Transcriptional profiling of mitochondria associated genes in prefrontal cortex of subjects with major depressive disorder

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Abstract

Objectives—Recent evidences suggest that mitochondrial dysfunction maybe involved in the pathophysiology of major depressive disorder (MDD); however, the role of mitochondrial genes in this disorder has not been studied systematically. In the present study, we profiled expression of mitochondrial genes in dorsolateral prefrontal cortex (dlPFC) of MDD and non-psychiatric control subjects.

Methods—Human mitochondrial RT^2 profile PCR array plates were used to examine differentially expressed genes in dlPFC of 11 MDD and 11 control subjects. Differentially expressed genes were validated independently by qRT-PCR. Biological relevance of differentially expressed genes was analyzed by Gene Ontology (GO) and ingenuity pathways analysis (IPA).

Results—We found that 16 genes were differentially expressed in the MDD group compared with control group. Among them, 3 genes were down-regulated and 13 genes up-regulated. None of these genes were affected by confounding variables, such as age, postmortem interval, brain pH, and antidepressant toxicology. Seven differentially expressed genes were successfully validated in MDD subjects. GO and IPA analyses identified several new regulatory networks associated with mitochondrial dysfunctions in MDD.

Conclusions—Our findings suggest abnormal mitochondrial systems in the brain of MDD subjects which could be involved in the etiopathogenesis of this disorder.

Keywords

Major depression; dorsolateral prefrontal cortex; mitochondrial genes; expression; gene network

Introduction

Major depressive disorder (MDD) is a common psychiatry disorder, the lifetime prevalence of which is 15%~20%. Due to considerable morbidity and mortality, MDD is considered to be the second leading causes of disability (Ferrari et al. 2013). Despite that medication and

psychotherapeutic treatments are available for clinical depression, it is urgent and critical to uncover its underlying pathogenic mechanism and find more effective drugs (Bondy 2002).

It is hypothesized that mitochondrial dysfunction in specific brain regions may be related to the cause or effect of MDD (Tobe 2013). Mitochondria are vital cellular organelles, which generate adenosine triphosphate (ATP) via mitochondrial respiratory chain (oxidative phosphorylation) in eukaryotic cells. ATP acts as the universal energy used for a wide range of cellular process (Perier and Vila 2012). For neurons, neuronal energy needs are primarily supplied by mitochondrial oxidative phosphorylation, as it is too sufficient to obtain energy through cytosolic glycolysis. That is why neuron related diseases are highly vulnerable to mitochondrial dysfunction (Ivanov et al. 2014). Besides acting as a power station, mitochondrial dynamics also regulate neuronal energy metabolism, Ca^{2+} homeostasis, and dendritic and axonal motility, which affect release of neurotransmitters and neurotropic factors (Mattson et al. 2008).

Mitochondrial dysfunction mainly takes part in the three metabolic processes: 1) reactive oxygen species (ROS), 2) calcium signaling, and 3) apoptosis. In ROS, mitochondria are one of the most important sources of reactive oxygen species in the mammalian cells. Moderate ROS can regulate synaptic plasticity and learning and memory. On the other hand, excessive ROS can damage mitochondrial structures and oxidative injury, which can produce free radicals, which in turn cause mitochondrial abnormalities (Sun and Chen 1998). Calcium ions act as messengers to activate intra-mitochondrial metabolic processes (McCORMACK et al. 1990). The level of calcium ions regulates the activation of mitochondrial permeability transition as well (Wong et al. 2012). Oxidative stress associated with mitochondrial dysfunction can induce cytochrome C which activates caspase dependent apoptotic pathway (Lin et al. 2012). Recently, it has been shown that apoptosis is associated with changes in mitochondrial mass consistent with mitochondrial fission (Chistiakov et al. 2014). Taken together, mitochondrial dysfunction involved in the number of vital cell physiological processes through ATP production, Ca^{2+}, and oxidative stress.

Several lines of evidence demonstrate that mitochondrial dysfunction underlie pathophysiology of MDD. For example, chronic stress-induced depression in rodents leads to inhibition of mitochondrial respiratory chain in the cortex and cerebellum (Rezin et al. 2008). Neuroimaging studies with PET (positron emission tomography) or SPECT (single-photon emission computed tomography) have found that MDD patients show the symptoms of reduced blood flow, energy, and glucose metabolism, notably in the prefrontal cortex and basal ganglia (Beauregard 2014). When mitochondrial functions were studied in muscle biopsy, it was found that both the production of ATP and activity of respiratory chain enzymes are decreased in MDD patients (Wibom et al. 2002). Chang et al. (2015) compared the alterations of mtDNA copy number, SNPs, and oxidative damage of mtDNA in leukocytes of MDD patients and found that mtDNA copy number in MDD patients was significantly lower than the control group, however, no common 4977-bp deletion in mtDNA was detected. On the other hand, Gardner et al. (2003) reported that deletions in the mtDNA, a 9.2 kbp product encompassing nt4336–13532, were more frequent in the MDD patients compared with control subjects. They also showed a significant decrease of mitochondrial ATP production rates and mitochondrial enzyme ratios in the MDD muscles compared to

controls. Two other groups investigated mitochondrial respiration via high-resolution respirometry in the intact and permeabilized platelets of MDD patients and found significant decrease of respiration efficiency in intact platelets in this population (Hroudová et al. 2013; Karabatsiakis et al. 2014). On the other hand, respiratory rates were unchanged in permeabilized platelets of MDD patients. Karabatsiakis et al. (2014) also confirmed that mitochondrial respiration of PBMCs is significantly decreased in MDD patients. Importantly, decline of respiratory activity is negatively correlated with the severity of depressive symptoms, such as loss of energy, difficulties concentrating and fatigue etc. (Karabatsiakis et al. 2014).

Recently, genetic studies have confirmed that mitochondrial genes contribute to risk of MDD. For example, using low-coverage whole-genome sequencing of 5,303 MDD and 5,337 controls, Cai et al. (2015) identified two genes to be associated with MDD: one is *SIRT1* and the other one is *LHPP. SIRT1* is involved in mitochondrial functions. These investigators also reported that MDD was associated with increased amounts of mtDNA (Cai et al. 2015). Another study examined the association between mtDNA sequence variations and heteroplasmy levels in the dorsolateral prefrontal cortex from a cohort of schizophrenia, bipolar, and MDD subjects and age-matched controls. Three mitochondrial variants (C10652T, T14668C and A15043G) showed a significant allelic association with MDD (Rollins et al. 2009). Altogether, these evidences clearly suggest that mitochondria dysfunction may participate in the pathophysiology of MDD.

DNA microarray analyses have been conducted to detect alteration of mitochondria-related gene expression in postmortem brain of schizophrenia and bipolar subjects (Iwamoto et al. 2005). A small fraction of genes encoding respiratory chain components was found to be downregulated in these subjects. However, there have been no studies examining the pattern of mitochondria gene expression in the MDD postmortem brain samples. In the present study, we performed quantitative analysis for expression of mitochondria-related genes in dorsolateral prefrontal cortex (dlPFC) to reveal difference between MDD and control subjects. In parallel, we conducted bioinformatics analysis to investigate corresponding pathway(s) associated with mitochondrial functions.

Material and Methods

Tissue preparation

The present studies were performed in dlPFC (Brodmann's area 9) obtained from the right hemisphere of the brain from 11 MDD subjects and 11 non-psychiatric control subjects (referred as control subjects). Brain samples were obtained from the Brain Collection Program of the Maryland Psychiatric Research Center, Baltimore, MD. Family members/ informants signed written informed consents. The prefrontal cortex was defined as the gray matter from the most anterior 1-cm coronal slice of the cortex and was further dissected according to the Brodmann atlas. All the tissues were screened for evidence of neuropathology by experienced neuropathologists. The presence of Alzheimer's disease, infarcts, demyelinating diseases, or atrophy (or clinical history of these disorders) disqualified subjects from the study. pH of the brain was measured as discussed earlier (Dwivedi et al. 2009).

Psychological autopsy

Psychological autopsies were performed using the Diagnostic Evaluation After Death (DEAD) (Salzman et al. 1983) and the Structured Clinical Interview for the DSM-IV (SCID) (Spitzer RL 1995) as described earlier (Dwivedi et al. 2009). Briefly, one or two family members, after giving verbal informed consent, underwent an interview. Two psychiatrists independently reviewed the write-up from this interview, as well as the SCID that was completed from it, as part of their diagnostic assessment of the case. Diagnoses were made from the data obtained in this interview, medical records from the case, and records obtained from the Medical Examiner's office. The two diagnoses were compared and discrepancies were resolved by means of a consensus conference. Control subjects were verified as free from mental illnesses using these consensus diagnostic procedures. Toxicology screening for alcohol, a comprehensive battery for illicit drug use, and screening for antidepressant or psychoactive drugs taken prior to death were performed in blood/urine of each subject. None of the subjects showed positive toxicology for alcohol or substance abuse. In each case, screening for the presence of HIV was done in blood samples, and all HIV-positive cases were excluded. This study was approved by the Institutional Review Board of the University of Alabama at Birmingham. Demographic and clinical characteristics of normal controls and MDD subjects are provided in Table 1.

RNA isolation

Total RNA was isolated using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to manufacturer's directions and treated with DNAse 1. The RNA yield was determined by absorbance at 260 nm and the purity of RNA was determined by measuring the optical density with an absorbance ratio of 260/280 using NanoDrop®ND-1000 (NanoDrop Technologies, DE, USA). Only RNA with >1.8 purity was used. The quality of RNA was assessed using Agilent Bioanalyzer 2100 as described earlier (Dwivedi et al. 2009) and only samples with an RNA integrity number >7 were used.

PCR array

RT reaction was performed with 400 ng of total RNA using an RT^2 First-strand Kit (SABiosciences, Qiagen Inc., Valencia, CA). The quality of RNA was confirmed by using an RT^2 RNA QC PCR Array Kit (SABiosciences, Qiagen Inc., Valencia, CA, USA). Two hundred nanograms of random-primed cDNAs were processed for quantitative real-time reverse-transcriptase PCR (qRT-PCR) of 84 genes involved in biogenesis and function of mitochondria and housekeeping genes by using an RT^2Profiler™ PCR Array Kit for human mitochondria (PAHS-087A, SABiosciences, Qiagen Inc., Valencia, CA, USA). An integrated web-based software package for the PCR Array System was used to calculate Ct-based fold-change from the raw threshold cycle data.

Real-time PCR validation

Seven genes which showed significant change were selected to verify the PCR array results. Quantitative real-time PCR expression analysis was carried out using MX3005P sequence detection system (Agilent Technologies, Santa Clara, CA, USA) in dlPFC of each depressed (n = 11) and control (n = 11) subjects in which PCR array was determined. Reaction mixture

(20 μl) was prepared, which included 10 μl of EvaGreen 2X qPCR MasterMix-ROX (Applied Biology Materials, Richmond, BC, Canada), 0.2 mM of each oligo primer and 1 μl of diluted cDNA. Gene transcripts were quantified and normalized using GeNorm algorithm. Following reference genes were used: *B2M*, *HPRT1*, *RPL13A*, *GAPDH*, *ACTB*. The primer sequences for these genes are mentioned in Supplementary Table 1. All biological samples were tested in triplicate, and SD values of the means were calculated using standard statistical methods. The $2^{-\Delta\Delta CT}$ method was used for quantification of the transcript expression (Schmittgen and Livak 2008). Specific annealing of the oligonucleotides was controlled by dissociation kinetics performed at the end of each PCR run. The efficiency of each primer pair was measured on a PCR product by serial dilution. Quantitative real-time PCR primer sequences are provided in Supplementary Table 1.

Statistical and bioinformatics analyses

All data were analyzed using Statistical Package for the Social Sciences (SPSS), version 23 (IBM, USA). The data are reported as the mean ± SD. Similar statistical procedure was followed to examine the difference in expression of genes that were validated. The differences in age, sex, pH of the brain and postmortem interval (PMI), between MDD subjects and controls were analyzed using the independent-sample "*t*"test. An α level 0.05 was considered statistically significant. The relationships between gene expression and PMI, age, and pH of the brain were determined by Pearson product-moment correlation analysis. The confounding variables were used as covariates during data analysis. The effects of gender on various measures were determined by an independent sample "*t*"test comparing males and females. Similarly, an independent sample "*t*"test was used to compare the depressed subjects who showed presence of antidepressant at the time of death with the depressed subjects who did not. To exploit the Ct obtained from PCR-Array, gene expression heatmaps were generated using the heatmap function of the gplots R package (v.2.16.0). PCR array results were analyzed using the $\Delta\Delta Ct$ method (PCR Array Data Analysis Webportal: http://www.sabiosciences.com/pcrarraydataanalysis.php (SABiosciences, Qiagen, Valencia, CA, USA). The fold change was normalized to the arithmetic mean of five reference genes (*RPL13A*, *GAPDH*, *ACTB*, *B2M*, and *HPRT1*). Mitochondria related gene expression with differences greater than 1.3-fold (p 0.05) was considered as differential expressed genes. Gene Ontology (GO) term enrichments were performed TopGO R package (v.2.18) with the classic Fisher method. The differentially expressed genes and fold change were uploaded into data analysis software (Ingenuity System Inc., Redwood City, CA, USA). To view the molecular connections and regulatory network, canonical pathway analysis were conducted with IPA software. In the analysis of validation in MDD, the housekeeping gene GAPDH was selected as the reference and the relative expressions value were expressed by $\Delta Ct = Ct$ target gene-Ct GAPDH, which means that the higher value of ΔCt of the mitochondrial genes, the lower its transcription level.

Results

Subject demography

The demographic characteristics of MDD subjects and normal control subjects are provided in Table 1. There were 8 males and 3 females in the control group, and 9 males and 2

females in the MDD group. The age range was 24–77 years; the PMI was in the range of 8–36 hr. There were no significant differences in age (t = 1.5, df = 20, p = 0.15) or PMI (t = 0.59, df = 20, p = 0.56) between MDD subjects and control subjects. The mean brain pH values of MDD subjects and controls were 6.8±0.13 and 6.8±0.2, respectively, which were not different between these groups (t = 0.37, df = 20, p = 0.71).

Transcriptional profiling of mitochondrial genes

The transcriptional profiling of 84 mitochondrial genes was obtained by the human mitochondrial RT^2 profiler PCR array and was used to produce a heatmap scheme (Figure 1a) and scatter plot (Figure 1b). The figure describes the difference between MDD and control groups. On the basis of Ct value of each gene in the heatmap figure, high Ct value represented the low expression.

There were a total of sixteen genes (fold change   1.3) that were differentially expressed in the MDD group compared with control group. Among them, three genes were down-regulated in the MDD subjects. These 3 genes included: *SLC25A23* (phosphate carrier), *SCL25A12* (aspartate/glutamate carrier), and *STARD3* (lipid trafficking proteins) (Table 2). There were also 13 mRNA upregulated changes in the MDD patients. These upregulated genes and related expression information are listed in the Table 3. The fold change of SFN (stratifin) was 2.24 and ranked the 1st among all the differentially expressed genes. In addition, *TIMM8B*, the p-value of which was 0.008453, was associated with MDD. *TIMM8B* encodes translocase of inner mitochondrial membrane 8 homolog B and shows moderate to high expression in human central nervous systems. The studies related to *TIMM8B* are rarely reported. Our study provides the first proof for the *TIMM8B* and *SFN* in MDD.

Effect of confounding variables

The effects of potential confounding variables, namely, age, gender, PMI, and brain pH were evaluated with respect to the expression of mitochondrial genes, in which we had found differences between control and MDD subjects. As shown in Supplemental Table 2, there were no significant effects of any of these variables on the expression of mitochondrial genes. There were 8 males and 3 females in the control group and 9 males and 2 females in the MDD group. Comparison of expression levels between males and females showed no effects in both control and MDD groups (Supplemental Table 3). Out of 11 MDD subjects, 7 had no antidepressant toxicology whereas 4 showed antidepressant toxicology at the time of death. Except *MIPEP* and *BDC3* genes, the expression levels of other mitochondrial genes were similar in groups with and without antidepressant toxicology (Supplemental Table 4).

Validation in the MDD samples by qRT-PCR

Seven differentially transcribed mitochondria genes were selected to validate in the MDD and control subjects (n =11 in each group). The results are shown in Figure 2. As can be seen, the expression of *TIMM8B* (t = 2.87, df = 20, p = 0.01) and *SLC25A23* (t = 2.04, df = 20, p = 0.02), SFN (t = 2.46, df = 20, p = 0.023), *SLC25A30* (t = 2.54, df = 20, p = 0.021), and *UCP2* (t = 3.0, df = 20, p = 0.007), were upregulated and *SLC25A12* (t = 4.03, df = 20, p = 0.001), and *SLC25A27* (t = 5.59, df = 20, p <0.001), were downregulated in MDD

subjects as compared with control subjects. The fold changes in in these genes were almost similar when replicated as compared with the qRT-PCR profile array data.

Gene ontology (GO) analysis

To obtain information about biological processes, we performed GO analysis with TopGO packages of R software. Gene ontology analysis was performed to identify significantly enriched biological processes, molecular functions, and cellular components. For biological processes, we found a total of 416 GO terms with p-value <0.01. These GO terms include direct or indirect functions towards mitochondria. The top 20 statistically significant GO terms associated with mitochondria biological processes are listed in Supplemental Table 5. The most significantly GO terms were related to mitochondrial transport, establishment of protein localization in mitochondrion, protein localization to mitochondrion, single-organism intracellular transport, intracellular transport, apoptotic mitochondrial changes, etc. For molecular functions, there were 29 GO terms, the p-value of which was<0.01. Supplemental Table 6 shows top 20 molecular function terms from GO enrichment analysis. Theses molecular function terms are involved in many multiple signaling and binding activities including MDM2/MDM4 family protein binding, protein phosphatase 2A binding, protein serine/threonine kinase inhibitor activity, etc. Twenty two GO terms of cellular component category reached significance level (p< 0.01) (Supplemental Table 7). Of these 20 GO terms include mitochondrion, mitochondrial part, organelle envelope, etc. GO term enrichment analysis provided a brief view of biological processes, cellular components and molecular functions associated with MDD.

Pathway analysis

We uploaded 16 differentially expressed genes into IPA analysis tool. Canonical pathway analysis was first performed to find these gene specific affected pathways. The results are shown in the Figure 3. The top canonical pathway for these gene sets includes p53 signaling, Myc-mediated apoptosis signaling, induction of apoptosis by HIV1, PEDF signaling, etc. These results indicate an association between apoptosis and mitochondria gene sets in MDD. There were 4 differentially expressed genes (*BBC3, Bcl2, CDKN2A, TP53*) enriched in P53 pathway (p $=3.16\times 10^{-7}$) and 3 genes (*Bcl2, CDKN2A, TP53*) in the Myc-mediated apoptosis signaling (6.44×10^{-6}).

Discussion

To our knowledge, this is the first study to explore the mitochondria gene expression in the postmortem brain of MDD subjects. In this study, we used mitochondrial PCR array profiling to find alterations in 84 mitochondria related gene expression. We found 16 specific genes differentially expressed between MDD and control subjects. In order to examine if the changes in mitochondrial genes in MDD subjects can be replicated, we independently examined expression of *TIMM8B, SLC25A23*, *SFN, SLC25A30, UCP2, SLC25A12*, and *SLC25A27* in dlPFC that we found to be altered in the MDD group. We were able to replicate the findings, suggesting that these changes are consistent. We did not find any effect of confounding variables such as age, sex, postmortem interval, and pH of the brain on any of the genes that showed significant change in the MDD group. Two genes

MIPEP and *BBC3* were downregulated in the MDD subjects who showed positive antidepressant toxicology. Interestingly, these two genes were upregulated in the MDD group, suggesting that antidepressant toxicology had no overall effect on the expression of these two genes.

Three downregulated genes, including *SLC25A27*, *SLC25A12*, and *STARD3* were significantly decreased in the dlPFC of MDD subjects. Of interest to this study was *SLC25A27*. The product of *SLC25A27* is uncoupling proteins 4 (UCP4), which is located on the inner mitochondrial membrane and plays an important role in reducing ROS and alleviation of oxidative stress, consequently, inhibition of apoptosis, ATP inadequacy, etc. (Ho et al. 2012). It has earlier been reported that downregulation of *SLC25A27* could negatively influence these processes. Abnormalities in *UCP4* have been reported in schizophrenia, autism spectrum disorder, and Huntington's disease (Anitha et al. 2012; Besson et al. 2010; Mouaffak et al. 2011; Yasuno et al. 2007). This paper provides evidence that downregulation of *SLC25A27* may contribute to neuropsychiatric disorders.

We also found that the expression of 13 genes was significantly increased in dlPFC of MDD subjects. These include: *SLC25A30, TIMM8B, SOD2, BCL2, UCP2, SLC25A23, MIPEP, TP53, SLC25A31, UCP1, CDKN2A, BBC3,* and *SFN*. Among these 13 genes, 3 genes *SLC25A30, SLC25A23, and SLC25A31* belong to the member of solute carrier family 25.*SLC25A23* encodes the human APC2 (ATP-Mg/Pi carrier), which is a part of Ca^{2+}sensitive mitochondrial carriers. *SLC25A23* mediates the influx of Ca^{2+} and regulates ATP levels in neurons (Rueda et al. 2015). Le-Niculescu et al. (2009) performed gene expression profiling studies in blood samples of mood disorder patients and identified *SLC25A23* as one of the candidate biomarker genes with convergent functional genomics, which can elevate low mood state (MDD). Our results in postmortem brain samples confirm this finding and suggest that upregulation of *SLC25A23* may be associated with MDD.

SLC25A31,which encodes ANT4 protein (adenine nucleotide translocases), helps in supplying ADP to mitochondria and in release of ATP into cytoplasm (Dolce et al. 2005). So far, there is no published report of *SLC25A31* and *SLC25A30* in MDD. Our study is the first one to demonstrate such changes in this disorder. Stratifin, also called 14-3-3 sigma, is encoded by SFN gene. Genetics studies have shown that *SFN* is associated with schizophrenia. A reduction in mRNA and protein expression of diverse 14-3-3 isoforms have been reported in different brain areas of subjects with schizophrenia (English et al. 2009; Middleton et al. 2005; Vawter et al. 2001). Recently, (Rivero et al. 2015) performed western blot analysis of this protein in the PFC of MDD and control subjects. They found that the immunoreactivity values of 14-3-3 sigma were not alteration in MDD subjects. Although data from in vitro, animal, and human postmortem studies supported that 14-3-3 protein plays critical roles in various neurodegenerative disorders including schizophrenia and bipolar, so far, there have been no report of SFN in pathophysiology of MDD. Our study provides strong evidence of altered SFN expression in brain of MDD subjects.

Our study showed that superoxide dismutase 2 (SOD2) was significantly increased in dlPFC of MDD subjects, which successfully replicated Szuster-Ciesielska et al. (2008) findings. SOD2 expresses proteins of mitochondrial antioxidant enzymes. ROS induces upregulation

of SOD2 through activation of cellular signaling pathways. Evidence shows that ROS or RNS increase and decrease antioxidant defense in depression (Maes et al. 2011). The increase of ROS/RNS and reduction of antioxidant defenses may cause damage of DNA and fatty acids (Aprioku 2013). We found *SOD2* upregulation bears further testimony to oxidative stress in the MDD patients.

In this study, we performed Gene Ontology analysis on the three categories ("Molecular function", "Biological process" and "Cellular component") and listed the top 20 significantly enriched GO terms. For molecular function part, there are 29 GO terms, the p-value of which is below 0.01. These 29 GO terms include MDM2/MDM4 family protein binding, protein kinase inhibitor activity, transporter activity, superoxide dismutase activity, etc. The results demonstrated the altered expression genes of mitochondria takes part in several crucial physiological functions. For cellular component part, the 22 GO terms, with p-value <0.01, were identified with topGO software. The significant GO terms were mainly associated with mitochondrial membrane, envelope, cytoplasm and mitochondrial matrix, etc. For biological process, we found 416 GO terms with p-value <0.01. These GO terms included cell apoptosis, cell aging, neuron death, B cell differentiation, chromatin assembly, etc. These results suggest that the mitochondrial associated genes may participate in MDD pathophysiology via several biological processes. Further studies will be needed to confirm the involvement of these biological processes.

When we analyzed the canonical pathways, we found that p53- and Myc-mediated apoptosis signaling pathways were significantly enriched in the brain of MDD subjects. These two signaling pathways were identified around *Bcl2*, *TP53* and *CDKN2A* genes. These results reveal strong possibility of apoptosis as pathophysiological process of cell death, which may significantly contribute to the understanding of the etiology of MDD. *Bcl2* is a member of apoptosis family genes, which is linked with other apoptotic-regulatory factors at the mitochondria membrane and regulates the apoptosis. Bcl-2 family members are activated by various types of stresses and form pores in the mitochondrial outer membranes (Gross et al. 1999). Cytochrome C and other pro-apoptotic factors are released from the intermembrane space and triggercaspase-3 pathway (Gogvadze et al. 2006). It has been reported that *BCL2* confer risk of MDD susceptibility and antidepressant treatment outcome in a Han Chinese population. A significant association between the rs2279115C allele associated with *BCL2* and treatment resistant depression in males (corrected p=0.048) were found (Zhang et al. 2014). On the other hand, resistance to the development of stress-induced behavioral despair in the forced swim test is associated with elevated hippocampal anti-apoptotic Bcl-xl expression (Shishkina et al. 2010). We speculate that apoptotic pathways interact with the other pathways including oxidative and inflammatory, which may result in the development of MDD phenotype.

Besides p53- and Myc-mediated apoptosis signaling pathways, we also found PI3K-AKT and Wnt/β-catenin signaling pathways were significantly enriched in the canonical pathway analysis. PI3k-mediated signaling plays a crucial role in many physiological functions in the brain, including cell survival, synaptic plasticity, protein synthesis, and membrane trafficking. The function of *PI3K* is to activate the downstream target Akt, which is central to the PI3K-AKT pathway. We have earlier reported reduced catalytic activation and altered

expression of specific PI3-kinase regulatory subunits in postmortem brain of depressed suicide subjects (Dwivedi et al. 2008). We have also reported that the catalytic activity and expression of the other 2 important genes in the PI3K/AKT pathway, PDK and PTEN, were altered in the prefrontal cortex and hippocampus of depressed suicide subjects (Dwivedi et al. 2010). Our study also supports the evidence that aberrant PI3K-kinase signaling may possible involved in the pathogenic mechanisms of depression.

Interestingly, canonical pathway analysis yielded Wnt signaling, which has been shown to be involved in affective disorders. For example, several genetics studies have found an association of Wnt signaling pathway genes in patients with MDD (Inkster et al. 2010). The mechanism of action of several mood stabilizing agents have been shown to be associated with Wnt signaling (Zhou et al. 2009). Dysregulated expression of several Wnt pathway-related genes has been reported in patients with bipolar disorder and suicide (Matigian et al. 2007; Ren et al. 2013). A recent study by explored the influence on GSK-3β expression, a critical component of Wnt signaling, in the rapid antidepressant action of ketamine in a mouse model of depression (Beurel 2011). The study demonstrated that ketamine can rapidly increase the phosphorylation of both GSK-3α and GSK-3β. Taken together, these studies confirmed the important role of the Wnt signaling system in depression and treatment response.

In summary, we identified 16 differentially expressed mitochondria genes by human RT^2 PCR array. These differentially genes not only contain mitochondrial transporter family proteins but also include specific genes related to oxidative stress and apoptotic pathways. We also conducted GO analysis and IPA analysis and found several new regulatory networks. Overall, our study provides strong evidence that mitochondrial genes play a critical role in the MDD and indicate potential therapeutic targets and biomarkers to predict the MDD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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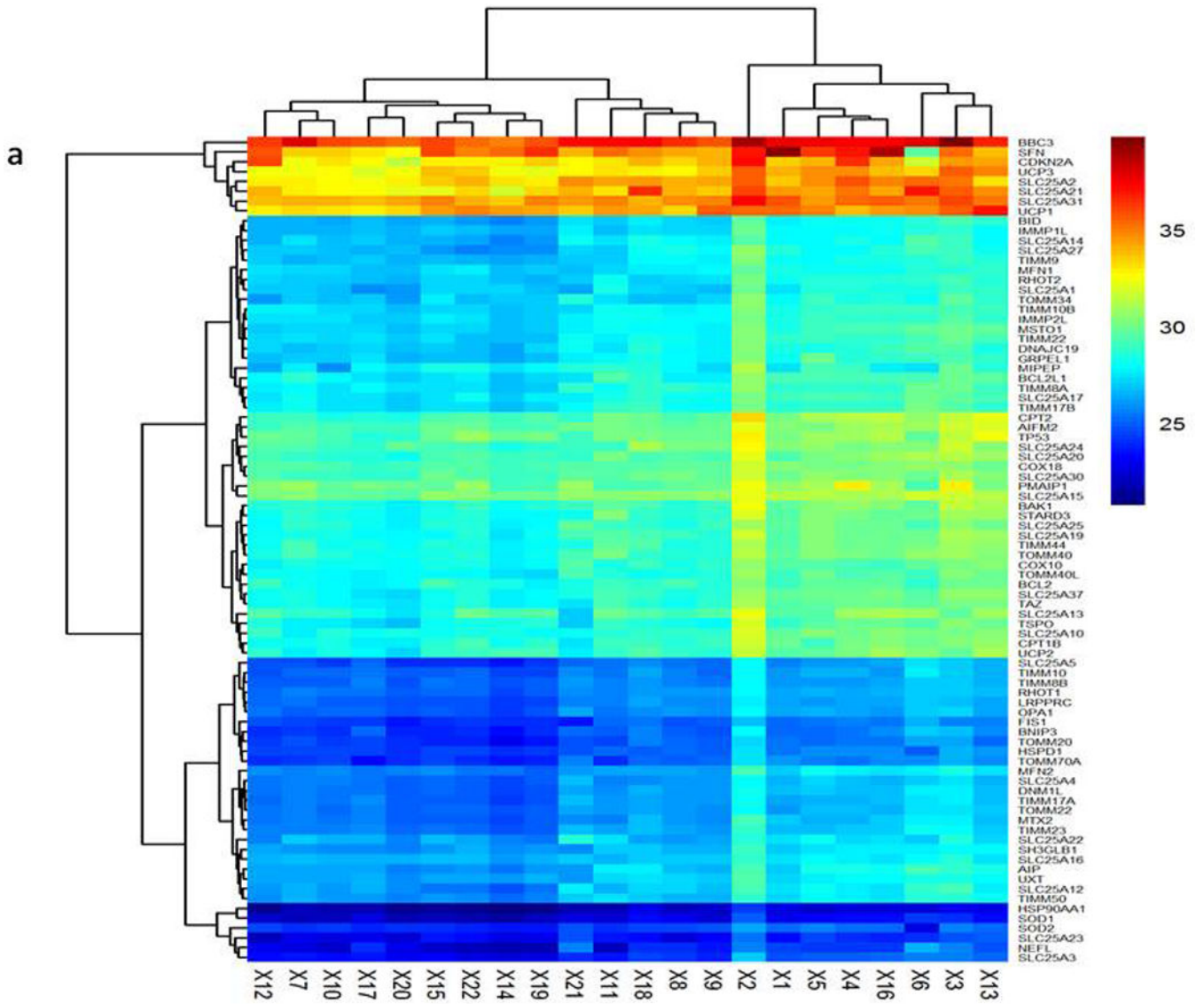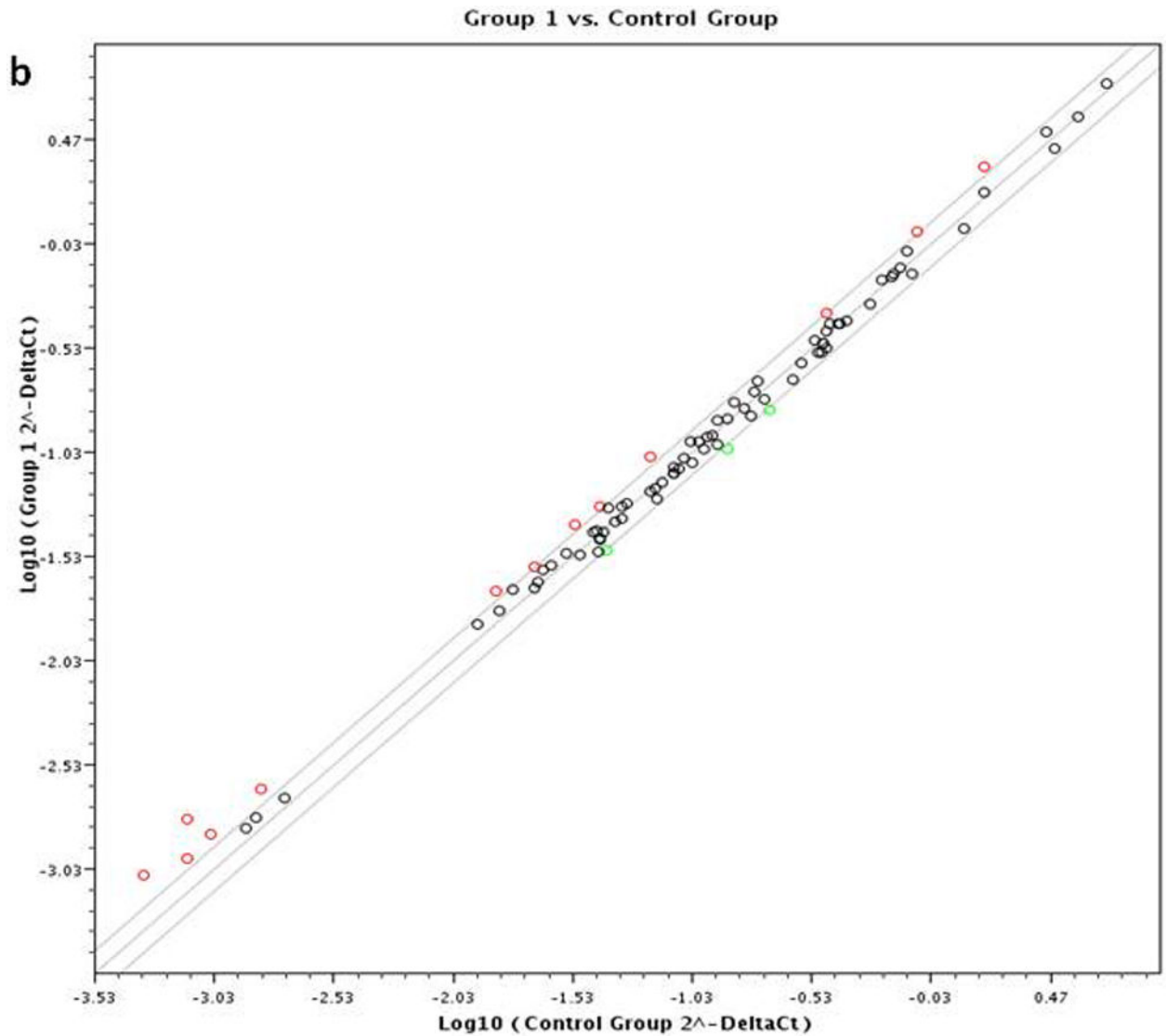
Figure 1.

Figure 1a: Hierarchical clustering analysis of mitochondria gene expression profile in MDD and control subjects. Each row represented different sample, each column represented different genes. Red represents high Ct value with low expression. Blue represent low Ct value with high expression. Figure 1b: Scatter plot based on the fold change of each gene. Group 1 means MDD Group; Control means healthy control.

Figure 2.
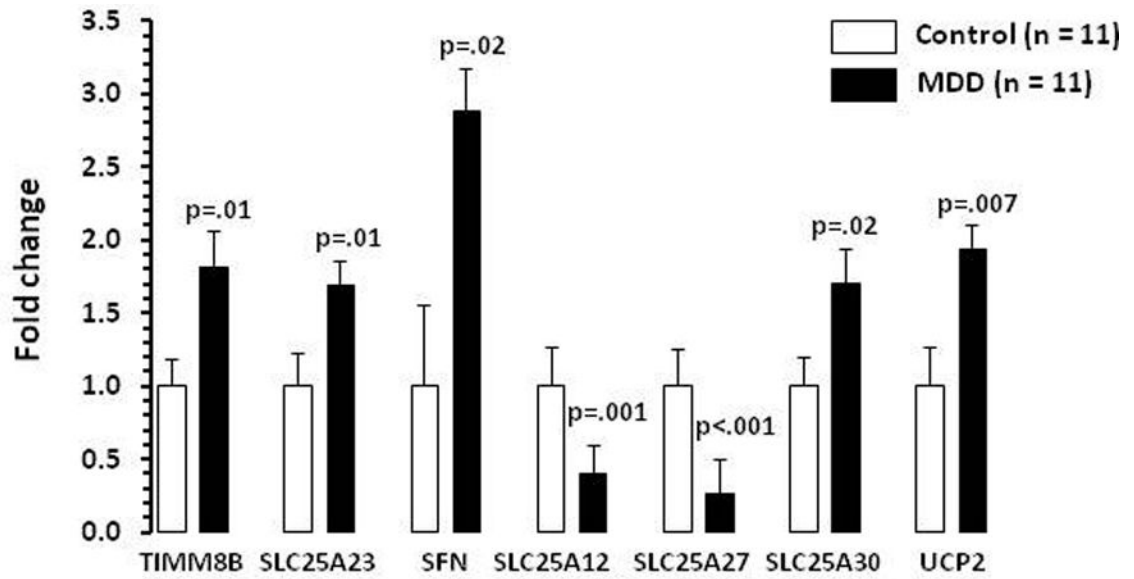Bar diagram showing expression levels of genes that were tested for validation analysis in dlPFC of control and MDD subjects. Data are the mean ± SD. N = 11 in each group.
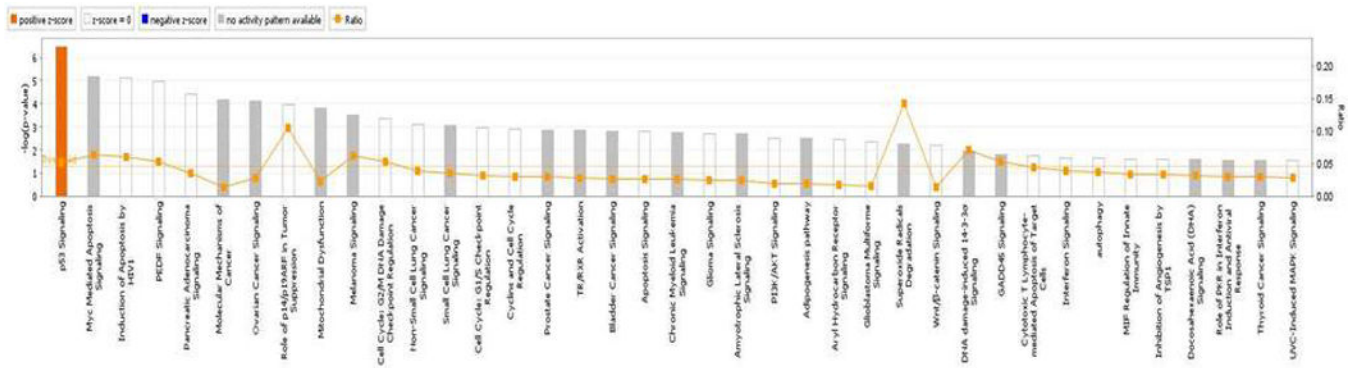
Figure 3.

Canonical pathway analysis of genes differentially expressed in dlPFC of MDD subjects. The line represents the ratio of the number of genes represented within each pathway to the total number of genes in the pathway.

Table 1

Demographic and clinical characteristics of control and MDD subjects

Case #	Age (yrs)	Race	Gender	PMI (hr)	Brain pH	Cause of Death	Drug Toxicology	Alcohol abuse	Diagnosis
1	37	White	Male	24	6.8	ASCVD	None	None	Control
2	35	White	Male	24	7.1	Accidental chest injury	None	None	Control
3	45	White	Male	22	6.8	ASCVD	None	None	Control
4	45	White	Female	12	6.8	Pneumonia	OTC cold medicine	None	Control
5	24	White	Female	23	6.7	Embolism	None	None	Control
6	47	White	Male	10	6.9	ASCVD	None	None	Control
7	37	White	Female	19	6.9	Hemopericardium	None	None	Control
8	31	White	Male	16	6.7	Vehicle injury	None	None	Control
9	28	White	Male	13	7.1	Electrocution	None	None	Control
10	28	White	Male	13	6.8	Cardiac arrhythmia, natural	None	None	Control
11	77	White	Male	16	6.9	Drowning	None	None	Control
Mean SD	39.4 14.6	11 White 0 Black	8 Males 3 Females	17.45 5.18	6.86 0.14				
12	65	White	Male	14	7.1	ASCVD	None	None	MDD
13	55	Black	Female	8	6.9	ASCVD	Fluoxetine, acetone	None	MDD
14	24	White	Male	11	6.8	Vehicle injury	Sertraline	None	MDD
15	39	White	Male	36	6.7	Fatty liver	None	None	MDD
16	46	Black	Male	20	6.6	Seizure	Fluoxetine	None	MDD
17	59	White	Male	20	7.2	ASCVD	Atropine, methadone	None	MDD
18	29	White	Female	22	6.8	Cardiomegaly	Fluoxetine, propoxyphene	None	MDD
19	49	White	Male	23	6.8	Undetermined	None	None	MDD
20	58	White	Male	24	6.5	ASCVD	None	None	MDD
21	59	White	Male	9	6.9	ASCVD	Lidocaine	None	MDD
22	49	White	Male	24	6.9	ASCVD	None	None	MDD
Mean SD	48.4 13.0	10 White 1 Black	9 Males 2 Females	19.2 8.2	6.8 0.2				

ASCVD = Atherosclerosis cardiovascular disease; Controls = non-psychiatric controls; MDD = major depressive disorder; PMI = postmortem interval

Table 2

Down-regulated mitochondria genes expressed in dlPFC of MDD subjects

#	Gene Symbol	UniGene ID	Functional Gene Grouping	Fold Change
1	SLC25A27	9481	Solute carrier family 25, member 27	−1.35
2	SLC25A12	8604	Solute carrier family 25 (aspartate/glutamate carrier), member 12	−1.31
3	STARD3	10948	StAR-related lipid transfer (START) domain containing 3	−1.29

Table 3

Up-regulated mitochondria genes expressed in dlPFC of MDD subjects

#	Gene Symbol	UniGene ID	Functional Gene Grouping	Fold Change
1	SLC25A30	253512	Solute carrier family 25, member 30	1.29
2	TIMM8B	26521	Translocase of inner mitochondrial membrane 8 homolog B (yeast)	1.29
3	SOD2	6648	Superoxide dismutase 2, mitochondrial	1.32
4	BCL2	596	B-cell CLL/lymphoma 2	1.35
5	UCP2	7351	Uncoupling protein 2 (mitochondrial, proton carrier)	1.4
6	SLC25A23	79085	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23	1.42
7	MIPEP	4285	Mitochondrial intermediate peptidase	1.43
8	TP53	7157	Tumor protein p53	1.45
9	SLC25A31	83447	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 31	1.46
10	UCP1	7350	Uncoupling protein 1 (mitochondrial, proton carrier)	1.53
11	CDKN2A	1029	Cyclin-dependent kinase inhibitor 2A	1.55
12	BBC3	27113	BCL2 binding component 3	1.86
13	SFN	2810	Stratifin	2.24