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Increased peripheral blood expression of electron transport chain genes in bipolar depression

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Abstract

Objective—To identify specific genetic pathways showing altered expression in peripheral blood of depressed subjects with bipolar disorder (BPD).

Methods—Illumina Sentrix BeadChip (Human-6v2) microarrays containing > 48,000 transcript probes were used to measure levels of gene expression in peripheral blood from 20 depressed subjects with BPD and in 15 healthy control subjects. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was used to confirm a subset of these differences.

Results—A total of 1,180 genes were differentially expressed between subjects with BPD and healthy controls (fold-change > 1.3, false discovery rate-corrected $p < 0.05$, covaried for age and sex). Of these, 559 genes were up-regulated in BPD subjects and 621 were down-regulated. Surprisingly, there was no difference between medicated ($n = 11$) and unmedicated ($n = 9$) subjects with BPD for any of these genes. Pathway analysis using GeneGo MetaCore software showed that the most significantly affected pathway was the mitochondrial electron transport chain (ETC). Of the 85 objects (genes or proteins) in this pathway, 22 were up-regulated and 2 down-regulated in subjects with BPD. qRT-PCR confirmed up-regulation of nuclear encoded ETC genes in complexes I, III, IV, and V and, in addition, demonstrated up-regulation of mitochondrially encoded genes in each of these complexes.

Conclusion—These results suggest that increased expression of multiple components of the mitochondrial ETC may be a primary deficit in bipolar depression, rather than an effect of medication.

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Keywords

bipolar disorder; gene expression; microarray; mitochondria; oxidative phosphorylation; qRT-PCR

Bipolar disorder (BPD) is a devastating neurobiological illness affecting from 0.8–1.2% of the population (1–3). Clinically, the disorder is characterized by episodes of mania and major depression. However, as with the vast majority of psychiatric illnesses, the nature of the underlying pathophysiology remains unknown (4). Neuroanatomical studies of patients with BPD have demonstrated structural abnormalities including reduced numbers of glia in the subgenual prefrontal cortex (5) and decreased neuronal and glial density in the dorsolateral prefrontal cortex (6), as well as alterations in neuropeptides and monoamine metabolites (7). However, the relationship between these findings and changes in mood state and treatment response remains unknown.

More recently, genome-wide association studies (GWAS) have been used to identify genetic variants that may contribute to the risk for the development of BPD (8–14). Genes with replicated associations to BPD from GWAS include the L-type voltage-dependent calcium channel, alpha 1C subunit (CACNA1C) and ankyrin 3 (ANK3) genes (15). However, effect sizes for these genes are small (e.g., odds ratios of ~1.4 at ANK3 and ~1.2 at CACNA1C), and polymorphisms in or near these genes are currently of no use in individual risk prediction (16). Moreover, we do not yet understand how these genetic variants relate to either the neuroanatomical changes discussed above or specific symptoms in patients with BPD.

Multiple lines of evidence have suggested that alterations in energy metabolism may play a role in the pathogenesis of BPD (17–20). These include studies showing decreased levels of phospho-creatine in the frontal lobes of patients with bipolar depression (19,20), increased levels of lactate in cerebrospinal fluid of patients with BPD (21), and decreased levels of messenger RNA (mRNA) for creatinine kinase (22). In mammalian cells, energy is generated by oxidative phosphorylation, which is carried out by the mitochondrial electron transport chain (ETC) [reviewed in Lenaz and Genova (23)]. Several groups have described abnormal expression of ETC genes in postmortem brain tissue of subjects with BPD (24–26). Abnormal expression of ETC genes has also been reported in response to glucose deprivation in cultured cells derived from patients with BPD (27).

While these studies all point to dysregulation of energy metabolism as a contributing factor in the pathogenesis of BPD, the reported direction of change has not always been consistent. For example, Konradi et al. (24), Iwamoto et al. (25), and Sun et al. (26) have all reported down-regulation of genes encoding components of the mitochondrial ETC in postmortem brain tissue of subjects with BPD. However, in the small subgroup of unmedicated subjects with BPD ($n = 4$) in the study by Iwamoto et al. (25), this trend was reversed, with most of the mitochondrial genes (85.2%) showing up-regulation compared with controls. This same study found that each of the medication groups examined (antidepressants, valproate, and antipsychotics) appeared to have a significant global repressive effect on the expression of mitochondria-related genes. Ben-Shachar and Karry (28) found that there was region-specific variation in the expression of mitochondrial genes in patients with BPD, with increased expression in the parieto-occipital cortex, but reductions in the cerebellum. Nakatani et al. (29) reported increased expression of one ETC gene (NDUFV2) in the dorsolateral cortex (BA 46) of subjects with BPD, but no change in any other ETC genes. Others have suggested that the differences in mitochondrial gene expression observed in these studies may be largely accounted for by differences in brain pH at the time of death

(30). Thus, whether and in which direction the expression of mitochondrial genes is altered in BPD remains an area of active investigation.

It is now broadly accepted that changes in gene expression in the brain are related to long-term neuroplasticity underlying both the pathophysiology of psychiatric disorders and the mechanism of action of many of the agents used to treat them (31–34). However, with current technologies it is impossible to assess the time course of such changes in the brains of living subjects. Peripheral cells, including lymphocytes, express many of the same signaling molecules implicated as possible mediators of the long-term effects of both antidepressants and mood stabilizers. These include the CREB (35), phosphatidylinositol (36), ERK (37), and EGFR (38) pathways. Lymphocytes also express a large number of neurotransmitter receptors including dopamine receptors, cannabinoid receptors, β -adrenergic receptors, mineralocorticoid and glucocorticoid receptors, muscarinic acetylcholine receptors, substance P receptors, GABA_A receptors, and serotonin receptors and transporters [reviewed in Gladkevich et al. (39)]. A microarray study evaluating the comparability of gene expression in blood and brain (40) found that whole blood shares significant gene expression similarities with multiple central nervous system (CNS) tissues.

These similarities in gene expression between neural tissue and peripheral lymphoid tissue, combined with the inability to directly sample gene expression in the living brain, have led investigators to examine the feasibility of using blood as a surrogate tissue in which to examine changes in gene expression that may be associated with specific psychiatric disorders. While tissue or region-specific changes will not be detected, changes that affect basic aspects of cellular physiology such as energy metabolism are likely to be seen in both peripheral and central tissues.

In this study, we used microarrays to measure levels of gene expression in peripheral blood in 20 depressed subjects with BPD and in 15 untreated healthy controls. While comparisons with BPD subjects in other mood states (including euthymic BPD subjects) and subjects with major depressive disorder would be of interest, in order to increase subject homogeneity in the current study we included only depressed BPD subjects. In addition, since lithium has been shown to regulate a large number of genes, many of which may be relevant to the pathogenesis of BPD (41–43), we specifically excluded subjects who were currently taking lithium. Pathway analysis was used to identify those functional pathways that differed most significantly between depressed subjects with BPD and healthy controls. To further characterize the differences in mitochondrial gene expression (the top-ranked pathway from the microarray study), we carried out quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analysis for a set of 16 genes, including 12 nuclear encoded ETC genes and 4 mitochondrially encoded genes, which were not interrogated by the microarray platform.

Materials and methods

Subjects

All procedures involving human subjects were approved by the Yale Human Investigation Committee and are in accordance with the Helsinki Declaration of 1975. All subjects provided written informed consent at the time of enrollment in the study. Inclusion criteria for subjects with BPD included: age between 18 and 65 years, diagnosis of BPD [type I (BPD-I) or II (BPD-II)], currently depressed as defined by the DSM-IV-TR (44), and not currently being treated with lithium. Diagnosis was determined by consensus of clinical interview by a board-certified psychiatrist (either RDB or ZB) and the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (45). Exclusion criteria included DSM-IV-TR diagnoses other than BPD-I or BPD-II and current or recent abuse of illicit substances (verified by

urine toxicology screening). Mood ratings for BPD subjects were performed using the Hamilton Depression Rating Scale (46,47), the Montgomery-Åsberg Depression Rating Scale (48), the Hamilton Anxiety Rating Scale (49), and the Young Mania Rating Scale (50). Study eligibility was based on clinical diagnosis rather than any predetermined severity criteria.

An additional group of healthy controls was recruited through advertising. None of the control subjects met criteria for any DSM-IV-TR Axis I diagnosis as determined by the SCID or current or recent abuse of illicit substances.

Sample preparation and microarray analysis

Blood samples were collected directly into PAXgene blood RNA tubes (QIAGEN, Valencia, CA, USA) and stored frozen at -80°C until processing to reduce variation due to differences in sample processing. The choice to use whole blood rather than peripheral blood mononuclear cells (PBMCs) or some other cell fraction for analysis of gene expression is based on several factors. Primary among these is the desire to capture gene expression profiles that are as close as possible to those that exist *in vivo*. Collecting blood samples directly into PAXgene blood RNA tubes, which lyse the cells and prevent degradation of the RNA present, has been shown to prevent changes in gene expression associated with differences in storage or handling of the samples prior to RNA extraction (51). Total RNA was isolated from 10 cc whole blood using the PAXgene Blood RNA Isolation kit (QIAGEN) per the manufacturer's instructions, and depleted of globin mRNA message using GLOBIN clear hybridization capture technology (Ambion, Austin, TX, USA). RNA quality was evaluated using the Agilent Bioanalyser (Agilent Technologies, Inc., Santa Clara, CA, USA) using both visual inspection and RNA integrity number (RIN) analysis (average RIN = 7.5, range 5.5–8.8). Globin-reduced total RNA underwent complementary DNA (cDNA) synthesis and overnight *in vitro* transcription utilizing the Illumina TotalPrep RNA Amplification Kit (Ambion). Biotinylated cRNA (1.5 μg) was hybridized onto an Illumina Sentrix BeadChip (Human-6v2) (Illumina, Inc., San Diego, CA, USA) then scanned on a BeadArray Reader (Illumina, Inc.).

Microarray hybridization and scanning were carried out at the National Institute of Health Neuroscience Microarray Center at Yale (<http://info.med.yale.edu/neuromicroarray>). Per the policies of the NIH microarray consortium, all raw data, including project annotation, generated by the project have been made publicly available. Raw and normalized data can be accessed at the National Center for Biotechnology Information Gene Expression Omnibus (NCBI-GEO) repository (<http://www.ncbi.nlm.nih.gov/geo/>). The GSE accession number is GSE23848.

Normalization and data analysis

Statistical analysis of microarray data was carried out at the Keck Foundation Biotechnology Biostatistics Resource (<http://keck.med.yale.edu/biostats>). Illumina BeadStudio software was used to generate probe and gene expression profiles of each sample. Quantile normalization was carried out using the package incorporated in the Illumina BeadStudio software package (52). Further statistical analysis was carried out on all genes with a detection p-value < 0.01 as determined using the Illumina BeadStudio software (i.e., a 99% probability that expression was above background), and normalized expression levels > 20 . A total of 17,240 genes on the array met these criteria. This is similar to the detection sensitivity seen in other studies of whole blood using the Illumina Sentrix BeadChip platform (53).

Gene expression levels were compared between subjects with BPD and healthy controls using analysis of variance, and were covaried for the effects of age and sex. The p-values were adjusted to control the group-wise false discovery rate (FDR) (54,55) at <0.05 using the statistical package R.

Network analysis was carried out using GeneGo MetaCore® software (GeneGo, Inc., Encinitas, CA, USA). The significance of biological pathways is estimated through a variation of Fisher's exact test as implemented in the MetaCore software package and adjusted for multiple testing using Benjamini-Hochberg FDR analysis (which is a built-in function of GeneGo MetaCore software). Additional information on the calculations of p-values for the various pathways included in MetaCore is available in the online publication: "Evaluating statistical significance of pathways and network in MetaCore" (available online at: http://portal.genego.com/help/P-value_calculations.pdf). Analysis done using this software package has been shown to produce results that are congruent, but not identical, to those generated using other commercially available systems (e.g., Ingenuity Pathways Analysis) and the DAVID Functional Classification tool (56).

qRT-PCR analysis

qRT-PCR was carried out using samples from a subset of the subjects and controls tested in the microarray (17 subjects with BPD and 10 controls; samples were not used for qRT-PCR when no more purified RNA was available) for a set of 16 genes including 12 nuclear encoded ETC genes: NADH dehydrogenase flavoprotein subunits A1, B3, S4, and S5 (NDUFA1, NDUFB3, NDUFS4, and NDUFS5); succinate dehydrogenase complex, subunit D (SDHD); ubiquinol-cytochrome c reductase binding protein (UQCRB); ubiquinol-cytochrome c reductase, complex III, subunit VII (UQCRCQ); cytochrome c oxidase subunits 6C, 7B and 7C (COX6C, COX7B, and COX7C); and ATP synthase, FO, subunits D, E (ATP5H, ATP5I); and four mitochondrially encoded genes: mitochondrially encoded NADH dehydrogenase 2 (MT-ND2), mitochondrially encoded cytochrome b (MT-CYB), mitochondrially encoded cytochrome c oxidase I (MT-COI), and mitochondrially encoded ATP synthase 6 (MT-ATP6). Normalization was carried out against the endogenous reference gene TNF receptor associated protein (TRAP1), which was recently shown to be stably expressed in RNA from peripheral blood (57). Similar results were obtained using a second housekeeping gene, peptidylprolyl isomerase B (PPIB) (58) for normalization. qRT-PCR was carried out using the TaqMan® Universal PCR Master Mix Protocol (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and Real-Time PCR probes listed on the NCBI Probe Database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=probe>).

Results

Microarray analysis was carried out using Illumina Sentrix BeadChip (Human-6v2) microarrays on peripheral blood RNA isolated from a group of 20 depressed subjects with either BPD-I (n = 17) or BPD-II (n = 3) and 15 healthy control subjects. Demographic and clinical information for both BPD subjects and healthy controls are summarized in Table 1. Depressed subjects with BPD included 9 unmedicated patients and 11 who were receiving one or more atypical antipsychotic medications (olanzapine, quetiapine, risperidone, or ziprasidone). Two subjects were taking valproic acid in addition to an atypical antipsychotic and one subject each was taking carbamazepine, oxcarbazepine, or topiramate in addition to an atypical antipsychotic (patients had been selected on the condition that they were not receiving lithium). None of the BPD subjects (or healthy controls) was taking antidepressants at the time of the study. Both the healthy controls and the BPD group were predominantly female (70%) and predominantly Caucasian (65%). There were no significant differences in age or ethnic background among the groups compared.

Analysis of the microarray data by ANOVA identified 1,180 genes that were differentially expressed between depressed subjects with BPD and healthy controls (fold-change >1.3, FDR-corrected $p < 0.05$, covaried for age and sex). Of these, 559 genes were up-regulated in BPD subjects and 621 were down-regulated. To assess whether these differences were related to medication usage, we compared gene expression in medicated ($n = 11$) and unmedicated ($n = 9$) subjects with BPD separately from healthy controls. Surprisingly, ANOVA showed no significant differences between medicated and unmedicated BPD subjects in the expression of any of the 1,180 genes where differences were seen in the microarray data between BPD subjects and controls. A complete list of the fold-changes and FDR corrected p -values for all of the genes showing differential expression between BPD subjects and controls, as well as the corresponding values when medicated and unmedicated BPD subjects were compared separately to controls, is provided in Supplemental Table 1.

To identify functional relationships among the differentially expressed genes, we carried out pathway analysis using GeneGo MetaCore software. Table 2 lists the top 10 functional pathways identified using this software as well as the p -value associated with the number of differentially expressed genes in each pathway. As shown in Table 2, the most significantly affected pathway was the mitochondrial ETC ($p < 8.64 \times 10^{-7}$). Of the 85 objects (genes or proteins) in this pathway, 22 were up-regulated and 2 down-regulated in subjects with BPD.

Table 3 lists the fold-change and FDR-adjusted p -values for each of the differentially expressed genes in the mitochondrial ETC pathway identified in the microarray analysis. Results are shown for BPD subjects as a group compared to controls and for medicated and unmedicated BPD subjects compared separately to controls. There were no significant differences between medicated and un-medicated subjects with BPD for any of these genes.

Up-regulated ETC genes include components of complexes I, III, IV, and V. The position of each of the differentially regulated ETC genes within the mitochondrial ETC is illustrated in Figure 1.

To further characterize the differences in mitochondrial gene expression, we carried out qRT-PCR analysis using peripheral blood RNA isolated from a set of 12 nuclear encoded ETC genes and 4 mitochondrially encoded genes (one each from complexes I, III, IV, and V —complex II is encoded by 4 nuclear encoded genes). Table 4 shows the fold-change and p -value for each of these genes as assessed by qRT-PCR after normalization against the endogenous reference gene TRAP1, which was recently shown to be stably expressed in RNA from peripheral blood (57). Similar results were obtained using a second housekeeping gene, PPIB (58), for normalization. There was no difference between BPD subjects and controls in expression of either of these genes as assessed by microarray hybridization or qRT-PCR. Findings from the microarray study are shown for comparison where available.

Discussion

These results extend previous reports demonstrating alterations in energy metabolism in subjects with BPD. Using whole genome microarrays, we identified a large number of genes that were differentially expressed in peripheral blood of subjects with BPD compared to healthy controls. Pathway analysis using GeneGo MetaCore software showed that the most significantly affected pathway was the mitochondrial ETC ($p < 8.6 \times 10^{-7}$), suggesting that this pathway may play an important role in the pathophysiology of depression in patients with BPD. Moreover, this number is probably an underestimate of the number of dysregulated genes in the ETC pathway, since the arrays used did not include any of the mitochondrially encoded components of the ETC (13 genes) (59).

Combining the results from the microarray and qRT-PCR studies, it appears that there is a general increase in the expression of both nuclear and mitochondrially encoded ETC genes in subjects with bipolar depression. Moreover, all of the top 10 functional pathways identified in the microarray analysis are interconnected, and relate directly or indirectly to mitochondrial functions, including energy metabolism and the regulation of apoptosis by mitochondrial proteins. In particular, production of energy via the ETC (the top-ranked pathway) is directly coupled to the regulation of apoptosis and cell survival (Table 2, pathways 6–8, 10) since mitochondrially formed oxidants activate a number of signaling pathways related to apoptosis. In particular, activation of the mitochondrial death pathways mediated by Bax and Bak is dependent upon oxidative phosphorylation (60,61). There is also evidence that the activity of the mitochondrial ETC is regulated directly by the Notch signaling pathway (Table 2, pathways 2, 4) (62,63) and indirectly by the pro-inflammatory/anti-apoptotic regulatory protein, nuclear factor kappa B (NF- κ B) (Table 2, pathways 3–5) (64,65). The macrophage migration inhibitory pathway (Table 2, pathway 9) also interacts with the NF- κ B pathway and has been implicated as a regulator of apoptosis (66). Thus, it appears likely that a dysregulation of mitochondrial function is central to the large number of gene expression changes observed in the current study.

Limitations of this study include the small sample size and possible confounding effects of differences among specific subpopulations of white blood cells between subjects with BPD and controls. Such differences could affect the relative expression of some genes, i.e., those specific to the over- or under-represented cell types. However, since ETC genes are expressed in all cell types, it is unlikely that the observed differences in ETC gene expression are related to differences in subpopulations of white cells between the two groups.

Several previous studies have examined the expression of mitochondrial genes, most notably NDUFV2, in transformed lymphocytes from patients with BPD. Washizuka et al. examined the expression of NDUFV2 (67) and 11 other mitochondria-related genes (68) in lymphoblastoid cells derived from a cohort of 21 Japanese subjects with BPD and 11 controls. They found that components of complex I and complex IV were down-regulated in the subset of subjects with BPD-I (n=13), although only the change in NDUFV2 was significant after correction for multiple testing. In contrast, Xu et al. (69) reported no difference in the level of expression of NDUFV2 in transformed lymphocytes from a group of 178 subjects with BPD of European Caucasian ancestry compared to a group of 120 controls. In a follow-up study, Washizuka et al. (70) confirmed the decrease of NDUFV2 in subjects with BPD-I of Japanese ancestry (n=25), but found increased expression of NDUFV2 in Japanese subjects with BPD-II (n=10) and no change in BPD (either BPD-I or BPD-II) in subjects of Caucasian ancestry. However, the significance of these studies with regard to the expression of ETC genes in lymphocytes *in vivo* is unclear, since transformation with Epstein-Barr virus has been shown to affect the expression of multiple genes in lymphocytes, including pathways regulating cell growth and division (71–74).

In contrast to our current findings, a number of postmortem studies (24–26) have suggested that the expression of ETC genes may be decreased in patients with BPD. There are several possible explanations for the difference between those findings and our findings in the current study. First, it is possible that there are state-related differences in the expression of ETC genes in subjects with BPD. In the current study, all BPD subjects were depressed at the time of study entry, while the postmortem studies may have included subjects who were depressed, manic, or euthymic at the time of death. Consistent with that hypothesis, Dror et al. (75) found evidence of state-dependent changes in mitochondrial complex I activity in platelets of schizophrenic patients, with increased activity in patients with active psychotic symptoms but decreased activity in patients with residual schizophrenia. Further studies will

be needed to determine if there is a similar state-dependence to the changes in ETC gene expression observed in this study. Second, the majority of patients included in the previous studies were receiving mood stabilizers and/or antipsychotic medications at the time of death. Thus, the decreased expression of ETC genes in those studies may be reflective of either acute or chronic effects of these medications on gene expression. Consistent with that hypothesis, Iwamoto et al. (25) found that each of the medication groups examined (antidepressants, valproate, and antipsychotics) appeared to have a significant global repressive effect on the expression of mitochondria-related genes. Moreover, in the same study, the small group of unmedicated subjects with BPD ($n=4$) showed an up-regulation of ETC genes consistent with the findings we have reported here. Third, the previous reports are based on postmortem brain samples, while our findings are based on blood samples isolated from living subjects with BPD. Thus, there may be a tissue-specific difference with ETC genes being up-regulated in blood, but down-regulated in some or all regions of the CNS of patients with BPD. Consistent with that hypothesis, Ben-Shachar and Karry (28) found that there was region-specific variation in the expression of mitochondrial genes in patients with BPD, with increased expression in the parieto-occipital cortex but reduced expression in the cerebellum. Finally, differences in brain pH at the time of death may explain a large part of the previously reported decrease in ETC gene expression (30). Further studies will be needed to clarify more precisely the relationships among diagnosis, mood state, treatment status, and ETC gene expression in BPD.

Studies in cell culture and animal models have shown that chronic treatment with lithium or valproate can enhance mitochondrial function and protect against mitochondrially mediated toxicity (76). Lithium has also been shown to increase the activity of ETC complexes I + III and II + III in extracts from human postmortem brain tissue at therapeutically relevant concentrations (77). Conversely, rats subjected to an experimental model of depression showed impaired mitochondrial function (78). Thus, enhancing mitochondrial ETC function may play a role in the therapeutic effects of lithium and other mood stabilizers. In the current study, we found that depressed BPD subjects had elevated levels of mRNA for several of the genes in ETC complexes I, III, IV, and V. Increased expression of these genes suggests that there may be increased mitochondrial turnover in depressed subjects with BPD (79). Consistent with the hypothesis that bipolar depression is characterized by impaired mitochondrial function, Andreazza et al. (80) have recently demonstrated that the activity of mitochondrial complex I is decreased and oxidative damage is increased in the postmortem prefrontal cortex of patients with BPD.

Abnormal expression of mitochondrial genes has also been suggested to play a role in other disorders, including schizophrenia (18,81,82) and type 2 diabetes (83,84). An emerging challenge for the field will be to establish how changes in mitochondrial gene expression, and by implication changes in mitochondrial function, relate to the specific symptoms observed in each of these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Merikangas KR, Akiskal HS, Angst J, et al. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. *Arch Gen Psychiatry*. 2007; 64:543–552. [PubMed: 17485606]
2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005; 62:593–602. [PubMed: 15939837]
3. Waraich P, Goldner EM, Somers JM, Hsu L. Prevalence and incidence studies of mood disorders: a systematic review of the literature. *Can J Psychiatry*. 2004; 49:124–138. [PubMed: 15065747]
4. Martinowich K, Schloesser RJ, Manji HK. Bipolar disorder: from genes to behavior pathways. *J Clin Invest*. 2009; 119:726–736. [PubMed: 19339764]
5. Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci USA*. 1998; 95:13290–13295. [PubMed: 9789081]
6. Rajkowska G, Halaris A, Selemon LD. Reductions in neuronal and glial density characterize the dorsolateral prefrontal cortex in bipolar disorder. *Biol Psychiatry*. 2001; 49:741–752. [PubMed: 11331082]
7. Vawter MP, Freed WJ, Kleinman JE. Neuropathology of bipolar disorder. *Biol Psychiatry*. 2000; 48:486–504. [PubMed: 11018222]
8. WTCCC. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature*. 2007; 447:661–678. [PubMed: 17554300]
9. Baum AE, Akula N, Cabanero M, et al. A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Mol Psychiatry*. 2008; 13:197–207. [PubMed: 17486107]
10. Ferreira MA, O'Donovan MC, Meng YA, et al. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet*. 2008; 40:1056–1058. [PubMed: 18711365]
11. Sklar P, Smoller JW, Fan J, et al. Whole-genome association study of bipolar disorder. *Mol Psychiatry*. 2008; 13:558–569. [PubMed: 18317468]
12. Smith EN, Bloss CS, Badner JA, et al. Genome-wide association study of bipolar disorder in European American and African American individuals. *Mol Psychiatry*. 2009; 14:755–763. [PubMed: 19488044]
13. Scott LJ, Muglia P, Kong XQ, et al. Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry. *Proc Natl Acad Sci USA*. 2009; 106:7501–7506. [PubMed: 19416921]
14. Hattori E, Toyota T, Ishitsuka Y, et al. Preliminary genome-wide association study of bipolar disorder in the Japanese population. *Am J Med Genet B Neuropsychiatr Genet*. 2009; 150B:1110–1117. [PubMed: 19259986]
15. O'Donovan MC, Craddock NJ, Owen MJ. Genetics of psychosis; insights from views across the genome. *Hum Genet*. 2009; 126:3–12. [PubMed: 19521722]
16. Craddock N, Sklar P. Genetics of bipolar disorder: successful start to a long journey. *Trends Genet*. 2009; 25:99–105. [PubMed: 19144440]
17. Quiroz JA, Gray NA, Kato T, Manji HK. Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder. *Neuropsychopharmacology*. 2008; 11:2551–2565. [PubMed: 18235426]
18. Shao L, Martin MV, Watson SJ, et al. Mitochondrial involvement in psychiatric disorders. *Ann Med*. 2008; 40:281–295. [PubMed: 18428021]
19. Kato T. Mitochondrial dysfunction as the molecular basis of bipolar disorder: Therapeutic implications. *CNS Drugs*. 2007; 21:1–11. [PubMed: 17190525]
20. Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. *Mol Psychiatry*. 2005; 10:900–919. [PubMed: 16027739]
21. Regenold WT, Phatak P, Marano CM, Sassan A, Conley RR, Kling MA. Elevated cerebrospinal fluid lactate concentrations in patients with bipolar disorder and schizophrenia: Implications for

- the mitochondrial dysfunction hypothesis. *Biol Psychiatry*. 2009; 65:489–494. [PubMed: 19103439]
22. 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. [PubMed: 16696827]
  23. Lenaz G, Genova ML. Structural and functional organization of the mitochondrial respiratory chain: a dynamic super-assembly. *Int J Biochem Cell Biol*. 2009; 41:1750–1772. [PubMed: 19711505]
  24. 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. [PubMed: 14993118]
  25. Iwamoto K, Bundo M, Kato T. Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Hum Mol Genet*. 2005; 14:241–253. [PubMed: 15563509]
  26. Sun X, Wang JF, Tseng M, Young LT. Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *J Psychiatry Neurosci*. 2006; 31:189–196. [PubMed: 16699605]
  27. Naydenov AV, MacDonald ML, Ongur D, Konradi C. Differences in lymphocyte electron transport gene expression levels between subjects with bipolar disorder and normal controls in response to glucose deprivation stress. *Arch Gen Psychiatry*. 2007; 64:555–564. [PubMed: 17485607]
  28. Ben-Shachar D, Karry R. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. *PLoS ONE*. 2008; 3:e3676. [PubMed: 18989376]
  29. Nakatani N, Hattori E, Ohnishi T, et al. Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: Relevance to neuronal network perturbation. *Hum Mol Genet*. 2006; 15:1949–1962. [PubMed: 16687443]
  30. Vawter MP, Tomita H, Meng F, et al. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: Implications for brain disorders. *Mol Psychiatry*. 2006; 11(615):63–79.
  31. Beech RD. Thyroid hormones, affective disorders, and gene-regulation: a reexamination. *Psychiatric Annals*. 1997; 27:773–778.
  32. Beech, RD.; Duman, RS. The role of transcription factors in the biology of depression. In: Licinio, J.; Wong, M-L., editors. *Biology of Depression: Towards a Novel Understanding and Therapeutic Strategies*. Weinheim: Wiley-VCH; 2005. p. 823-854.
  33. Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch Gen Psychiatry*. 1997; 54:597–606. [PubMed: 9236543]
  34. Manji HK, Moore GJ, Chen G. Clinical and preclinical evidence for the neurotrophic effects of mood stabilizers: implications for the pathophysiology and treatment of manic-depressive illness. *Biol Psychiatry*. 2000; 48:740–754. [PubMed: 11063971]
  35. Kuo CT, Leiden JM. Transcriptional regulation of T lymphocyte development and function. *Annu Rev Immunol*. 1999; 17:149–187. [PubMed: 10358756]
  36. Jumaa H, Hendriks RW, Reth M. B cell signaling and tumorigenesis. *Annu Rev Immunol*. 2005; 23:415–445. [PubMed: 15771577]
  37. Fischer AM, Katayama CD, Pages G, Pouyssegur J, Hedrick SM. The role of erk1 and erk2 in multiple stages of T cell development. *Immunity*. 2005; 23:431–443. [PubMed: 16226508]
  38. Feistritzer C, Mosheimer BA, Sturn DH, Riewald M, Patsch JR, Wiedermann CJ. Endothelial protein C receptor-dependent inhibition of migration of human lymphocytes by protein C involves epidermal growth factor receptor. *J Immunol*. 2006; 176:1019–1025. [PubMed: 16393989]
  39. Gladkevich A, Kauffman HF, Korf J. Lymphocytes as a neural probe: potential for studying psychiatric disorders. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2004; 28:559–576.
  40. 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. [PubMed: 16526044]

41. Chetcuti A, Adams LJ, Mitchell PB, Schofield PR. Microarray gene expression profiling of mouse brain mRNA in a model of lithium treatment. *Psychiatr Genet*. 2008; 18:64–72. [PubMed: 18349697]
42. McQuillin A, Rizig M, Gurling HM. A microarray gene expression study of the molecular pharmacology of lithium carbonate on mouse brain mRNA to understand the neurobiology of mood stabilization and treatment of bipolar affective disorder. *Pharmacogenet Genomics*. 2007; 17:605–617. [PubMed: 17622937]
43. Seelan RS, Khalyfa A, Lakshmanan J, Casanova MF, Parthasarathy RN. Deciphering the lithium transcriptome: Microarray profiling of lithium-modulated gene expression in human neuronal cells. *Neuroscience*. 2008; 151:1184–1197. [PubMed: 18222042]
44. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4. Washington, DC: American Psychiatric Association; 2000. Text Revision (DSM-IV-TR)
45. First, MB.; Spitzer, RL.; Gibbon, M.; Williams, J. *Structured Clinical Interview for DSM-IV Axis I Disorders*. New York: Biometrics Research Department, New York State Psychiatric Institute; 1996. Patient Edition (SCID-I/P, Version 2.0)
46. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*. 1960; 23:56–62. [PubMed: 14399272]
47. Mazure C, Nelson JC, Price LH. Reliability and validity of the symptoms of major depressive illness. *Arch Gen Psychiatry*. 1986; 43:451–456. [PubMed: 3964024]
48. Montgomery SA, Åsberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry*. 1979; 134:382–389. [PubMed: 444788]
49. Hamilton M. The assessment of anxiety states by ratings. *Br J Med Psychol*. 1959; 32:50–55. [PubMed: 13638508]
50. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry*. 1978; 133:429–435. [PubMed: 728692]
51. QIAGEN. *PAXgene Blood RNA Kit Handbook*. Valencia: QIAGEN; 2005.
52. Illumina, Inc. Technical Support. San Diego: Illumina, Inc; 2009. Chapter 4: Normalization and Differential Analysis. *BeadStudio Gene Expression Module v32 User Guide*.
53. Barnes M, Freudenberg J, Thompson S, Aronow B, Pavlidis P. Experimental comparison and cross-validation of the Affymetrix and Illumina gene expression analysis platforms. *Nucleic Acids Res*. 2005; 33:5914–5923. [PubMed: 16237126]
54. Reiner A, Yekutieli D, Benjamini Y. Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics*. 2003; 19:368–375. [PubMed: 12584122]
55. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci USA*. 2003; 100:9440–9445. [PubMed: 12883005]
56. Ptitsyn AA, Weil MM, Thamm DH. Systems biology approach to identification of biomarkers for metastatic progression in cancer. *BMC Bioinformatics*. 2008; 9 (Suppl 9):S8. [PubMed: 18793472]
57. Stamova BS, Apperson M, Walker WL, et al. Identification and validation of suitable endogenous reference genes for gene expression studies in human peripheral blood. *BMC Med Genomics*. 2009; 2:49. [PubMed: 19656400]
58. Pachot A, Blond JL, Mouglin B, Miossec P. Peptidylpropyl isomerase B (PPIB): a suitable reference gene for mRNA quantification in peripheral whole blood. *J Biotechnol*. 2004; 114:121–124. [PubMed: 15464605]
59. Hock MB, Kralli A. Transcriptional control of mitochondrial biogenesis and function. *Annu Rev Physiol*. 2009; 71:177–203. [PubMed: 19575678]
60. Niizuma K, Endo H, Chan PH. Oxidative stress and mitochondrial dysfunction as determinants of ischemic neuronal death and survival. *J Neurochem*. 2009; 1091(Suppl):133–138. [PubMed: 19393019]
61. Tomiyama A, Serizawa S, Tachibana K, et al. Critical role for mitochondrial oxidative phosphorylation in the activation of tumor suppressors Bax and Bak. *J Natl Cancer Inst*. 2006; 98:1462–1473. [PubMed: 17047195]
62. Mantel CR, Wang RH, Deng C, Broxmeyer HE. Sirt1, notch and stem cell “age asymmetry. *Cell Cycle*. 2008; 7:2821–2825. [PubMed: 18797187]

63. de la Pena P, Bornstein B, del Hoyo P, et al. Mitochondrial dysfunction associated with a mutation in the Notch3 gene in a CADASIL family. *Neurology*. 2001; 57:1235–1238. [PubMed: 11591842]
64. Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: Fifteen years later. *Biochem Pharmacol*. 2006; 72:1493–1505. [PubMed: 16723122]
65. Jones WK, Brown M, Ren X, He S, McGuinness M. NF-kappaB as an integrator of diverse signaling pathways: the heart of myocardial signaling? *Cardiovasc Toxicol*. 2003; 3:229–254. [PubMed: 14555789]
66. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol*. 2003; 3:791–800. [PubMed: 14502271]
67. Washizuka S, Kakiuchi C, Mori K, et al. Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2003; 120B: 72–78. [PubMed: 12815743]
68. 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. [PubMed: 15762855]
69. Xu C, Li PP, Kennedy JL, et al. Further support for association of the mitochondrial complex I subunit gene NDUFV2 with bipolar disorder. *Bipolar Disord*. 2008; 10:105–110. [PubMed: 18199248]
70. Washizuka S, Iwamoto K, Kakiuchi C, Bundo M, Kato T. Expression of mitochondrial complex I subunit gene NDUFV2 in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia. *Neurosci Res*. 2009; 63:199–204. [PubMed: 19135101]
71. Carter KL, Cahir-McFarland E, Kieff E. Epstein-Barr virus-induced changes in B-lymphocyte gene expression. *J Virol*. 2002; 76:10427–10436. [PubMed: 12239319]
72. Chen A, Zhao B, Kieff E, Aster JC, Wang F. EBNA-3B- and EBNA-3C-regulated cellular genes in Epstein-Barr virus-immortalized lymphoblastoid cell lines. *J Virol*. 2006; 80:10139–10150. [PubMed: 17005691]
73. Niller HH, Wolf H, Minarovits J. Epigenetic dysregulation of the host cell genome in Epstein-Barr virus-associated neoplasia. *Semin Cancer Biol*. 2009; 19:158–164. [PubMed: 19429479]
74. Faumont N, Durand-Panteix S, Schlee M, et al. c-Myc and Rel/NF-kappaB are the two master transcriptional systems activated in the latency III program of Epstein-Barr virus-immortalized B cells. *J Virol*. 2009; 83:5014–5027. [PubMed: 19264782]
75. Dror N, Klein E, Karry R, et al. State-dependent alterations in mitochondrial complex I activity in platelets: a potential peripheral marker for schizophrenia. *Mol Psychiatry*. 2002; 7:995–1001. [PubMed: 12399953]
76. Bachmann RF, Wang Y, Yuan P, et al. Common effects of lithium and valproate on mitochondrial functions: protection against methamphetamine-induced mitochondrial damage. *Int J Neuropsychopharmacol*. 2009; 12:805–822. [PubMed: 19149911]
77. Maurer IC, Schippel P, Volz HP. Lithium-induced enhancement of mitochondrial oxidative phosphorylation in human brain tissue. *Bipolar Disord*. 2009; 11:515–522. [PubMed: 19624390]
78. Rezin GT, Cardoso MR, Goncalves CL, et al. Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. *Neurochem Int*. 2008; 53:395–400. [PubMed: 18940214]
79. Diaz F, Moraes CT. Mitochondrial biogenesis and turnover. *Cell Calcium*. 2008; 44:24–35. [PubMed: 18395251]
80. Andreazza AC, Shao L, Wang JF, Young LT. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. *Arch Gen Psychiatry*. 2010; 67:360–368. [PubMed: 20368511]
81. Rezin GT, Amboni G, Zugno AI, Quevedo J, Streck EL. Mitochondrial dysfunction and psychiatric disorders. *Neurochem Res*. 2009; 34:1021–1029. [PubMed: 18979198]
82. Rollins B, Martin MV, Sequeira PA, et al. Mitochondrial variants in schizophrenia, bipolar disorder, and major depressive disorder. *PLoS ONE*. 2009; 4:e4913. [PubMed: 19290059]
83. Mootha VK, Handschin C, Arlow D, et al. Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. *Proc Natl Acad Sci USA*. 2004; 101:6570–6575. [PubMed: 15100410]

84. Soyat S, Krempler F, Oberkofler H, Patsch W. PGC-1alpha: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. *Diabetologia*. 2006; 49:1477–1488. [PubMed: 16752166]

Fig. 1.

Diagram of the mitochondrial electron transport chain showing genes with altered expression in subjects with bipolar disorder (BPD). Complex I (NADH dehydrogenase) catalyzes the removal of two electrons from NADH and transfers them to the electron carrier ubiquinone (Q). The reduced product is ubiquinol (QH₂). Complex II (succinate dehydrogenase) catalyzes the conversion of succinate to fumarate and in the process transfers two electrons to ubiquinone via FAD. Complex III catalyzes the removal of two electrons from QH₂ and transfers them to two molecules of the electron carrier, cytochrome c. Complex IV removes two electrons from the two molecules of cytochrome c and transfers them to molecular oxygen (O₂), producing water. The proton gradient established by pumping hydrogens across the inner mitochondrial membrane is then utilized by Complex V (ATP synthase) to catalyze the formation of ATP from ADP and P_i. Genes encoding various subunits of complexes I, III, IV, and V that were up-regulated in peripheral blood of subjects with BPD are shown as red hexagons next to the corresponding complex.

Table 1

Summary of demographic and clinical information of study participants

	Microarray		qRT-PCR	
	Bipolar disorder	Healthy control	Bipolar disorder	Healthy control
Gender, n (%)				
Male	6 (30)	5 (33)	6 (35)	6 (60)
Female	14 (70)	10 (67)	11 (65)	4 (40)
Ethnicity, n (%)				
Caucasian	13 (65)	10 (67)	12 (70)	7 (70)
Non-Caucasian	7 (35)	5 (33)	5 (30)	3 (30)
Age (years), mean (SD)	38.4 (9.5)	29.0 (9.6)	38.5 (9.7)	28.1 (6.7)
HAM-D score, mean (SD)	27.7 (15.0)	NA	27.9 (14.8)	NA
HAM-A score, mean (SD)	14.6 (7.6)	NA	14.4 (8.2)	NA
MADRS score, mean (SD)	19.3 (11.3)	NA	18.6 (11.7)	NA
YMRS score, mean (SD)	6.1 (5.0)	NA	6.5 (5.3)	NA

qRT-PCR = quantitative real-time reverse transcription polymerase chain reaction; HAM-D= Hamilton Depression Rating Scale; HAM-A= Hamilton Anxiety Rating Scale; MADRS= Montgomery-Åsberg Depression Rating Scale, YMRS = Young Mania Rating Scale.

Align values by left parenthesis.

Table 2

Top 10 functional pathways identified by microarray analysis as differing significantly between bipolar disorder subjects and healthy controls

Rank	Pathway	Cell process	p-value	Differentially expressed genes, n	Genes or proteins in pathway, n
1	Oxidative phosphorylation/electron transport chain	Energy metabolism	8.64E-07	24	85
2	Development: Notch signaling pathway	Response to extracellular stimulus	2.73E-04	12	40
3	Transcription: NF- κ B signaling pathway	Transcription, immune response	5.36E-04	11	37
4	Development: NOTCH1-mediated pathway for NF- κ B activity modulation	Transcription, response to extracellular stimulus	6.18E-04	10	32
5	Bacterial infections in cystic fibrosis airways	Lung diseases/cystic fibrosis	7.20E-04	12	44
6	Development: EPO-induced Jak-STAT pathway	Apoptosis, response to extracellular stimulus	1.35E-03	10	35
7	Apoptosis and survival: Lymphotoxin-beta receptor signaling	Apoptosis, immune response	1.39E-03	11	41
8	Apoptosis and survival: BAD phosphorylation	Apoptosis and survival	1.71E-03	10	36
9	Immune response: MIF – the neuroendocrine-macrophage connector	Immune response	2.07E-03	9	31
10	Apoptosis and survival: Anti-apoptotic TNFs/NF- κ B/Bcl-2 pathway	Apoptosis and survival	4.84E-03	10	41

Table 3

Mitochondrial electron transport chain (ETC) genes showing differential expression in bipolar disorder (BPD) subjects versus healthy controls by microarray analysis

Gene symbol	Fold-change (BPD overall versus control)	p-value	Fold-change (unmedicated BPD versus control)	p-value	Fold-change (medicated BPD versus control)	p-value
Complex I (NADH dehydrogenase)^a						
NDUFA1	1.50	0.0075		1.49	0.0226	1.51 0.0241
NDUFA4	1.46	0.0181		1.37	NS	1.58 0.0246
NDUFA6	1.31	0.0124		1.27	NS	1.37 0.0217
NDUFB3	1.81	0.0017		1.62	0.0137	2.09 0.0032
NDUFB9	-1.42	0.0269		-1.53	0.0277	-1.29 NS
NDUFS4	1.67	0.0028		1.64	0.0129	1.72 0.01
NDUFS5	2.01	0.0009		1.98	0.0061	2.04 0.0049
Complex II (succinate dehydrogenase)^b						
SDHA	1.27	NS		1.38	0.0463	1.15 NS
SDHD	-1.63	0.0029		-1.6	0.0137	-1.68 0.01
LOC642502 ^c	-1.25	NS		-1.31	0.0265	-1.19 NS
Complex III (ubiquinol cytochrome c reductase)^d						
LOC644969 ^e	2.00	0.0181		1.82	NS	2.25 0.0281
UQCRCB	1.63	0.002		1.55	0.0121	1.74 0.0053
Complex IV (cytochrome c oxidase)						
COX6C	2.17	0.0027		2.05	0.014	2.32 0.0085
COX7B	2.21	0.005		1.79	0.037	2.90 0.0049
COX7C	1.56	0.0148		1.54	0.0379	1.58 0.0371
Complex V (ATP synthase)						
ATP5D ^f	1.33	0.024		1.39	0.0298	1.25 NS
ATP5E ^g	1.43	0.0028		1.38	0.0152	1.49 0.007
ATP5H ^h	1.33	0.0043		1.33	0.0146	1.33 0.0171
ATP5I ⁱ	1.33	0.0385		1.23	NS	1.48 0.0301
ATP5J ^j	1.34	0.0249		1.29	NS	1.40 0.0383

The genes listed do not correspond precisely with the objects included in the GeneGO database since two of the genes listed (LOC642502 and LOC644969) are included based on sequence homology to other known ETC genes, but are not included in the GeneGO database. UQCRB = ubiquinol-cytochrome c reductase binding protein.

- ^a Also known as NADH-coenzyme Q oxidoreductase.
- ^b Also known as succinate-Q oxidoreductase.
- ^c Predicted similar to SDHC.
- ^d Also known as Q-cytochrome c oxidoreductase.
- ^e Predicted similar to ubiquinol-cytochrome c reductase complex 14 kDa protein.
- ^f ATP synthase, F1, subunit delta.
- ^g ATP synthase, F1, subunit epsilon.
- ^h ATP synthase, F0, subunit D.
- ⁱ ATP synthase, F0, subunit E.
- ^j ATP synthase, F1, subunit F6.

Table 4

Comparison of microarray and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) results for selected electron transport chain genes

Gene symbol	Fold-change qRT-PCR	p-value qRT-PCR	Fold-change microarray	p-value microarray
Complex I (NADH dehydrogenase)^a				
NDUFA1–nuclear encoded	2.35 fold	0.0069	1.49 fold	0.0068
NDUFB3–nuclear encoded	2.79 fold	0.0128	1.80 fold	0.0022
NDUFS4–nuclear encoded	2.78 fold	0.0011	1.67 fold	0.0007
NDUFS5–nuclear encoded	2.58 fold	0.0066	1.94 fold	0.0024
MT-ND2	1.96 fold	0.0361	n/a	n/a
Complex II (succinate dehydrogenase)				
SDHD–nuclear encoded	1.98 fold	0.0128	(-)1.64	0.0125
Complex III (ubiquinol cytochrome c reductase)				
UQCRB–nuclear encoded	4.78 fold	6.98E-05	1.62 fold	0.0007
UQCRQ–nuclear encoded	1.28 fold	0.4270 (NS)	1.63 fold	0.002
MT-CYB ^b	1.23 fold	0.4408 (NS)	n/a	n/a
Complex IV (cytochrome c oxidase)				
COX6C–nuclear encoded	1.91 fold	0.0546 (NS)	2.16 fold	0.0036
COX7B–nuclear encoded	3.86 fold	0.0032	2.19 fold	0.0097
COX7C–nuclear encoded	2.24 fold	0.0055	1.56 fold	0.0039
MT-COI ^c	3.06 fold	0.0002	n/a	n/a
Complex V (ATP synthase)				
ATP5H–nuclear encoded	1.52 fold	0.1821 (NS)	1.33 fold	0.0053
ATP5I–nuclear encoded	1.35 fold	0.2856 (NS)	1.33 fold	0.0385
MT-ATP6	2.38 fold	0.0066	n/a	n/a

NDUFA1 = NADH dehydrogenase flavoprotein A1; NDUFB3 = NADH dehydrogenase flavoprotein B3; NDUFS4 = NADH dehydrogenase flavoprotein S4; NDUFS5 = NADH dehydrogenase flavoprotein S5; MT-ND2 = mitochondrially encoded NADH dehydrogenase 2; SDHD = succinate dehydrogenase complex, subunit D; UQCRB = ubiquinol-cytochrome c reductase binding protein; UQCRQ = ubiquinol-cytochrome c reductase, complex III subunit VII; MT-CYB = mitochondrially encoded cytochrome b; COX6C = cytochrome c oxidase subunit VIc; COX7C = cytochrome c oxidase subunit VIIb; COX7C = cytochrome c oxidase subunit VIIc; MT-COI = mitochondrially encoded cytochrome c oxidase I; ATP5H = ATP synthase, FO, subunit d; ATP5I = ATP synthase, FO, subunit E; MT-ATP6 = mitochondrially encoded ATP synthase 6.

^a Also known as NADH-coenzyme Q oxidoreductase.

^b Also known as CYTB.

^c Also known as COX1.