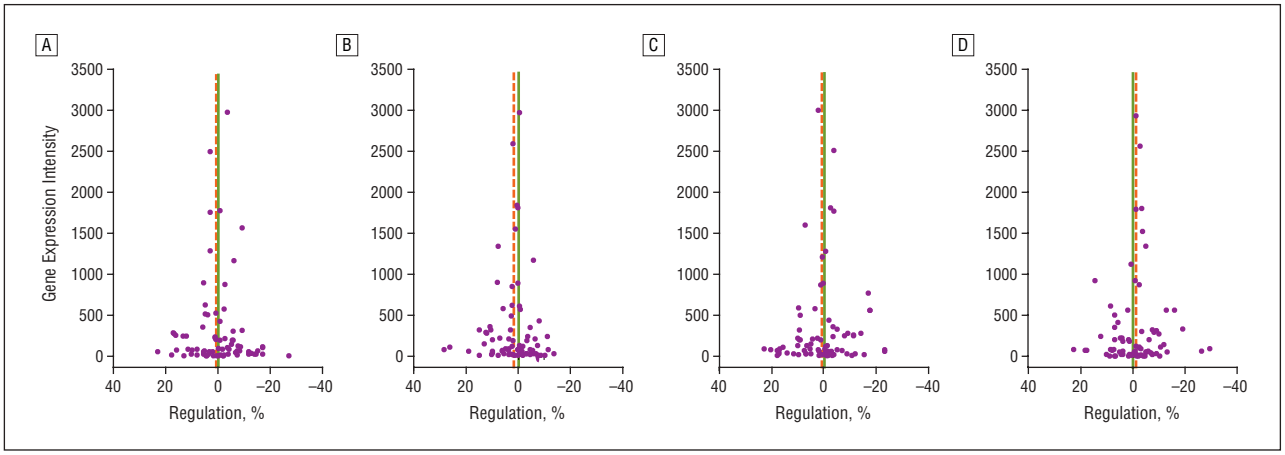

Figure 9. Regulation of the entire group of 77 T-cell markers. Expression levels of each individual probe set were compared between low glucose for bipolar disorder lymphocytes and low glucose for normal control lymphocytes (A), normal glucose for bipolar disorder lymphocytes and normal glucose for normal control lymphocytes (B), low and normal glucose for normal control lymphocytes (C), and low and normal glucose for bipolar disorder lymphocytes (D). Green line indicates equal regulation; red line, actual average regulation of all transcripts. See eTable 3 (http://www.archgenpsychiatry.com) for all GeneID numbers.

To determine whether a shift between B and T cells had taken place in any of the comparisons, the expression levels of 54 B-cell–specific transcripts and 77 T-cell–specific transcripts were examined (**Figures 7**, **8**, and **9**; see eTable 3 [http://www.archgenpsychiatry.com] for transcripts). The percentage of individually regulated genes

did not surpass the chance expectations in any of the comparisons (Figure 7; see Figure 2 for chance expectations), and the group of B-cell–specific (Figure 8) and T-cell–specific (Figure 9) transcripts was not significantly shifted. In addition, 5 marker genes for natural killer lymphocytes and 5 marker genes for monocytes were unchanged in all of the comparisons. Sixteen marker genes for granulocytes were examined as well; however, most were under the detection limit and none were affected by any condition.

COMMENT

Lymphocytes of NCs responded to low-glucose stress with an up-regulation of nuclear transcripts of the mitochondrial respiratory chain. Individual transcripts were significantly up-regulated, and the group of all transcripts for proteins of the mitochondrial respiratory chain was shifted toward higher expression levels. Lymphocytes of patients with BPD did not have the same response. Indeed, a number of individual mitochondrial transcripts were down-regulated in BPD lymphocytes in response to low-glucose stress, a finding mirrored by the pairwise comparison, although the group of mitochondrial respiratory chain transcripts as a whole was not changed much. Because marker genes for B and T cells did not change in a similar manner, it is unlikely that the changes were owing to a shift between B and T cells.

Are lymphocytes a good model for the brain? Obviously, the best model tissue for psychiatric disorders is brain tissue. However, human brain tissue is not easily accessible, it does not permit experimental manipulations, and the results from molecular analyses can be adversely influenced by postmortem handling. Lymphocytes can be readily harvested, they are easier to handle than postmortem brain tissue, and their gene expression levels can be informative about gene expression in the central nervous system.[14] Thus, they could reveal biomarkers of psychiatric disorders.[10] Indeed, the lower expression of nuclear genes of the mitochondrial respiratory chain in lymphocytes in BPD, observed here under low-glucose stress, is similar to our previous findings in hippocampal tissue.[9] Although the data in the hippocampus originally led us to hypothesize that patients with BPD have lower expression levels of these genes, the lymphocyte data suggest that NCs might have had an up-regulation in response to energy deprivation of the tissue during or immediately after death.

A potential confounding effect on gene expression in the patients with BPD is the exposure to psychotropic drugs. However, no single agent was present in more than 30% of all patients with BPD and medications were diverse, including lithium carbonate, valproic acid, anticonvulsants, antidepressants, and antipsychotics. We also found no significant relationship between electron transfer transcript expression and medication. Three aspects of our finding make a confounding effect of psychotropic drugs doubtful: (1) both the fresh (uncultured) lymphocytes and lymphocytes cultured in normal-glucose medium showed no difference between NCs and subjects with BPD; (2) in the pairwise comparison, the same medication profile is present in low and normal glucose samples; and (3) the lymphocytes in culture were cultured for 5 days in the absence of any drugs and had been washed 3 times before plating. The latter reasoning, however, is not particularly strong as it is well established that many psychotropic medications have a long half-life, at least in the brain.

In conclusion, we have found evidence of a functionally different response to glucose deprivation between subjects with BPD and NCs. This response is reflected in specific expression patterns of genes coding for electron transfer protein transcripts and suggests that the normal molecular adaptation to energy stress, present in NCs, is deficient in patients with BPD. No differences were observed in basal expression levels of electron transfer transcripts. Although we have only examined patients with BPD at this point, the findings raise hope that gene expression analysis in easily accessible tissue might provide helpful insights into molecular abnormalities in psychiatric disorders.

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Author Contributions: Dr Konradi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All of the statistical analyses were carried out by Dr Konradi.
Financial Disclosure: Dr Konradi and McLean Hospital have submitted a patent application on the findings presented here.
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REFERENCES

1. Post RM, Leverich GS, Altshuler LL, Frye MA, Suppes TM, Keck PE Jr, McElroy SL, Kupka R, Nolen WA, Grunze H, Walden J. An overview of recent findings of the Stanley Foundation Bipolar Network (part I). *Bipolar Disord*. 2003;5:310-319.
2. Chen YW, Dilsaver SC. Lifetime rates of suicide attempts among subjects with bipolar and unipolar disorders relative to subjects with other Axis I disorders. *Biol Psychiatry*. 1996;39:896-899.
3. Wyatt RJ, Henter I. An economic evaluation of manic-depressive illness: 1991. *Soc Psychiatry Psychiatr Epidemiol*. 1995;30:213-219.
4. MacDonald ML, Naydenov A, Chu M, Matzilevich D, Konradi C. Decrease in creatine kinase messenger RNA expression in the hippocampus and dorsolateral prefrontal cortex in bipolar disorder. *Bipolar Disord*. 2006;8:255-264.
5. Deicken RF, Weiner MW, Fein G. Decreased temporal lobe phosphomonoesters in bipolar disorder. *J Affect Disord*. 1995;33:195-199.

6. Kato T, Inubushi T, Kato N. Magnetic resonance spectroscopy in affective disorders. *J Neuropsychiatry Clin Neurosci*. 1998;10:133-147.
7. Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch Gen Psychiatry*. 2004;61:450-458.
8. Kato T, Kato N. Mitochondrial dysfunction in bipolar disorder. *Bipolar Disord*. 2000;2:180-190.
9. Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry*. 2004;61:300-308.
10. Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sasik R, Khanlou N, Han M, Liew CC, Tsuang MT. Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. *Proc Natl Acad Sci U S A*. 2005;102:15533-15538.
11. Vawter MP, Ferran E, Galke B, Cooper K, Bunney WE, Byerley W. Microarray screening of lymphocyte gene expression differences in a multiplex schizophrenia pedigree. *Schizophr Res*. 2004;67:41-52.
12. Middleton FA, Pato CN, Gentile KL, McGann L, Brown AM, Trauzzi M, Diab H, Morley CP, Medeiros H, Macedo A, Azevedo MH, Pato MT. Gene expression analysis of peripheral blood leukocytes from discordant sib-pairs with schizophrenia and bipolar disorder reveals points of convergence between genetic and functional genomic approaches. *Am J Med Genet B Neuropsychiatr Genet*. 2005;136:12-25.
13. Washizuka S, Kakiuchi C, Mori K, Tajima O, Akiyama T, Kato T. Expression of mitochondria-related genes in lymphoblastoid cells from patients with bipolar disorder. *Bipolar Disord*. 2005;7:146-152.
14. Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141:261-268.
15. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV Axis I Disorders*. New York: Biometrics Research Dept, New York State Psychiatric Institute; 1995.
16. MacDonald ML, Eaton ME, Dudman JT, Konradi C. Antipsychotic drugs elevate mRNA levels of presynaptic proteins in the frontal cortex of the rat. *Biol Psychiatry*. 2005;57:1041-1051.
17. Zhong S, Li C, Wong WH. ChipInfo: software for extracting gene annotation and gene ontology information for microarray analysis. *Nucleic Acids Res*. 2003;31:3483-3486.
18. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*. 2003;4:249-264.
19. Li C, Wong WH. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc Natl Acad Sci U S A*. 2001;98:31-36.
20. Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol*. 2003;4:R7. doi:10.1186/gb-2003-4-1-r7.
21. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 2002;3:RESEARCH0034. doi:10.1186/gb-2002-3-7-research0034.

eTable 1. All Nuclear Transcripts of the Mitochondrial Respiratory Chain Used for Analyses in Figures 1, 2, 3, 4, and 5

Gene	GenBank Accession No.	Locus Link ID No.	Affymetrix Probe Set ID No.	Low Glucose Comparison in BPD vs NC		Normal Glucose Comparison in BPD vs NC	
				Natural-Scale Fold Change	Log_2-Transformed *P* Value*	Natural-Scale Fold Change	Log_2-Transformed *P* Value*
Complex I							
NADH dehydrogenase 1 alpha, 1, 7.5 kDa	NM_004541	4694	202298_at	–1.00	.94	–1.01	.75
NADH dehydrogenase 1 alpha, 2, 8 kDa	BC003674	4695	209224_s_at	–1.06	.32	1.17	**.04**
NADH dehydrogenase 1 alpha, 3, 9 kDa	NM_004542	4696	218563_at	–1.01	.91	–1.00	.97
NADH dehydrogenase 1 alpha, 4, 9 kDa	NM_002489	4697	217773_s_at	–1.03	.39	1.02	.60
NADH dehydrogenase 1 alpha, 5, 13 kDa	NM_005000	4698	201304_at	–1.15	**.04**	–1.08	**.04**
NADH dehydrogenase 1 alpha, 5, 13 kDa	AK022209	4698	215850_s_at	–1.06	.53	–1.06	.15
NADH dehydrogenase 1 alpha, 6, 14 kDa	BC002772	4700	202000_at	–1.10	**.02**	–1.06	.34
NADH dehydrogenase 1 alpha, 6, 14 kDa	BC002772	4700	202001_s_at	–1.01	.83	1.05	.22
NADH dehydrogenase 1 alpha, 7, 14.5 kDa	NM_005001	4701	202785_at	–1.05	.61	–1.02	.70
NADH dehydrogenase 1 alpha, 8, 19 kDa	NM_014222	4702	218160_at	–1.04	.48	1.01	.83
NADH dehydrogenase 1 alpha, 9, 39 kDa	AF050641	4704	208969_at	–1.01	.97	–1.01	.63
NADH dehydrogenase 1 alpha, 10, 42 kDa	NM_004544	4705	217860_at	–1.01	.89	1.02	.73
NADH dehydrogenase 1 alpha, assembly factor 1	NM_016013	51103	204125_at	–1.02	.67	1.08	.09
NADH dehydrogenase 1 beta, 1, 7 kDa	NM_004545	4707	206790_s_at	–1.09	**.01**	–1.04	.47
NADH dehydrogenase 1 beta, 2, 8 kDa	NM_004546	4708	218200_s_at	1.02	.62	1.02	.58
NADH dehydrogenase 1 beta, 2, 8 kDa	NM_004546	4708	218201_at	–1.02	.74	1.03	.22
NADH dehydrogenase 1 beta, 3, 12 kDa	NM_002491	4709	203371_s_at	–1.05	.22	–1.00	.94
NADH dehydrogenase 1 beta, 4, 15 kDa	NM_004547	4710	218226_s_at	–1.03	.30	–1.02	.52
NADH dehydrogenase 1 beta, 5, 16 kDa	NM_002492	4711	203621_at	–1.05	.12	1.02	.50
NADH dehydrogenase 1 beta, 6, 17 kDa	NM_002493	4712	203613_s_at	–1.05	.17	–1.01	.78
NADH dehydrogenase 1 beta, 7, 18 kDa	NM_004146	4713	202839_s_at	1.00	.86	–1.08	.05
NADH dehydrogenase 1 beta, 7, 18 kDa	M33374	4713	211407_at	1.02	.60	1.02	.57
NADH dehydrogenase 1 beta, 8, 19 kDa	NM_005004	4714	201226_at	–1.02	.69	1.02	.55
NADH dehydrogenase 1 beta, 8, 19 kDa	NM_005004	4714	201227_s_at	–1.05	.34	1.00	.99
NADH dehydrogenase 1 beta, 8, 19 kDa	AA723057	4714	214241_at	1.14	.36	–1.01	.86
NADH dehydrogenase 1 beta, 11, 17.3 kDa	NM_019056	54539	218320_s_at	–1.07	.20	1.05	.22
NADH dehydrogenase 1, alpha/beta, 1, 8 kDa	NM_005003	4706	202077_at	–1.04	.31	1.00	.89
NADH dehydrogenase 1, unknown, 1, 6 kDa	NM_002494	4717	203478_at	–1.08	.07	–1.01	.70
NADH dehydrogenase 1, unknown, 2, 14.5 kDa	NM_004549	4718	218101_s_at	–1.06	.22	1.02	.79
NADH dehydrogenase Fe-S protein 1, 75 kDa	NM_005006	4719	203039_s_at	–1.05	.23	–1.04	.43
NADH dehydrogenase Fe-S protein 2, 49 kDa	NM_004550	4720	201966_at	–1.01	.54	–1.12	.18
NADH dehydrogenase Fe-S protein 3, 30 kDa	NM_004551	4722	201740_at	–1.01	.77	1.02	.44
NADH dehydrogenase Fe-S protein 4, 18 kDa	BC005270	4724	209303_at	–1.08	.08	–1.00	.98
NADH dehydrogenase Fe-S protein 5, 15 kDa	NM_004552	4725	201757_at	–1.06	.20	–1.03	.28
NADH dehydrogenase Fe-S protein 6, 13 kDa	NM_004553	4726	203606_at	–1.02	.77	1.02	.74
NADH dehydrogenase Fe-S protein 7, 20 kDa	BC005954	374291	211752_s_at	–1.02	.71	–1.05	.19
NADH dehydrogenase Fe-S protein 8, 23 kDa	NM_002496	4728	203189_s_at	–1.03	.38	–1.00	.98
NADH dehydrogenase Fe-S protein 8, 23 kDa	NM_002496	4728	203190_at	–1.03	.30	–1.01	.66
NADH dehydrogenase flavoprotein 1, 51 kDa	AF092131	4723	208714_at	1.02	.56	1.04	.39
NADH dehydrogenase flavoprotein 2, 24 kDa	NM_021074	4729	202941_at	1.03	.46	–1.03	.61
Complex II							
Succinate dehydrogenase complex, A, flavoprotein (Fp)	NM_004168	6389	201093_x_at	1.04	.34	1.01	.82
Succinate dehydrogenase complex, A, flavoprotein (Fp)	AI348006	255812, 6389	222021_x_at	–1.02	.58	–1.03	.50
Succinate dehydrogenase complex, B, iron sulfur (Ip)	NM_003000	6390	202675_at	1.03	.49	1.00	.90
Succinate dehydrogenase complex, B, iron sulfur (Ip)	AW294107	6390	214166_at	1.02	.87	1.07	.18
Succinate dehydrogenase complex, C, 15 kDa	NM_003001	6391	202004_x_at	1.02	.74	–1.12	.10
Succinate dehydrogenase complex, C, 15 kDa	BG110532	6391	215088_s_at	–1.05	.28	1.04	.33
Succinate dehydrogenase complex, C, 15 kDa	AF080579	6391	216591_s_at	–1.15	.44	–1.16	.43
Succinate dehydrogenase complex, D	NM_003002	6392	202026_at	–1.04	.34	1.10	.07

(continued)

eTable 1. All Nuclear Transcripts of the Mitochondrial Respiratory Chain Used for Analyses in Figures 1, 2, 3, 4, and 5 (cont)

Gene	GenBank Accession No.	Locus Link ID No.	Affymetrix Probe Set ID No.	Low Glucose Comparison in BPD vs NC: Natural-Scale Fold Change	Low Glucose Comparison in BPD vs NC: Log_2-Transformed *P* Value*	Normal Glucose Comparison in BPD vs NC: Natural-Scale Fold Change	Normal Glucose Comparison in BPD vs NC: Log_2-Transformed *P* Value*
Complex III							
Ubiquinol–cytochrome c reductase binding protein	NM_006294	7381	205849_s_at	–1.06	.06	1.02	.39
Ubiquinol–cytochrome c reductase binding protein	BC005230	7381	209065_at	–1.20	<**.001**	1.07	.29
Ubiquinol–cytochrome c reductase binding protein	M26700	7381	209066_x_at	–1.06	**.03**	1.01	.69
Ubiquinol–cytochrome c reductase complex (7.2 kDa)	NM_013387	29796	218190_s_at	–1.02	.67	–1.01	.83
Ubiquinol–cytochrome c reductase core protein I	NM_003365	7384	201903_at	1.07	.21	–1.07	.12
Ubiquinol–cytochrome c reductase core protein II	NM_003366	7385	200883_at	–1.09	**.04**	–1.04	.56
Ubiquinol–cytochrome c reductase core protein II	AV727381	7385	212600_s_at	–1.07	.07	–1.05	.33
Ubiquinol–cytochrome c reductase hinge protein	NM_006004	7388	202233_s_at	–1.09	**.03**	1.01	.85
Ubiquinol–cytochrome c reductase, 6.4 kDa	NM_006830	10975	202090_s_at	–1.06	.17	1.03	.43
Ubiquinol–cytochrome c reductase, Rieske iron-sulfur 1	BC000649	7386	208909_at	–1.01	.56	–1.04	.16
Complex IV							
Cytochrome c oxidase IV	AA854966	1327	200086_s_at	–1.08	<**.001**	1.01	.72
Cytochrome c oxidase IV	NM_001861	1327	202698_x_at	–1.05	**.01**	–1.04	.10
Cytochrome c oxidase IV	AW337510	1327	213758_at	–1.03	.73	1.13	**.01**
Cytochrome c oxidase Va	NM_004255	9377	203663_s_at	–1.00	.98	–1.02	.70
Cytochrome c oxidase Vb	NM_001862	1329	202343_x_at	1.01	.75	–1.02	.52
Cytochrome c oxidase Vb	BC006229	1329	211025_x_at	–1.02	.86	–1.03	.53
Cytochrome c oxidase Vb	AI557312	1329	213735_s_at	–1.04	.57	1.01	.84
Cytochrome c oxidase Vb	AI557312	1329	213736_at	–1.25	.19	1.07	.69
Cytochrome c oxidase VIa 1	NM_004373	1337	200925_at	1.00	.86	1.02	.56
Cytochrome c oxidase VIa 2	NM_005205	1339	206353_at	1.01	.75	–1.03	.62
Cytochrome c oxidase VIb 1 (ubiquitous)	NM_001863	1340	201441_at	–1.03	.56	–1.01	.75
Cytochrome c oxidase VIc	NM_004374	1345	201754_at	–1.06	.10	–1.03	.38
Cytochrome c oxidase VIIa 2 (liver)	NM_001865	1347	201597_at	–1.02	.68	1.11	**.02**
Cytochrome c oxidase VIIa 2 like	NM_004718	9167	201256_at	–1.08	**.01**	–1.05	**.04**
Cytochrome c oxidase VIIb	NM_001866	1349	202110_at	–1.00	.96	1.05	.27
Cytochrome c oxidase VIIc	NM_001867	1350	201134_x_at	–1.05	.14	–1.01	.77
Cytochrome c oxidase VIIc	AA382702	1350	213846_at	–1.09	.16	–1.01	.82
Cytochrome c oxidase VIIc	AF042165	1350	217491_x_at	–1.07	**.02**	1.01	.75
Cytochrome c oxidase 8A (ubiquitous)	NM_004074	1351	201119_s_at	1.01	.80	1.03	.34
Cytochrome c, somatic	BC005299	54205	208905_at	–1.03	.57	–1.00	.98
Cytochrome c-1	NM_001916	1537	201066_at	1.07	.16	1.02	.49
COX10 homolog	NM_001303	1352	203858_s_at	1.00	.99	1.03	.56
COX11 homolog	NM_004375	1353	203551_s_at	–1.15	.13	–1.00	.94
COX11 homolog	BC005895	1353	211727_s_at	–1.12	**.01**	1.03	.59
COX15 homolog	NM_004376	1355	219547_at	–1.04	.39	1.06	.13
COX15 homolog	BC002382	1355	221550_at	–1.15	**.04**	–1.02	.95
Complex V							
ATP synthase mitochondrial F1 complex assembly factor 2	AW118608	91647	213057_at	1.09	.16	1.09	.09
ATP synthase mitochondrial F1 complex assembly factor 2	AF070584	91647	214330_at	1.03	.55	1.02	.62
ATP synthase, alpha, cardiac muscle	AI587323	498	213738_s_at	–1.03	.15	–1.01	.69
ATP synthase, b	BC005960	515	211755_s_at	–1.01	.69	–1.02	.48
ATP synthase, beta	NM_001686	506	201322_at	1.03	.27	–1.02	.57
ATP synthase, c (subunit 9)	AL080089	516	208972_s_at	–1.01	.90	1.02	.79

(continued)

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				Natural-Scale Fold Change	Log_2-Transformed *P* Value*	Natural-Scale Fold Change	Log_2-Transformed *P* Value*
Complex V (cont)							
ATP synthase, c (subunit 9) isoform 2	D13119	517	208764_s_at	–1.07	**.04**	1.00	.97
ATP synthase, c (subunit 9) isoform 3	NM_001689	518	207507_s_at	–1.02	.73	–1.01	.92
ATP synthase, c (subunit 9) isoform 3	NM_001689	518	207508_at	–1.00	.93	1.01	.80
ATP synthase, d	AF061735	10476	210149_s_at	–1.05	.29	–1.01	.73
ATP synthase, delta	NM_001687	513	203926_x_at	1.01	.90	1.02	.73
ATP synthase, delta	BE798517	513	213041_s_at	1.05	.40	1.06	.28
ATP synthase, e	NM_007100	521	207335_x_at	–1.08	.14	–1.01	.89
ATP synthase, e	BC003679	521	209492_x_at	–1.05	.31	–1.02	.51
ATP synthase, epsilon	NM_006886	514	217801_at	–1.06	.07	1.06	.10
ATP synthase, f, isoform 2	NM_004889	9551	202961_s_at	–1.00	.97	–1.04	.38
ATP synthase, F6	NM_001685	522	202325_s_at	–1.04	.38	1.02	.51
ATP synthase, g	NM_006476	10632	207573_x_at	–1.03	.37	1.02	.66
ATP synthase, g	AA917672	10632	208745_at	–1.14	**.001**	1.03	.54
ATP synthase, g	AF070655	10632	208746_x_at	–1.04	.23	1.00	.93
ATP synthase, g	AL050277	10632	210453_x_at	–1.04	.25	1.00	.98
ATP synthase, gamma 1	NM_005174	509	205711_x_at	–1.00	.89	–1.05	.06
ATP synthase, gamma 1	BC000931	509	208870_x_at	1.01	.72	–1.03	.18
ATP synthase, gamma 1	AV711183	509	213366_x_at	–1.02	.65	–1.03	.21
ATP synthase, gamma 1	BG232034	509	214132_at	–1.12	.43	1.05	.40
ATP synthase, O (oligomycin sensitivity–conferring protein)	NM_001697	539	200818_at	–1.09	**.001**	–1.04	.29
ATP synthase, O (oligomycin sensitivity–conferring protein)	S77356	539	216954_x_at	–1.16	**.001**	1.01	.74
ATP synthase, s (factor B)	NM_015684	27109	206992_s_at	–1.08	.08	–1.09	.09
ATP synthase, s (factor B)	NM_015684	27109	206993_at	–1.15	**.01**	1.08	.22
ATP synthase, s (factor B)	AW195882	27109	213995_at	–1.11	.10	1.03	.56

Abbreviations: ATP, adenosine triphosphate; BPD, bipolar disorder; COX, cytochrome c oxidase; Fe-S, iron-sulfur; ID, identification; NADH, reduced nicotinamide adenine dinucleotide; NC, normal control.

*Boldface type indicates statistical significance.

eTable 2. Fifteen Mitochondrial Transcripts Used for Paired Comparisons

Gene	Locus Link ID No.	Affymetrix Probe Set No.	BPD Comparison of Normal vs Low Glucose		NC Comparison of Normal vs Low Glucose		ANOVA, Diagnosis × Glucose Concentration	
			Fold Change	*P* Value*	Fold Change	*P* Value*	F Statistic	*P* Value*
Complex I								
NADH dehydrogenase 1 alpha, 5, 13 kDa	4698	201304_at	–1.15	**.02**	1.25	.06	13.0	**.001**
NADH dehydrogenase 1 beta, 1, 7 kDa	4707	206790_s_at	–1.04	.18	1.07	.08	6.9	**.01**
Complex II								
Succinate dehydrogenase, D	6392	202026_at	–1.09	.07	1.07	.20	5.0	**.03**
Complex III								
Ubiquinol–cyt c reductase binding protein	7381	205849_s_at	–1.04	.12	1.07	.08	7.6	**.009**
Ubiquinol–cyt c reductase binding protein	7381	209065_at	–1.09	.07	1.18	**.03**	11.4	**.002**
Ubiquinol–cyt c reductase core protein I	7384	201903_at	1.07	.13	–1.11	.17	5.3	**.03**
Complex IV								
COX11	1353	211727_s_at	–1.04	.45	1.14	**.007**	5.4	**.03**
COX IV-1	1327	200086_s_at	–1.01	.79	1.11	**.04**	7.6	**.009**
COX VIIa-1 (muscle)	1346	204570_at	1.12	.08	–1.11	.17	5.4	**.03**
COX VIIa-2 (liver)	1347	201597_at	–1.06	.10	1.1	.08	7.3	**.01**
COX VIIc	1350	217491_x_at	–1.02	.50	1.08	.07	5.4	**.03**
Complex V								
ATP synthase, F0 complex, g	10632	208745_at	–1.05	.23	1.11	**.03**	7.1	**.01**
ATP synthase, F0 complex, s (factor B)	27109	206993_at	–1.12	**.03**	1.02	.78	5.9	**.02**
ATP synthase, F1 complex, epsilon	514	217801_at	–1.1	**.001**	1.07	.12	14.6	**.001**
ATP synthase, F1 complex, O (OSCP)	539	216954_x_at	–1.07	**.04**	1.16	**.02**	6.4	**.02**

Abbreviations: ANOVA, analysis of variance; ATP, adenosine triphosphate; BPD, bipolar disorder; COX, cytochrome c oxidase; cyt c, cytochrome c; ID, identification; NADH, reduced nicotinamide adenine dinucleotide; NC, normal control; OSCP, oligomycin sensitivity–conferring protein.

*Boldface type indicates statistical significance.

eTable 3. All Transcripts Specific for B and T Cells Used for Analyses in Figures 7, 8, and 9

Gene	GenBank Accession No.	Gene ID No.	Affymetrix Probe Set ID No.
B-Cell Markers			
B-cell CLL/lymphoma 10	AF082283	8915	205263_at
B-cell CLL/lymphoma 11A (zinc finger protein)	AF080216	53335	210347_s_at
B-cell CLL/lymphoma 11A (zinc finger protein)	NM_018014	53335	219497_s_at
B-cell CLL/lymphoma 11A (zinc finger protein)	NM_018014	53335	219498_s_at
B-cell CLL/lymphoma 11B (zinc finger protein)	NM_022898	64919	219528_s_at
B-cell CLL/lymphoma 2	M13994	596	203684_s_at
B-cell CLL/lymphoma 2	NM_000633	596	203685_at
B-cell CLL/lymphoma 3	NM_005178	602	204908_s_at
B-cell CLL/lymphoma 6 (zinc finger protein 51)	NM_001706	604	203140_at
B-cell CLL/lymphoma 6 (zinc finger protein 51)	S67779	604	215990_s_at
B-cell CLL/lymphoma 7A	NM_020993	605	203795_s_at
B-cell CLL/lymphoma 7A	NM_020993	605	203796_s_at
B-cell CLL/lymphoma 7B	NM_001707	9275	202518_at
B-cell CLL/lymphoma 7C	NM_004765	9274	219072_at
B-cell CLL/lymphoma 9	NM_004326	607	204129_at
B-cell linker	NM_013314	29760	207655_s_at
B-cell receptor-associated protein 29	NM_018844	55973	205084_at
B-cell receptor-associated protein 29	AL583687	55973	217657_at
B-cell receptor-associated protein 29	AI393960	55973	217662_x_at
B-cell receptor-associated protein 31	NM_005745	10134	200837_at
B-cell scaffold protein with ankyrin repeats 1	NM_017935	55024	219667_s_at
B-cell translocation gene 1, antiproliferative	AL535380	694	200920_s_at
B-cell translocation gene 1, antiproliferative	NM_001731	694	200921_s_at
Cardiotrophin-like cytokine factor 1	NM_013246	23529	219500_at
CD19 antigen	NM_001770	930	206398_s_at
CD22 antigen	NM_001771	4099, 933	204581_at
CD40 antigen (TNF receptor superfamily member 5)	NM_001250	958	205153_s_at
CD40 antigen (TNF receptor superfamily member 5)	BF664114	958	215346_at
CD40 antigen (TNF receptor superfamily member 5)	X60592	958	35150_at
CD48 antigen (B-cell membrane protein)	NM_001778	962	204118_at
CD80 antigen (CD28 antigen ligand 1, B7-1 antigen)	NM_005191	941	207176_s_at
CD83 antigen (activated B lymphocytes, immunoglobulin)	NM_004233	9308	204440_at
CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	BG236280	942	205685_at
CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	NM_006889	942	205686_s_at
CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	L25259	942	210895_s_at
Interleukin 4 receptor	NM_000418	3566	203233_at
Membrane-spanning 4-domains, subfamily A, member 1	BC002807	931	210356_x_at
Membrane-spanning 4-domains, subfamily A, member 1	X12530	931	217418_x_at
Musculin (activated B-cell factor-1)	AF060154	9242	209928_s_at
Paired box gene 5 (B-cell lineage specific activator)	BF510692	5079	221969_at
Pre-B-cell colony enhancing factor 1	NM_005746	10135	217738_at
Pre-B-cell colony enhancing factor 1	NM_005746	10135	217739_s_at
Pre-B-cell leukemia transcription factor 1	BF967998	5087	212151_at
Pre-B-cell leukemia transcription factor 2	BE397715	5089	202875_s_at
Pre-B-cell leukemia transcription factor 2	NM_002586	5089	202876_s_at
Pre-B-cell leukemia transcription factor 2	BC003111	5089	211096_at
Pre-B-cell leukemia transcription factor 2	BC003111	5089	211097_s_at
Pre-B-cell leukemia transcription factor 3	NM_006195	5090	204082_at
Pre-B-cell leukemia transcription factor interacting protein 1	NM_020524	57326	207838_x_at
Pre-B-cell leukemia transcription factor interacting protein 1	BF344265	57326	212259_s_at
Pre-B-cell leukemia transcription factor interacting protein 1	AI348545	57326	214176_s_at
Pre-B-cell leukemia transcription factor interacting protein 1	AI935162	57326	214177_s_at
Prohibitin 2	NM_007273	11331	201600_at
Tumor necrosis factor receptor superfamily, member 17	NM_001192	608	206641_at
T-Cell Markers			
CD2 antigen (p50), sheep red blood cell receptor	NM_001767	914	205831_at
CD28 antigen (Tp44)	NM_006139	940	206545_at
CD28 antigen (Tp44)	AF222341	940	211856_x_at
CD28 antigen (Tp44)	AF222343	940	211861_x_at
CD3Z antigen, zeta polypeptide (TiT3 complex)	J04132	919	210031_at
CD4 antigen (p55)	U47924	920	203547_at

(continued)

eTable 3. All Transcripts Specific for B and T Cells Used for Analyses in Figures 7, 8, and 9 (cont)

Gene	GenBank Accession No.	Gene ID No.	Affymetrix Probe Set ID No.
T-Cell Markers (cont)			
CD5 antigen (p56-62)	NM_014207	921	206485_at
CD6 antigen	NM_006725	923	208602_x_at
CD6 antigen	U66145	923	211893_x_at
CD6 antigen	U66146	923	211900_x_at
CD6 antigen	AW134823	923	213958_at
CD69 antigen (p60, early T-cell activation antigen)	L07555	969	209795_at
CD8 antigen, alpha polypeptide (p32)	AW006735	925	205758_at
Cutaneous T-cell lymphoma-associated antigen 1	NM_022663	64693	220957_at
Expressed in T-cells and eosinophils in atopic dermatitis	AB020694	23197	212106_at
Expressed in T-cells and eosinophils in atopic dermatitis	AB020694	23197	212108_at
Frequently rearranged in advanced T-cell lymphomas	NM_005479	10023	219889_at
Frequently rearranged in advanced T-cell lymphomas 2	AB045118	23401	209864_at
Granulysin	NM_006433	10578	205495_s_at
Granulysin	M85276	10578	37145_at
Human T-cell leukemia virus enhancer factor	NM_002158	3344	206708_at
IL2-inducible T-cell kinase	D13720	3702	211339_s_at
Inducible T-cell costimulator	AB023135	29851	210439_at
Inducible T-cell costimulator ligand	AL355690	23308	211197_s_at
Mal, T-cell differentiation protein	NM_002371	4118	204777_s_at
Mature T-cell proliferation 1	NM_014221	4515	205106_at
Mature T-cell proliferation 1	BC002600	4515	210212_x_at
Mature T-cell proliferation 1	Z24459	4515	216862_s_at
Pre-T-cell antigen receptor alpha	U36759	171558	211252_x_at
Pre-T-cell antigen receptor alpha	AL035587	171558	215492_x_at
Rearranged T-cell receptor alpha chain mRNA, variable region	AE000659	NA	217412_at
Sirtuin (silent mating type information regulation 2 homolog) 6	NM_016539	51548	219613_s_at
T-cell receptor alpha constant	M12959	28755	209670_at
T-cell receptor alpha locus	L34703	6955	211902_x_at
T-cell receptor alpha locus	AW873544	6955	215769_at
T-cell receptor alpha locus	X61070	6955	217056_at
T-cell receptor alpha locus	AE000659	6955	217394_at
T-cell receptor alpha locus	AW966434	28517, 28663, 28738, 28755, 348035, 6955	215524_x_at
T-cell receptor alpha locus	M15565	28517, 28663, 28738, 28755, 6955	210972_x_at
T-cell receptor alpha locus	M12423	28755, 6955	209671_x_at
T-cell receptor alpha locus, T-cell receptor delta locus	X72501	6955, 6964	216191_s_at
T-cell receptor alpha variable 20	BF976764	28663	215796_at
T-cell receptor-associated transmembrane adaptor 1	AJ240085	50852	217147_s_at
T-cell receptor beta constant 1	M15564	28568, 28639	210915_x_at
T-cell receptor gamma constant 2	M30894	6967	211144_x_at
T-cell receptor gamma constant 2	M16768	442532, 442670, 445347, 6967, 6983	209813_x_at
T-cell receptor gamma constant 2	M13231	442532, 442670, 445347, 6967, 6983	215806_x_at
T-cell receptor gamma constant 2	M27331	442532, 442670, 445347, 6967, 6983	216920_s_at
T-cell receptor V alpha gene segment V-alpha-w23, clone IGRa01	AA284903	NA	216133_at
T-cell receptor V alpha gene segment V-alpha-w24, clone IGRa02	AE000659	NA	217397_at
Tax1 (human T-cell leukemia virus type I) binding protein 1	AF090891	8887	200976_s_at
Tax1 (human T-cell leukemia virus type I) binding protein 1	AF090891	8887	200977_s_at
Tax1 (human T-cell leukemia virus type I) binding protein 1	AI935415	8887	213786_at
Tax1 (human T-cell leukemia virus type I) binding protein 3	AF234997	30851	209154_at
Tax1 (human T-cell leukemia virus type I) binding protein 3	AK001327	30851	215459_at
Tax1 (human T-cell leukemia virus type I) binding protein 3	AK001327	30851	215464_s_at

(continued)

eTable 3. All Transcripts Specific for B and T Cells Used for Analyses in Figures 7, 8, and 9 (cont)

Gene	GenBank Accession No.	Gene ID No.	Affymetrix Probe Set ID No.
T-Cell Markers (cont)			
T-cell acute lymphocytic leukemia 1	NM_003189	6886	206283_s_at
T-cell immunomodulatory protein	NM_030790	81533	221449_s_at
T-cell leukemia translocation altered gene	NM_022171	6988	203054_s_at
T-cell leukemia/lymphoma 1A	BC003574	8115	209995_s_at
T-cell leukemia/lymphoma 1A	X82240	8115	39318_at
T-cell lymphoma invasion and metastasis 1	NM_003253	7074	206409_at
T-cell lymphoma invasion and metastasis 1	U90902	7074	213135_at
T-cell receptor active alpha-chain V-region	L34698	NA	211667_x_at
T-cell receptor active alpha-chain V-region	AE000659	NA	217170_at
T-cell receptor active beta-chain (V10-D-J-C) mRNA, clone PL3.9	L48728	NA	216857_at
T-cell receptor alpha chain	X61079	NA	217063_x_at
T-cell receptor V alpha 14.1/J alpha 32/C alpha	X61072	NA	216540_at
Transcription factor 7 (T-cell specific, HMG-box)	AW027359	6932	205254_x_at
Transcription factor 7 (T-cell specific, HMG-box)	NM_003202	6932	205255_x_at
Transcription factor 7-like 2 (T-cell specific, HMG-box)	AI703074	6934	212761_at
Transcription factor 7-like 2 (T-cell specific, HMG-box)	AI375916	6934	212762_s_at
Transcription factor 7-like 2 (T-cell specific, HMG-box)	AV721430	6934	216035_x_at
Transcription factor 7-like 2 (T-cell specific, HMG-box)	AA664011	6934	216037_x_at
Transcription factor 7-like 2 (T-cell specific, HMG-box)	AJ270770	6934	216511_s_at
TSPY-like 2	NM_022117	64061	218012_at
Vac14 homolog	U25801	55697	216407_at

Abbreviations: CLL, chronic lymphocytic leukemia; HMG, high-mobility group; ID, identification; IL2, interleukin 2; mRNA, messenger RNA; NA, not available; TNF, tumor necrosis factor; TSPY, testis-specific protein, Y-linked; Vac, vacuole morphology.