Human Molecular Genetics, 2005, Vol. 14, No. 2 ***241–253***
doi:10.1093/hmg/ddi022
Advance Access published on November 24, 2004

Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis

Kazuya Iwamoto, Miki Bundo and Tadafumi Kato*

Laboratory for Molecular Dynamics of Mental Disorders, Brain Science Institute, RIKEN, Wako, Saitama 351-0198, Japan

Received August 18, 2004; Revised October 15, 2004; Accepted November 9, 2004

Accumulating evidence suggests that mitochondrial dysfunction underlies the pathophysiology of bipolar disorder (BD) and schizophrenia (SZ). We performed large-scale DNA microarray analysis of postmortem brains of patients with BD or SZ, and examined expression patterns of mitochondria-related genes. We found a global down-regulation of mitochondrial genes, such as those encoding respiratory chain components, in BD and SZ samples, even after the effect of sample pH was controlled. However, this was likely due to the effects of medication. Medication-free patients with BD showed tendency of up-regulation of subset of mitochondrial genes. Our findings support the mitochondrial dysfunction hypothesis of BD and SZ pathologies. However, it may be the expression changes of a small fraction of mitochondrial genes rather than the global down-regulation of mitochondrial genes. Our findings warrant further study of the molecular mechanisms underlying mitochondrial dysfunction in BD and SZ.

INTRODUCTION

Bipolar disorder (BD) and schizophrenia (SZ) are the two major psychiatric disorders, each affecting ~1% of the population worldwide. Despite decades of extensive genetic and pharmacological studies, the etiology or pathophysiology of these disorders remains unclear. Twin, adoption, family and linkage studies suggest that they are complex genetic diseases (1). Although the two disorders are typically distinct in that BD is a disorder of emotion whereas SZ is a disorder of thought or cognition, they have common pathophysiological profiles such as a chronic and relapsing course of illness and some psychotic symptoms. They also have overlapping genetic profiles, such as common susceptibility loci, as suggested by linkage studies (2). Recently, DNA microarray analysis using postmortem brains of patients has revealed many differences and commonalities between BD and SZ at the molecular level, such as altered expression of genes encoding receptors, channels, transcription factors and molecular chaperones (3), as well as altered expression of oligodendrocyte genes (4,5) and mitochondrial genes (6,7).

In BD, we have previously proposed the mitochondrial dysfunction hypothesis (8) based on the following evidence: (i) altered energy metabolism in brains of patients with BD, as detected by phosphorus-31 magnetic resonance spectroscopy (9), (ii) increased ratio of mitochondrial DNA (mtDNA) deletion in postmortem brains of patients with BD (10) and (iii) genetic association between mtDNA polymorphisms and BD (11). Importantly, an *in vivo* study has revealed elevated gray matter lactate levels in medication-free patients with BD, supporting the mitochondrial dysfunction hypothesis in BD (12). A number of studies have also suggested that mitochondrial dysfunction underlies the pathophysiology of SZ (13,14). Altered activity and expression of mitochondrial respiratory chain components in lymphocyte and postmortem brains of patients with SZ have been reported (15–19). An electron micrograph study reported a decreased number of mitochondria per axon in the striatum of medication-free SZ patients (20).

Among the available genomewide techniques, DNA microarray is the most suitable tool to survey the expression levels of almost all known mitochondrial genes in a given sample.

*To whom correspondence should be addressed at: Laboratory for Molecular Dynamics of Mental Disorders, Brain Science Institute, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan. Tel: +81 484676949; Fax: +81 484676947; Email: kato@brain.riken.go.jp

Several DNA microarray studies of postmortem brains of patients with BD or SZ have already been reported. These studies have differed in subjects, sample size, brain region and type of array, and their results seem to differ widely with regard to the expression profiles of mitochondrial genes. Konradi *et al.* (6) reported a global down-regulation of mitochondrial genes, such as those encoding the respiratory chain components, in BD, and they did not find such down-regulation in SZ. In our previous study, in contrast, we did not find such alterations in patients with BD, SZ or major depression (3). Other DNA microarray studies also reported no global down-regulation of mitochondrial genes in BD (21) or SZ (4,22–24), whereas some DNA microarray, proteomics and metabolomics approaches have revealed the global alterations of mitochondrial function in SZ (7,25).

There are several issues of concern about interpreting data from clinical samples, as it is difficult to control the experimental conditions, especially in studies using postmortem brains. Among the confounding factors in postmortem brain studies, sample pH and terminal condition of the subject (referred to as the agonal state) have been extensively addressed (26–30). The subjects who had prolonged agonal states, such as death by respiratory failure, or coma, tended to show effects of hypoxia or acidosis in the brain, both of which typically decrease brain pH. Although the mechanism by which a decrease in brain pH affects gene expression remains unclear, the subjects with low-pH brain tissue exhibited a distinct gene expression pattern from that seen in high-pH samples. Li *et al.* (29) found that brain pH affects gene expression systematically, according to functional categories of genes. One of the largest effects was the global down-regulation of mitochondrial genes, which was strikingly similar to the expression changes previously reported in BD and SZ (6,7). This raises a serious question that the down-regulation of mitochondrial genes observed in BD and SZ could be an artifact of sample pH rather than indicative of disease characteristics, because patients tend to show more complex causes of death than controls do, and many of these complications also lead to low-pH brain. The other concern is the effect of medication, because most drugs that are used to treat psychiatric disorders have mitochondrial toxicity (31–41).

Here, we performed large-scale DNA microarray analysis of postmortem brains of patients with BD or SZ, as well as controls, and we focused our analysis on the expression profiles of mitochondria-related genes. The goal of this study was to reveal whether mitochondrial dysfunction in BD or SZ can be detected from a gene-expression standpoint, after the effects of pH and medication are taken into consideration.

RESULTS

Overall expression changes of mitochondria-related genes

From a total of 105 RNA samples, we obtained expression profiles from 102 samples (Table 1). No difference in the percentage of presence call, which indicates the proportion of probe sets that were reliably detected in a sample, was found in BD (0.50 ± 0.06, $n = 33$, $P = 0.34$) or in SZ (0.52 ± 0.03, $n = 35$, $P = 0.86$) brain samples, when compared with controls (0.51 ± 0.01, $n = 34$).

Among the 676 mitochondria-related probe sets (mt-probe sets) analyzed, only those differentially expressed in BD or SZ compared with controls were selected by Student's *t*-test. In BD, 15.1% of mt-probe sets were significantly altered, whereas 11.2% were altered in SZ (Table 2). To examine whether these changes occurred in a diagnosis-specific manner, we used the case information concerning suicide status. We found that only 3.6% of mt-probe sets were different between suicide and non-suicide subjects (patients and controls). There was an extensive deviation toward down-regulation in both BD (94.1% of differentially expressed mt-probe sets, chi-square test for goodness-of-fit, $\chi^2 = 79.4$, $P = 5.65 \times 10^{-22}$) and SZ (81.6%, $\chi^2 = 30.3$, $P = 2.32 \times 10^{-8}$). In contrast, there was no significant deviation in the direction of expression changes corresponding with suicide status. Similar results were obtained when we performed the same analysis using the data normalized by robust multiarray average (RMA) method, although the number of differentially expressed mt-probe sets was slightly decreased in SZ (Table 2). Because of the suitability to the purpose of handling the different sets of samples, we used the data normalized by MAS5 for further analyses.

False discovery rate (FDR) analysis using the QVALUE software revealed that among the differentially expressed mt-probe sets, 24 showed significant changes with a *q*-value of 12.3% in BD ($\pi_0 = 0.588$) and 15 showed significant changes with a *q*-value of 15.3% in SZ ($\pi_0 = 0.730$); no significant changes were detected in relation to suicide status ($\pi_0 = 1$). FDR analysis using the significance analysis of microarrays (SAM) software also yielded similar results (data not shown). Although these results supported our initial findings, they clearly yielded conservative results, which prevented us from testing mitochondrial gene expressions further. We thus used standard statistical analyses to explore the relationships between mental disorders and expression of mt-probe sets.

Effect of pH

Expression levels of mt-probe sets showing down-regulation in BD or SZ compared with controls (139 probe sets) were remarkably correlated with sample pH (Table 3). In addition, the average sample pH values of BD and SZ were slightly but significantly decreased compared with controls in our sample sets (Table 4). To deal with this, we first performed non-biased hierarchical clustering analysis, based on the expression levels of 676 mt-probe sets (Fig. 1). This clustering analysis yielded two major groups: one group showed up-regulation and the other group, which mainly consisted of low-pH samples, showed global down-regulation of mt-probe sets. In agreement with the observation of 'critical pH threshold' (29), below which point transcriptional changes of mt-probe sets occurs, there seemed to be a pH boundary between 6.4 and 6.5 that separated groups in our sample set. Other variables, such as diagnosis, side of brain, gender and suicide status, could not account well for the clustering results. Although Li *et al.* (29) also pointed out that the agonal state affects the expression of mitochondrial genes, this did not help to explain the clustering results (see Discussion). Clustering

Table 1. Characteristics of the subjects

DSM-IV diagnosis	Age/gender	Cause of death	PMI	Brain pH	Side	Brain weight	Age of onset	Medication
Bipolar disorder								
297	29/M	Suicide: jumped	48	6.39	L	1570	22	Risperidone, lithium
297	29/M	Suicide: CO	60	6.70	L	1430	17	
296	45/M	Cardiac	28	6.35	R	1480	35	Thioridazine, risperidone, lithium, valproate and benztropine
296	29/F	OD	62	6.74	L	1330	18	Trazodone
297	44/M	Suicide: hanging	19	6.74	R	1660	33	Valproate
296	49/F	Suicide: MVA	19	5.87	R	1380	22	Perphenazine, lithium
297	48/F	Cardiac	18	6.50	L	1205	33	Fluoxetine, trazodone, doxepin
297	42/M	Drowning	32	6.65	R	1470	18	
297	35/M	Cardiac	35	6.30	L	1490	19	Haloperidol, lithium
297	59/F	Suicide: OD	53	6.20	R	1410	48	Valproate, paroxetine, trazodone
297	54/M	Suicide: OD	44	6.50	L	1510	45	Valproate, paroxetine
297	35/F	Suicide: CO	17	6.10	R	1250	21	Olanzapine, amitriptyline
296	45/M	Ketoacidosis	35	6.03	L	1300	16	Thioridazine, olanzapine, gabapentin
296	42/F	OD	49	6.65	L	1335	20	Risperidone, trazodone
297	58/F	Suicide: GSW	35	6.50	L	1440	27	Haloperidol, lithium
297	41/M	OD	39	6.60	L	1375	21	
296	64/M	Pneumonia	16	6.10	R	1340	19	Clozapine, valproate
297	59/M	Sleep apnea	84	6.65	R	1300	25	Valproate, gabapentin, trazodone, zolpidem
296	55/F	Cardiac	41	5.76	R	1270	40	Thiothixene
297	51/F	Suicide: bleeding	77	6.42	L	1120	35	Risperidone, valproate, paroxetine
296	51/M	Cardiac	23	6.67	R	1590	23	Lithium, carbamazepine, valproate
297	63/F	Cardiac	32	6.97	L	1290	43	Mirtazapine
297	44/F	Myocarditis	37	6.37	R	1200	26	Quetiapine, olanzapine, valproate, venlafaxine
297	56/F	Drowning	26	6.58	L	1170	14	Valproate, gabapentin, trazodone, sertraline
297	43/F	Suicide: OD	39	6.74	L	1505	25	Quetiapine, carbamazepine, gabapentin, venlafaxine
296	35/M	Drowning	22	6.58	R	1390	14	
297	50/F	Suicide: OD	62	6.51	L	1400	25	Amitriptyline
297	49/F	OD	38	6.39	R	1190	20	Amitriptyline, venlafaxine
297	33/F	Suicide: hanging	24	6.51	L	1450	15	Risperidone, lithium, fluoxetine
296	41/F	Cardiac	28	6.44	L	1360	14	Risperidone, valproate, trazodone
296	56/M	Suicide: OD	23	6.07	R	1670	28	Olanzapine, carbamazepine, fluoxetine, doxepin
296	48/M	Suicide: hanging	23	6.90	L	1466	31	Lithium, fluoxetine
296	19/M	OD	12	5.97	R	1484	17	Quetiapine, topiramate
Controls								
	44/F	Cardiac	28	6.59	R	1330		
	49/M	Cardiac	46	6.50	R	1605		
	53/M	Cardiac	9	6.40	L	1500		
	37/M	Cardiac	13	6.50	L	1600		
	51/M	Cardiac	31	6.70	R	1400		
	53/M	Cardiac	28	6.00	L	1340		
	38/F	Cardiac	33	6.00	R	1120		
	38/F	Cardiac	28	6.70	R	1350		
	60/M	Cardiac	47	6.80	R	1460		
	35/M	Myocarditis	52	6.70	R	1700		
	34/M	Cardiac	22	6.48	R	1480		
	45/M	Cardiac	29	6.94	R	1405		
	34/F	Cardiac	24	6.87	R	1255		
	42/M	Cardiac	37	6.91	L	1340		
	44/F	Cardiac	10	6.20	R	1305		
	57/M	Cancer	26	6.40	R	1470		
	45/M	Cardiac	18	6.81	L	1585		
	49/M	Cardiac	23	6.93	L	1390		
	35/M	Cardiac	24	7.03	L	1415		
	55/M	Cardiac	31	6.70	L	1515		
	49/F	Cardiac	45	6.72	L	1435		
	33/F	Asthma	29	6.52	L	1360		
	48/M	Cardiac	31	6.86	R	1580		
	50/M	Cardiac	49	6.75	R	1645		
	32/M	Cardiac	13	6.57	R	1410		
	47/M	Cardiac	11	6.60	L	1495		

Continued

Table 1. *Continued*

DSM-IV diagnosis	Age/gender	Cause of death	PMI	Brain pH	Side	Brain weight	Age of onset	Medication
	46/M	Cardiac	31	6.67	L	1360		
	40/M	Cardiac	38	6.67	L	1498		
	51/M	Cardiac	22	6.71	L	1900		
	31/M	Pulm embol	11	6.13	R	1335		
	48/M	Cardiac	24	6.91	R	1321		
	39/F	Cardiac	58	6.46	R	1260		
	47/M	Cardiac	36	6.57	L	1535		
	41/F	Cardiac	50	6.17	R	1290		
Schizophrenia								
296	43/M	Pneumonia	26	6.42	L	1620	21	Fluphenazine, valproate, benztropine
295	31/M	Cardiac	33	6.20	R	1480	22	Clozapine, benztropine
295	45/F	Suicide: jumped	52	6.51	R	1510	34	
296	40/M	Pneumonia	34	6.18	R	1480	21	Thiothixene, clozapine, valproate, benztropine
296	51/M	Cardiac	43	6.63	L	1390	23	Fluphenazine
296	19/M	OD	28	6.73	L	1465	18	Thioridazine, olanzapine, valproate
296	53/F	Cardiac	13	6.49	L	1345	29	Haloperidol, lithium
296	37/M	Cardiac	30	6.80	R	1550	13	Thioridazine, thiothixene, fluoxetine
295	52/M	Cardiac	10	6.10	R	1450	28	Haloperidol, benztropine, diphenhydramine
296	24/M	Suicide: OD	15	6.20	R	1505	20	Olanzapine, valproate
296	44/M	Exhaustive mania	9	5.90	L	1415	19	Haloperidol
295	39/M	MVA	80	6.60	L	1355	17	Fluphenazine, benztropine
296	33/M	Cardiac	29	6.50	L	1470	19	Haloperidol, lithium
295	50/M	Cardiac	9	6.20	R	1400	31	Thiothixene
296	43/M	Cirrhosis	18	6.30	R	1520	18	Haloperidol, benztropine
296	32/F	Suicide: jumped	36	6.80	L	1340	29	Risperidone
295	35/M	Cardiac	47	6.40	R	1370	14	Fluphenazine, trihexyphenidyl
295	44/M	Cardiac	32	6.67	L	1560	9	Risperidone, fluvoxamine
296	47/M	Acute pancreat	13	6.30	L	1310	20	Haloperidol, olanzapine, valproate
296	45/M	Cardiac	35	6.66	L	1390	15	
296	36/F	Suicide: hanging	27	6.49	L	1480	33	Risperidone, paroxetine, trihexyphenidyl
296	53/M	Cardiac	38	6.17	R	1400	23	Risperidone
296	54/F	Pneumonia	42	6.65	R	1170	17	Haloperidol, chlorpromazine, benztropine
296	44/F	Poss pulm thromb	26	6.58	L	1490	16	Thiothixene
296	47/F	OD	30	6.47	R	1430	23	Quetiapine, valproate, mirtazapine, bupropion, amitriptyline
296	39/M	Suicide: hanging	26	6.80	R	1470	34	Ziprasidone, risperidone, olanzapine, haloperidol
296	38/M	OD	35	6.68	L	1210	17	Quetiapine, haloperidol, gabapentin, trazodone
296	41/M	Cardiac	54	6.18	L	1629	20	risperidone, quetiapine, lithium, procyclidine
296	43/M	Suicide: hanging	65	6.67	R	1490	25	Haloperidol
296	42/M	Suicide: jumped	26	6.19	R	1410	24	Olanzapine, paroxetine, buspirone, clonazepam
295	47/F	Cardiac	35	6.50	L	1575	20	Risperidone, haloperidol
295	42/M	Cardiac	19	6.48	R	1310	18	Fluphenazine
296	46/M	Pneumonia	30	6.72	L	1630	22	Haloperidol, risperidone, carbamazepine
296	59/F	Cardiac	38	6.93	R	1515	14	Risperidone, trazodone
296	52/M	Pneumonia	16	6.52	R	1340	19	Thiothixene, fluoxetine, benztropine

CO, carbon monoxide poisoning; OD, overdose; GSW, gun shot wound; MVA, motor vehicle accident; PMI, postmortem interval (h).

Table 2. Differential expression of mt-probe sets in BD, SZ or suicide status

	BD		SZ		Suicide status[c]	
	MAS5	RMA	MAS5	RMA	MAS5	RMA
Differentially expressed[a]	102 (15.1%)	95 (14.1%)	76 (11.2%)	49 (7.2%)	24 (3.6%)	18 (2.7%)
Down-regulated[b]	96 (94.1%)	77 (81.1%)	62 (81.6%)	32 (65.3%)	15 (62.5%)	9 (50.0%)
Up-regulated[b]	6 (5.9%)	18 (18.9%)	14 (18.4%)	17 (34.7%)	9 (37.5%)	9 (50.0%)

[a]The number of differentially expressed probe sets and its percentage of the 676 mt-probe sets are given.
[b]The number of up- or down-regulated probe sets and their percentage of the total number of differentially expressed probe sets are given.
[c]All samples were divided into two groups by their cause of death (suicide or non-suicide).

Table 3. Correlation between expression levels of 139 down-regulated mt-probe sets and sample pH

	Number of samples	Average *R*	Average *P*
All samples	102	0.345 ± 0.145	0.060 ± 0.167
pH ≥ 6.4	73	0.211 ± 0.112	0.168 ± 0.234
pH ≥ 6.5	61	0.080 ± 0.113	0.465 ± 0.286

analysis by different calculations (based on Pearson's correlation and the measurement of Euclidean distance) using GeneSpring software yielded similar results that indicated the presence of pH thresholds between 6.4 and 6.5 (data not shown). To ensure the robustness of our analyses, we used two pH points (6.4 and 6.5) as the critical pH thresholds.

To confirm further the presence of pH thresholds, we performed same clustering analysis using only control subjects ($n = 34$). The results also showed the presence of pH thresholds between 6.4 and 6.5 (Supplementary Material, Fig. S1). We next divided control subjects into two groups: low-pH (pH $<$ 6.4) and high-pH (pH $\geq$ 6.4) groups. Statistical analysis revealed that a total of 219 probe sets showed significantly altered expressions among 676 mt-probe sets (Supplementary Material, Table S1). As expected from the clustering analysis, large part of probe sets (75.8%) showed down-regulations in the low-pH group.

We next assessed the effect of pH by omitting the low-pH samples sequentially from the statistical analyses (Fig. 2; Supplementary Material, Table S2). The total number of differentially expressed mt-probe sets in BD or SZ progressively decreased as the selection pH level was increased. Additionally, several new differentially expressed mt-probe sets appeared. At the critical pH thresholds, correlations between expression levels of mt-probe sets and sample pH were almost lost, and the average sample pH did not show statistical differences (Tables 3 and 4).

At these pH levels, we found that 52 probe sets (51.0% of the initially identified mt-probe sets in BD) were still differentially expressed and 24 probe sets were newly identified in BD, whereas 58 probes (76.3%) were still differentially expressed and 24 probe sets were newly found in SZ (Fig. 2; Supplementary Material, Table S3). Although a few exceptions were observed, there was a clear tendency for down-regulation in these mt-probe sets. They included genes responsible for a wide range of mitochondrial functions such as cell respiration, tricarboxylic acid (TCA) cycle, membrane function and transport, mitochondrial transcription and translation and metabolism and catabolism of various substrates. Among the differentially expressed mt-probe sets in BD or SZ at the critical pH thresholds, 57 were shared in both diseases. Although the rest of the probe sets showed differential expression in either BD or SZ, but not both, there was an apparent tendency for the same expression changes in the two diseases (Supplementary Material, Table S3), suggesting that the overall pattern of differential gene expression was very similar in BD and SZ.

We found that about half of differentially expressed mt-probe sets in BD or SZ at critical pH thresholds were commonly altered in the low-pH control group compared with the high-pH control group (Supplementary Material, Table S1). In addition, when we performed similar pH-shift analysis using the samples that showed lower pH than the selection pH level, we could not find any trends in the altered expression of mt-probe sets in BD or SZ (Fig. 2). In these analyses, the average sample pH in each diagnostic group did not show significant differences compared with control (Table 5). These findings demonstrate the importance of sample pH matching at the high pH levels when analyzing mitochondrial gene expression profiles. We next selected control subjects and subjects with BD such that the average pH was ~6.6 and 6.8, respectively (Supplementary Material, Table S4). Statistical analysis between these two groups (low-pH control and high-pH BD groups) showed the significant altered expressions of 51 probe sets among 676 mt-probe sets. About 80.4% of these probe sets showed down-regulation in the high-pH BD group. A total of 16 probe sets (15 down-regulation and 1 up-regulation) out of 51 probe sets were shared with the altered expressed mt-probes sets in BD at the thresholds of pH 6.4 and 6.5. None of these probe sets reversed their directions of expression changes (Supplementary Material, Table S5). Taken together, these results suggest that expression of mt-probe sets were critically influenced by sample pH, but the global down-regulation of mt-probe sets still existed in BD and SZ, even after the effect of sample pH was controlled.

To confirm the relationship between pH and expression levels of mitochondrial genes, we measured the expression levels of *CASQ1* by quantitative RT–PCR (qRT–PCR). *CASQ1* showed positive correlation with sample pH when all samples were used for calculation. Progressive decrease of correlation at the critical pH thresholds was observed, whereas significant correlation between expression levels of *CASQ1* measured by qRT–PCR and microarray was maintained (Table 6). In accordance with our previous study, correlation between the two methods was modest (3). We also confirmed the inverse relationship between sample pH and expression level by measuring the expression level of *AK2*. Progressive decrease of correlation at the critical pH thresholds was also observed (Table 6).

Effects of drugs in BD

To assess the gene expression effects of drugs in BD, we analyzed samples from four medication-free patients with BD. As the number of medication-free patients ($n = 4$) was too small to directly apply statistical analysis at this stage, we assessed the effects of drugs in an indirect way using robust statistical approaches. We first performed statistical analysis between control subjects and all BD samples or only medicated patients with BD. If the expression changes were considerably influenced by the medication, then the *P*-value in the statistical test using only medicated patients with BD should be lower or unchanged when compared with the test using all BD samples. The obtained *P*-value was further evaluated by a permutation test performing the repeated *t*-test using the BD samples, which were selected by random deletion of four samples from all BD samples, and all control subjects. When decrease of *P*-value by deletion of medication-free patients was statistically significant by the permutation test,

Table 4. The average sample pH and sample size in the selected samples through the pH-shift analysis

	All	pH ≥ 6.0	pH ≥ 6.1	pH ≥ 6.2	pH ≥ 6.3	pH ≥ 6.4	pH ≥ 6.5
Average pH (SD)							
Control	6.60 (0.27)	6.60 (0.27)	6.64 (0.23)	6.67 (0.19)	6.69 (0.16)	6.69 (0.17)	6.73 (0.15)
BD	6.44 (0.29)[a]	6.50 (0.24)	6.53 (0.21)	6.56 (0.18)[a]	6.57 (0.17)[a]	6.63 (0.14)	6.65 (0.14)
SZ	6.47 (0.24)[a]	6.49 (0.22)	6.49 (0.22)[a]	6.55 (0.19)[a]	6.59 (0.16)[a]	6.61 (0.14)	6.66 (0.12)
Sample number							
Control	34	34	32	30	29	29	25
BP	33	30	28	26	25	20	18
SZ	35	34	34	29	26	24	18

[a]Indicates that the average sample pH is significantly different ($P < 0.05$ in the *t*-test) from that in controls.

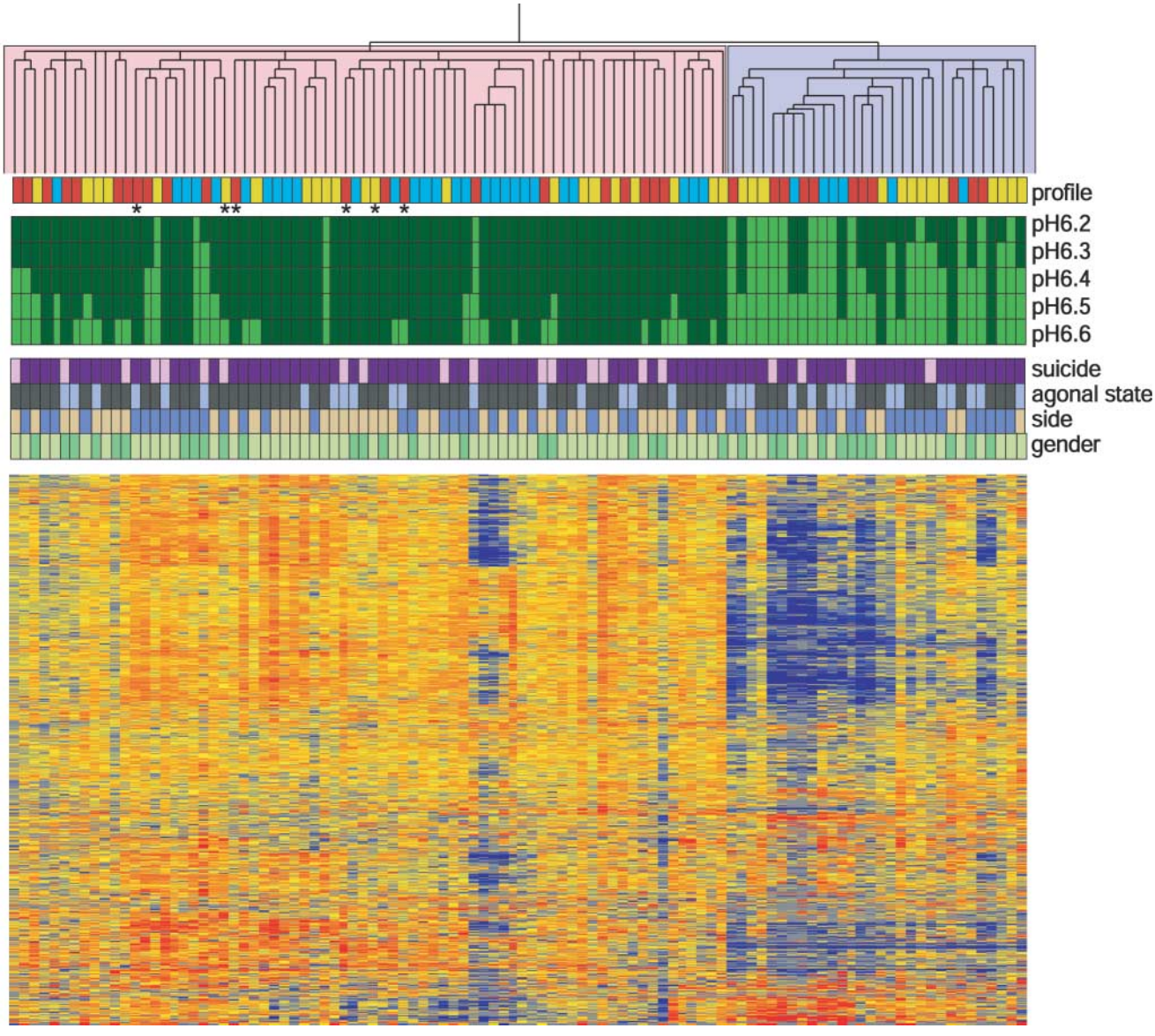

Figure 1. Two-way hierarchical clustering based on the expression levels of 676 mt-probe sets. Each column represents expression levels in individuals, and each row represents the individual probe sets. The expression value of the individual probe sets is normalized by its median value. Blue and red indicate that values are less than and greater than the median (yellow), respectively. The demographic variables of each individual are colored as follows. Diagnosis: yellow, SZ; red, BD; blue, control. Suicide: dark purple, death by non-suicide; light purple, death by suicide. Agonal state: dark gray, type-I sample; light gray, type-II sample. Side: dark blue, left hemisphere; yellow, right hemisphere. Gender: greenish yellow, male; green, female. Samples whose pH was greater than the given selection pH level are colored in dark green. Asterisks indicate medication-free patients.

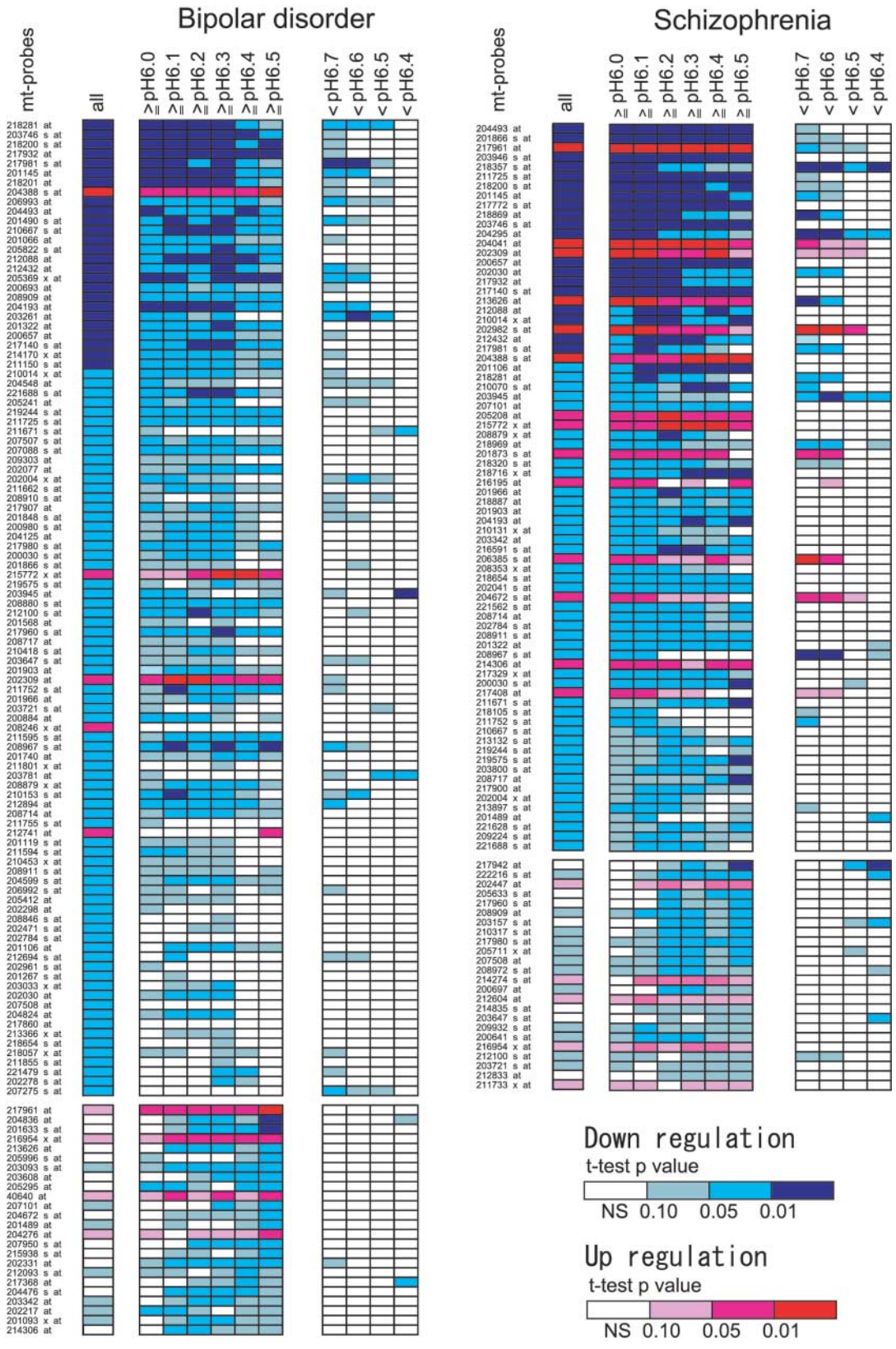

Figure 2. pH-shift analysis in BD and SZ. Each column represents the *P*-value in the *t*-test at the given selection pH level, and each row represents the individual probe sets. The probe sets were chosen ($P < 0.05$) and sorted by their *P*-values in the *t*-test using all samples. Newly appeared differentially expressed mt-probe sets through pH-shift analysis were also listed. Blue and red indicate the significant differential expression changes of down- and up-regulations, respectively. White indicates that the expression level is not statistically significant between two groups.

Table 5. The average sample pH and sample size in the omitted samples through the pH-shift analysis

	pH < 6.7	pH < 6.6	pH < 6.5	pH < 6.4
Average pH (SD)				
Control	6.41 (0.22)	6.37 (0.21)	6.25 (0.19)	6.10 (0.09)
BD	6.36 (0.26)	6.29 (0.24)	6.18 (0.22)	6.15 (0.21)
SZ	6.41 (0.21)	6.33 (0.18)	6.27 (0.16)	6.17 (0.11)
Sample number				
Control	18	15	9	5
BP	27	22	15	13
SZ	29	22	17	11

All the average sample pH in BD and SZ are not significantly different from those in controls.

this was regarded as reflecting an effect of drugs on the expression of that gene. This analysis was independently performed at the critical pH thresholds 6.4 and 6.5. The average sample pH did not show statistical differences at these pH thresholds (Table 7). Among the 76 probe sets differentially expressed in BD at the critical pH thresholds, 15 probe sets were apparently influenced by medication (Supplementary Material, Table S3). Most of them were down-regulated probe sets for respiratory chain components or mitochondrial transcription/translation genes, and none of the up-regulated probe sets appeared in this analysis, suggesting the general repressive effects of medication on the expression of mitochondria-related genes in the brain.

These results prompted us to examine the effect of medication on the expression of all of the 676 mt-probe sets. We divided the BD samples into medicated and non-medicated groups with regard to each of the three classes of drugs, antidepressants, valproate and antipsychotics, and then we compared their average expression levels with control samples as the selection pH level of 6.4. Among the 676 mt-probe sets tested, lower expression levels in the medicated groups were observed in 65.5% (antidepressant group), 75.7% (valproate group) and 81.0% (antipsychotic group) of mt-probe sets when compared with control subjects (Fig. 3). These observations imply that these drugs have similar and global repressive effects on the expression of mitochondria-related genes. Statistical analyses [one-way analysis of variance (ANOVA) followed by Dunnett's test] confirmed the repressive effects in each drug-treated group, especially in the antipsychotics-treated group. Among the 76 mt-probe sets identified in BD at the critical pH thresholds, expressions of 49 (64.5%) probe sets were influenced by at least one of the drug classes (Supplementary Material, Table S3). Notably, all probe sets identified by the permutation test appeared again in this analysis, suggesting the appropriateness of our analytical approaches.

We then directly compared the expression levels of mt-probe sets between medication-free patients with BD and control subjects. Among the 676 mt-probe sets, 27 showed significantly altered expressions at the critical pH thresholds (Table 8). Only three mt-probe sets (*GLDC*, *AK2* and *MRPL48*) were common to the two gene lists; 76 probe sets altered in all BD patients (Supplementary Material, Table S3) and 27 probe sets altered in medication-free BD patients (Table 8), suggesting the dissimilar altered mitochondrial gene expressions between medicated and medication-free patients with BD. In contrast to the global down-regulation of mt-probe sets found in all BD patients, most of the mt-probe sets (85.2%) showed up-regulations in samples from medication-free patients with BD, when compared with controls. The number of up-regulated genes was significantly larger than that expected by random variation ($\chi^2 = 13.4$, $P = 0.03 \times 10^{-2}$). By qRT–PCR analysis, we were able to confirm the up-regulation of *CASQ1* in medication-free patients with BD when compared with controls at the critical pH thresholds (Table 9).

DISCUSSION

Effect of pH and medication on mitochondrial gene expression

A global down-regulation of mitochondria-related genes in BD and SZ was found when all samples were used for analysis without the consideration of sample pH. The results obtained at this stage were very similar to those reported previously by others (7,29). This finding was confirmed even after the effect of sample pH was controlled. The resultant probe sets contained those genes responsible for a wide range of mitochondrial proteins such as respiratory chain components and various enzymes. We thus demonstrated the presence of mitochondrial dysfunction in postmortem brains of BD and SZ patients.

Non-biased clustering analysis revealed that there is a critical pH threshold between 6.4 and 6.5 in our sample set. These pH values are apparently lower than those reported by Li *et al.* (pH 6.8). This is most likely due to the different method of measuring pH, because the average sample pH differed in different brain collections (29). This suggests that critical pH threshold should be independently determined for each set of brain samples. In addition to sample pH, the agonal state of subjects, which may be closely linked to sample pH, has also been shown to influence mitochondrial gene expression profiles (30). However, in our case, the agonal state did not seem to affect the results of clustering analysis. This is partly accounted for by the difficulty of evaluating the exact agonal state from the given information of cause of death (Table 1). More detailed information about the course of death in each subject would be needed to assess the exact agonal state. Tomita *et al.* (30) proposed the average correlation index (ACI) to evaluate the pH and agonal state in postmortem brains. The ACI has the advantage in that this index could be calculated without the knowledge of sample pH and agonal state of subjects. In our case, the ACI analysis did identify the low-pH samples. However, this also identified samples without low-pH but with atypically down-regulated mitochondrial gene expressions (data not shown). We think that ACI would be a better index for the detection of outliers within a given sample set.

The global down-regulation of mitochondria-related genes identified by DNA microarray in postmortem samples may be largely influenced by the effect of medication. It is well known that antidepressants, valproate and antipsychotics have inhibitory effects on mitochondrial functions, especially on the activity of respiratory chain components. For example, valproate is a well-known mitochondrial toxin, inhibiting

Table 6. Correlation between qRT–PCR data and sample pH or microarray data

	All ($n = 101$)		pH $\geq$ 6.4 ($n = 72$)		pH $\geq$ 6.5 ($n = 60$)	
	R	*P*	*R*	*P*	*R*	*P*
Correlation with sample pH						
CASQ1 (*CFL1*-normalized)	0.350	<0.001	0.017	0.884	−0.001	0.992
CASQ1 (*GAPDH*-normalized)	0.394	<0.001	0.007	0.956	−0.014	0.917
AK2 (*CFL1*-normalized)	−0.445	<0.001	−0.322	0.006	−0.168	0.199
AK2 (*GAPDH*-normalized)	−0.478	<0.001	−0.316	0.007	−0.173	0.185
Correlation with microarray data						
CASQ1 (*CFL1*-normalized)	0.589	<0.001	0.473	<0.001	0.336	0.012
CASQ1 (*GAPDH*-normalized)	0.599	<0.002	0.474	<0.001	0.350	0.008
AK2 (*CFL1*-normalized)	0.295	0.003	0.525	<0.001	0.586	<0.001
AK2 (*GAPDH*-normalized)	0.485	<0.001	0.534	<0.001	0.547	<0.001

Table 7. The average sample pH and sample size in medicated and non-medicated BD groups at the critical pH thresholds

	All	pH $\geq$ 6.4	pH $\geq$ 6.5
Average pH (SD)			
Control	6.60 (0.27)	6.69 (0.17)	6.73 (0.15)
Medicated BD	6.40 (0.30)	6.63 (0.16)	6.65 (0.15)
Non-medicated BD	6.63 (0.05)	6.63 (0.05)	6.63 (0.05)
Sample number			
Control	34	29	25
Medicated BD	29	16	14
Non-medicated BD	4	4	4

All the average sample pH are not statistically different from those in controls.

mitochondrial oxidative phosphorylation (31–33). Antidepressants, both tricyclic antidepressants such as imipramine (34) and selective serotonin reuptake inhibitors (SSRI) such as fluoxetine (35), have roles in inhibiting oxidative phosphorylation and mitochondrial ATPase activity. Most antipsychotics, including both typical ones such as chlorpromazine and haloperidol, and atypical ones such as risperidone and quetiapine, are strong inhibitors of electron transfer activity at complex I of the respiratory chain (36–41). The relationship between the inhibitory effects of drugs on the respiratory chain and down-regulation of expression of its components at the mRNA or protein levels has not often been addressed, but Barrientos *et al.* (38) have reported that chronic haloperidol treatment in rats leads to the inhibition of the respiratory chain, which is associated with decreased protein synthesis in mitochondria.

As most medicated patients were treated with more than two classes of drugs, it was difficult to examine the effects of each class of drugs separately in our samples. It is also unclear whether the inhibitory effects of the drugs on mitochondria reflects their side effects or some efficacies. Considering the up-regulated nature of mitochondrial gene expression in medication-free patients with BD, as reported here, the inhibitory effects of the drugs may have a partially normalizing effect in these patients.

Inconsistent results among several DNA microarray studies with regard to mitochondrial gene expression may thus be attributable to differences in sample pH and/or differences in drug treatment profiles of the patients. Importantly, it is not enough to match the average pH among groups, but it is necessary to match the pH using a critical pH threshold. Other possible sources of discrepancy are the different methods of data analysis and the different selection criteria used in microarray for detecting differentially expressed genes. However, we did not observe any signature of the global down-regulation of the mt-probe sets when we reanalyzed a previously reported data set (3) ($P < 0.05$, Student's *t*-test, Welch's *t*-test or Mann–Whitney *U*-test, with no fold change (FC) selection using MAS4, MAS5 or RMA normalized data on 466 mt-probes of the Affymetrix HU95A chip) (data not shown). Thus, in our case, the difference in data analysis method and definition of the differentially expressed genes were not the major causes for inconsistency with other studies.

Mitochondrial dysfunction in BD

We found that brain samples from medication-free BD patients showed dissimilar altered expression patterns of mitochondria-related genes when compared with those of medicated BD patients. Interestingly, they did not show concerted down-regulations. Rather, they showed the altered expression of a small subset of mitochondria-related genes, such as those encoding respiratory chain components, with a tendency toward up-regulation. According to the mitochondrial dysfunction hypothesis in BD (8), altered expression patterns of mitochondria-related genes in BD were caused by the mutations/polymorphisms of mtDNA or chromosomal loci responsible for mitochondrial functions. We recently found that the expression level of *NDUFV2*, a complex I subunit gene, was altered in the lymphoblastoid cells derived from patients with BD (42). In this case, we found that the altered expression level of *NDUFV2* was due to the promoter polymorphism associated with BD. Although our preliminary analysis could not identify the evidence of accumulation of multiple deletions of mtDNA in BD or SZ patients used in this study (Kakiuchi *et al.*, in preparation), it would be interesting to explore whether other SNPs or mutations in nuclear genes or mtDNA are involved in the altered expressions of mitochondrial genes.

Although we were unable to assess the effect of medication in SZ due to the limited number ($n = 2$) of medication-free patients with SZ, these two patients showed the tendency of high levels of expression of mt-probe sets (Fig. 1). Considering the fact that similar mt-probe sets showed down-regulation in both BD and SZ, down-regulation of mitochondrial genes in SZ may also be a result of drug treatment.

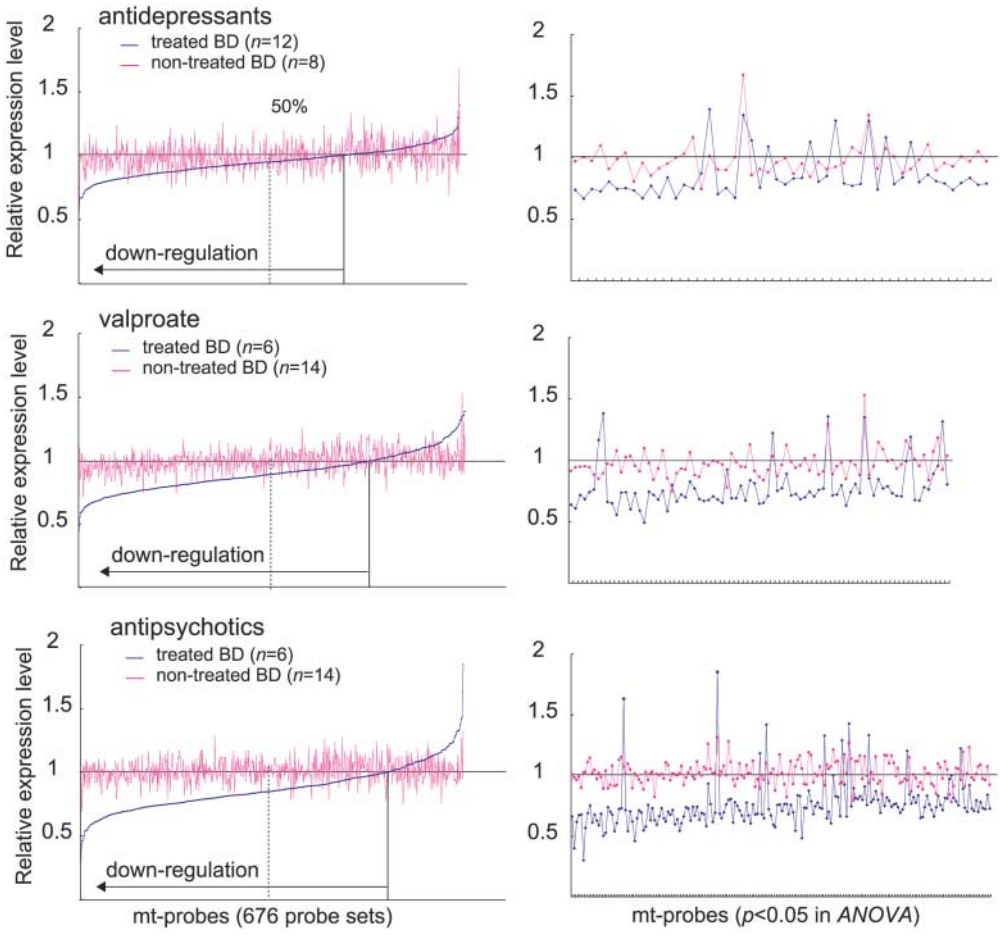

Figure 3. Effect of medication on mitochondrial gene expression in BD at the critical pH threshold. (Left panels) Expression levels of the 676 mt-probe sets were plotted. In each probe set, average expression level of medicated or non-medicated groups was normalized by dividing by those of controls. The probe sets are sorted from left to right by their normalized expression levels of drug-treated groups. For all three drugs, down-regulated probe sets were >50% of all probe sets indicated by dotted lines. (Right panels) Expression levels of the mt-probe sets whose expression was altered in drug-treated patients with BD compared with controls ($P < 0.05$ in ANOVA and Dunnett's test with the controls as a reference group). The probe sets are sorted from left to right by their ANOVA *P*-values.

CONCLUSION

Mitochondrial dysfunction in BD and SZ was observed even after the effect of pH was controlled. However, we conclude that this apparent dysfunction was due to the patients' medication, especially antipsychotics. Dissimilar altered expressions of mitochondria-related genes in medication-free patients with BD compared with medicated patients suggest that a small fraction of the mitochondria-related genes were differentially expressed in BD. Our findings warrant further study of the molecular mechanisms underlying mitochondrial dysfunction in BD and SZ.

MATERIALS AND METHODS

Subjects and RNA samples

RNA samples extracted from the prefrontal cortices (Broadmann's Area 46) were donated by the Stanley Array Collection (Bethesda, MD, USA). They contain total RNA samples from 35 individuals in each of the three diagnostic groups (BD, SZ and controls). Diagnoses had been made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association). Detailed information about the diagnosis and selection criteria for sample collection, and summary of demographic variables of each diagnostic group, can be found in Table 1 and at the Stanley web site (http://www.stanleyresearch.org/programs/brain_collection.asp). The method for estimation of RNA quality can also be found at this same web site. Briefly, brain pH was determined with a Sentron pH-meter (Topac Inc., Hingham, MA, USA) using homogenized occipital cortex, and RNA integrity and purity were determined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). We found that among 105 samples, three samples did not pass the Test2Chip analysis as described subsequently. Thus, a total of 102 subjects were examined (Table 1). Agonal state of these subjects was predicted using information about the cause of death as stated in the previous reports (29,30).

DNA microarray

DNA microarray experiments using an Affymetrix HU133A chip (Affymetrix, Santa Clara, CA, USA), which contains

Table 8. Altered expression of mt-probe sets in medication-free patients with BD at the critical pH thresholds

Probe set ID	Accession no.	Symbol	Product	Functional category	FC
212568_s_at	BF978872	DLAT	Dihydrolipoamide *S*-acetyltransferase	Pyruvate metabolism	1.51
205045_at	NM_007202	AKAP10	A kinase (PRKA) anchor protein 10	Others	1.40
205851_at	BC001808	NME6	Non-metastatic cells 6 (nucleoside-diphosphate kinase)	Others	1.39
221550_at	BC002382	COX15	COX15 homolog, cytochrome *c* oxidase assembly protein (yeast)	Respiratory chain	1.33
218273_s_at	NM_018444	PPM2C	Protein phosphatase 2C, magnesium dependent, catalytic subunit	Others	1.33
203328_x_at	NM_004969	IDE	Insulin-degrading enzyme	Others	1.31
219645_at	NM_001231	CASQ1	Calsequestrin 1	Others	1.31
204041_at	NM_000898	MAOB	Monoamine oxidase B	Neurotransmitter catabolism	1.28
221865_at	BF969986	—	DKFZp547P234 protein	Others	1.27
213396_s_at	AA456929	AKAP10	A kinase (PRKA) anchor protein 10	Others	1.27
204836_at	NM_000170	GLDC	Glycine dehydrogenase	Glycine catabolism	1.26
200979_at	BF739979	—	cDNA FLJ16053 fis	Others	1.25
204059_s_at	NM_002395	ME1	Malic enzyme 1, NADP(+) dependent, cytosolic	TCA cycle	1.25
201036_s_at	NM_005327	HADHSC	L-3-Hydroxyacyl-Coenzyme A dehydrogenase, short chain	Others	1.25
204672_s_at	NM_014942	ANKRD6	Ankyrin repeat domain 6	Others	1.25
200883_at	NM_003366	UQCRC2	Ubiquinol-cytochrome *c* reductase core protein II	Respiratory chain	1.25
208773_s_at	AL136943	FLJ20288	FLJ20288 protein	Others	1.24
208787_at	BC003375	MRPL3	Mitochondrial ribosomal protein L3	Transcription/translation	1.24
218558_s_at	NM_017446	MRPL39	Mitochondrial ribosomal protein L39	Transcription/translation	1.24
222014_x_at	AI249752	MTO1	Mitochondrial translation optimization 1 homolog (yeast)	Transcription/translation	1.23
205530_at	NM_004453	ETFDH	Electron-transferring flavoprotein dehydrogenase	Respiratory chain	1.22
203039_s_at	NM_005006	NDUFS1	NADH dehydrogenase (ubiquinone) Fe–S protein 1, 75 kDa	Respiratory chain	1.19
219220_x_at	NM_020191	MRPS22	Mitochondrial ribosomal protein S22	Transcription/translation	1.15
218281_at	NM_016055	MRPL48	Mitochondrial ribosomal protein L48	Transcription/translation	0.85
205760_s_at	NM_016821	OGG1	8-Oxoguanine DNA glycosylase	Carbohydrate metabolism	0.81
208967_s_at	U39945	AK2	Adenylate kinase 2	Others	0.71
219184_x_at	NM_013337	TIMM22	Translocase of inner mitochondrial membrane 22 homolog (yeast)	Membrane protein	0.58

Probe sets were selected by the *t*-test ($P < 0.05$ at the selection pH levels of 6.4 and 6.5 in BD) and sorted by their FC expressions.

22 284 probe sets, were performed as described previously (3). In brief, 8–10 μg of total RNA of each brain sample was used for reverse-transcription into cDNA. Biotinylated cRNA was synthesized from cDNA by *in vitro* transcription. Fragmented cRNA was first applied to the Test2Chip (Affymetrix), and then applied to the HU133A chip. The hybridization signal on the chip was scanned using an HP GeneArray scanner (Hewlett-Packard, Palo Alto, CA, USA) and was processed by GeneSuite software (Affymetrix).

Table 9. Up-regulation of *CASQ1* in the medication-free patients with BD at the critical pH thresholds

	pH ≥ 6.4		pH ≥ 6.5	
	FC	*t*-Test *P*	FC	*t*-Test *P*
CASQ1 (*CFL1*-normalized)	1.48	0.021	1.45	0.026
CASQ1 (*GAPDH*-normalized)	1.47	0.020	1.45	0.022

Data analysis

The microarray raw data were processed by MAS5 (Affymetrix) and imported into GeneSpring 6.1 software (Silicon-Genetics, Redwood, CA, USA). Data normalization was then performed by dividing each microarray data set by its median value, using GeneSpring 6.1 software. Alternatively, the normalization was performed by the RMA method, which was implemented in the module Affy (43) of the Bioconductor microarray analysis software (http://www.bioconductor.org/). The probe sets for mitochondria-related genes were selected either by the NetAffx database (44) with the keywords, 'mitochondria' or 'mitochondrion', or by the gene list of 'mitochondria' in the GeneSpring software, which classifies probe sets based on the information from GeneOntology (http://www.geneontology.org/). Only the probe sets that were called as present in more than half of the samples were analyzed. A total of 966 probe sets were selected as mt-probe sets, and 676 of these mt-probe sets were called as present in more than half of the samples. The list of mt-probe sets used in this study is available upon request.

Differentially expressed probe sets were selected by two-tailed Student's *t*-test ($P < 0.05$). FDR analysis was performed using QVALUE software (45) and SAM software (46). For estimating *q*-values by QVALUE software, π_0

(an estimate of the total proportion of true null hypotheses) was determined based on the distribution of 676 *P*-values using a smoothing method (45). Pearson's correlations between expression level and sample pH or between expression level of RT–PCR and microarray data were calculated. For pH-shift analysis, we omitted the samples whose pH was lower than a threshold pH level, and performed *t*-tests using either the remaining samples or the omitted samples. We used a *P*-value of <0.10 with threshold values of pH 6.4 and 6.5 to test for statistical significance.

The permutation test was performed by a script written in Microsoft Excel Visual Basic. In this test, repeated two-sample *t*-test was performed using the randomly resampled BD samples and all control subjects. Random resampling was done by four-delete jackknife method (47). After the 1000 times repetition, the rank of *P*-values derived from the analysis with the medicated patients with BD and control subjects was examined. This test was independently performed at the selection thresholds pH 6.4 and 6.5. The script is available upon request. ANOVA was applied to the three groups (medicated patients, non-medicated patients and controls), and Dunnett's test with the controls as a reference group was used to estimate the effect of medication. When the ANOVA revealed a significant difference among the three groups ($P < 0.05$), and Dunnett's test showed statistical differences between controls and medicated patients ($P < 0.05$), it was considered to be the evidence of a significant effect of medication. Statistical analyses were performed using GeneSpring or SPSS10.0J software (SPSS Japan, Tokyo, Japan).

Real-time quantitative RT–PCR

For qRT–PCR, 3–5 μg of total RNA was used for cDNA synthesis by oligo (dT) and SuperScript II reverse transcriptase (Invitrogen). qRT–PCR using SYBER/GREEN I dye (Applied Biosystems, Foster city, CA, USA) was performed with ABI PRISM 7900HT (Applied Biosystems). The comparative Ct method was employed for quantification of transcripts according to the manufacture's protocol (Applied Biosystems). Measurement of delta Ct was performed at least in triplicate. Amplification of the single product was confirmed by monitoring the dissociation curve and by gel electrophoresis. We used two control genes (*GAPDH* and *CFL1*) for normalization to control for possible fluctuations in quantitative values of the target transcripts. The validity of the use of *CFL1* as an internal control gene in postmortem brain samples has been shown previously (3,48). Among the mt-probe sets, expression levels of *AK2* and *CASQ1* were examined because they showed inverse and positive correlations, respectively, with sample pH in microarray data. Primer pairs used in this study were as follows: *AK2*, 5′-CAGCCTTCTCCAAAGCCACAT-3′ and 5′-CCTATCA TTCCCACCCATTGC-3′; *CASQ1*, 5′-AGCCCAATAGCGA AGAGGAGA-3′ and 5′-TCTGCGAAGGCCACAATGT-3′. Primer pairs for *GAPDH* and *CFL1* were as described in a previous report (3). Among the 102 samples tested, one healthy control sample showing the improper amplification curves in both *CASQ1* and *AK2* measurements was omitted from data analyses.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

ACKNOWLEDGEMENTS

RNA extracts from the human postmortem brains were donated by the Stanley Microarray Collection, courtesy of Drs Michael B. Knable, E. Fuller Torrey, Maree J. Webster and Robert H. Yolken. We are indebted to the Research Resource Center at the RIKEN institute for microarray analysis.

REFERENCES

1. Gershon, E.S. and Cloninger, C.R. (1994) *Genetic Approaches to Mental Disorders.* American Psychiatric Press, Washington, DC.
2. Berrettini, W.H. (2000) Are schizophrenic and bipolar disorders related? A review of family and molecular studies. *Biol. Psychiatry*, **48**, 531–538.
3. Iwamoto, K., Kakiuchi, C., Bundo, M., Ikeda, K. and Kato, T. (2004) Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol. Psychiatry*, **9**, 406–416.
4. Hakak, Y., Walker, J.R., Li, C., Wong, W.H., Davis, K.L., Buxbaum, J.D., Haroutunian, V. and Fienberg, A.A. (2001) Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. *Proc. Natl Acad. Sci. USA*, **98**, 4746–4751.
5. Tkachev, D., Mimmack, M.L., Ryan, M.M., Wayland, M., Freeman, T., Jones, P.B., Starkey, M., Webster, M.J., Yolken, R.H. and Bahn, S. (2003) Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet*, **362**, 798–805.
6. Konradi, C., Eaton, M., MacDonald, M.L., Walsh, J., Benes, F.M. and Heckers, S. (2004) Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch. Gen. Psychiatry*, **61**, 300–308.
7. Prabakaran, S., Swatton, J.E., Ryan, M.M., Huffaker, S.J., Huang, J.J., Griffin, J.L., Wayland, M., Freeman, T., Dudbridge, F., Lilley, K.S. *et al.* (2004) Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol. Psychiatry*, **9**, 684–697.
8. Kato, T. and Kato, N. (2000) Mitochondrial dysfunction in bipolar disorder. *Bipolar Disord.*, **2**, 180–190.
9. Kato, T., Takahashi, S., Shioiri, T. and Inubushi, T. (1993) Alterations in brain phosphorous metabolism in bipolar disorder detected by *in vivo* 31P and 7Li magnetic resonance spectroscopy. *J. Affect. Disord.*, **27**, 53–59.
10. Kato, T., Stine, O.C., McMahon, F.J. and Crowe, R.R. (1997) Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. *Biol. Psychiatry*, **42**, 871–875.
11. Kato, T., Kunugi, H., Nanko, S. and Kato, N. (2001) Mitochondrial DNA polymorphisms in bipolar disorder. *J. Affect. Disord.*, **62**, 151–164.
12. Dager, S.R., Friedman, S.D., Parow, A., Demopulos, C., Stoll, A.L., Lyoo, I.K., Dunner, D.L. and Renshaw, P.F. (2004) Brain metabolic alterations in medication-free patients with bipolar disorder. *Arch. Gen. Psychiatry*, **61**, 450–458.
13. Ben-Shachar, D. (2002) Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine. *J. Neurochem.*, **83**, 1241–1251.
14. Ben-Shachar, D. and Laifenfeld, D. (2004) Mitochondria, synaptic plasticity, and schizophrenia. *Int. Rev. Neurobiol.*, **59**, 273–296.
15. Whatley, S.A., Curti, D. and Marchbanks, R.M. (1996) Mitochondrial involvement in schizophrenia and other functional psychoses. *Neurochem. Res.*, **21**, 995–1004.
16. Prince, J.A., Blennow, K., Gottfries, C.G., Karlsson, I. and Oreland, L. (1999) Mitochondrial function is differentially altered in the basal ganglia of chronic schizophrenics. *Neuropsychopharmacology*, **21**, 372–379.
17. Ben-Shachar, D., Zuk, R., Gazawi, H., Reshef, A., Sheinkman, A. and Klein, E. (1999) Increased mitochondrial complex I activity in platelets of schizophrenic patients. *Int. J. Neuropsychopharmcol.*, **2**, 245–253.
18. Maurer, I., Zierz, S. and Moller, H. (2001) Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. *Schizophr. Res.*, **48**, 125–136.

19. Dror, N., Klein, E., Karry, R., Sheinkman, A., Kirsh, Z., Mazor, M., Tzukerman, M. and Ben-Shachar, D. (2002) State-dependent alterations in mitochondrial complex I activity in platelets: a potential peripheral marker for schizophrenia. *Mol. Psychiatry*, **7**, 995–1001.
20. Kung, L. and Roberts, R.C. (1999) Mitochondrial pathology in human schizophrenic striatum: a postmortem ultrastructural study. *Synapse*, **31**, 67–75.
21. Bezchlibnyk, Y.B., Wang, J.F., McQueen, G.M. and Young, L.T. (2001) Gene expression differences in bipolar disorder revealed by cDNA array analysis of post-mortem frontal cortex. *J. Neurochem.*, **79**, 826–834.
22. Mirnics, K., Middleton, F.A., Marquez, A., Lewis, D.A. and Levitt, P. (2000) Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron*, **28**, 53–67.
23. Vawter, M.P., Crook, J.M., Hyde, T.M., Kleinman, J.E., Weinberger, D.R., Becker, K.G. and Freed, W.J. (2002) Microarray analysis of gene expression in the prefrontal cortex in schizophrenia: a preliminary study. *Schizophr. Res.*, **58**, 11–20.
24. Chung, C., Tallerico, T. and Seeman, P. (2003) Schizophrenia hippocampus has elevated expression of chondrex glycoprotein gene. *Synapse*, **50**, 29–34.
25. Middleton, F.A., Mirnics, K., Pierri, J.N., Lewis, D.A. and Levitt, P. (2002) Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *J. Neurosci.*, **22**, 2718–2729.
26. Barton, A.J., Pearson, R.C., Najlerahim, A. and Harrison, P.J. (1993) Pre- and postmortem influences on brain RNA. *J. Neurochem.*, **61**, 1–11.
27. Kingsbury, A.E., Foster, O.J., Nisbet, A.P., Cairns, N., Bray, L., Eve, D.J., Lees, A.J. and Marsden, C.D. (1995) Tissue pH as an indicator of mRNA preservation in human post-mortem brain. *Brain Res. Mol. Brain Res.*, **28**, 311–318.
28. Bahn, S., Augood, S.J., Ryan, M., Standaert, D.G., Starkey, M. and Emson, P.C. (2001) Gene expression profiling in the post-mortem human brain—no cause for dismay. *J. Chem. Neuroanat.*, **22**, 79–94.
29. Li, J.Z., Vawter, M.P., Walsh, D.M., Tomita, H., Evans, S.J., Choudary, P.V., Lopez, J.F., Avelar, A., Shokoohi, V., Chung, T. *et al.* (2004) Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. *Hum. Mol. Genet.*, **13**, 609–616.
30. Tomita, H., Vawter, M.P., Walsh, D.M., Evans, S.J., Choudary, P.V., Li, J., Overman, K.M., Atz, M.E., Myers, R.M., Jones, E.G. *et al.* (2004) Effect of agonal and postmortem factors on gene expression profile: quality control in microarray analyses of postmortem human brain. *Biol. Psychiatry*, **55**, 346–352.
31. Haas, R., Stumpf, D.A., Parks, J.K. and Eguren, L. (1981) Inhibitory effects of sodium valproate on oxidative phosphorylation. *Neurology*, **31**, 1473–1476.
32. Rumbach, L., Warter, J.M., Rendon, A., Marescaux, C., Micheletti, G. and Waksman, A. (1983) Inhibition of oxidative phosphorylation in hepatic and cerebral mitochondria of sodium valproate-treated rats. *J. Neurol. Sci.*, **61**, 417–423.
33. Keller, B.J., Yamanaka, H. and Thurman, R.G. (1992) Inhibition of mitochondrial respiration and oxygen-dependent hepatotoxicity by six structurally dissimilar peroxisomal proliferating agents. *Toxicology*, **71**, 49–61.
34. Weinbach, E.C., Costa, J.L., Nelson, B.D., Claggett, C.E., Hundal, T., Bradley, D. and Morris, S.J. (1986) Effects of tricyclic antidepressant drugs on energy-linked reactions in mitochondria. *Biochem. Pharmacol.*, **35**, 1445–1451.
35. Curti, C., Mingatto, F.E., Polizello, A.C., Galastri, L.O., Uyemura, S.A. and Santos, A.C. (1999) Fluoxetine interacts with the lipid bilayer of the inner membrane in isolated rat brain mitochondria, inhibiting electron transport and F1F0-ATPase activity. *Mol. Cell. Biochem.*, **199**, 103–109.
36. Burkhardt, C., Kelly, J.P., Lim, Y.H., Filley, C.M. and Parker, W.D., Jr (1993) Neuroleptic medications inhibit complex I of the electron transport chain. *Ann. Neurol.*, **33**, 512–517.
37. Maurer, I. and Moller, H.J. (1997) Inhibition of complex I by neuroleptics in normal human brain cortex parallels the extrapyramidal toxicity of neuroleptics. *Mol. Cell. Biochem.*, **174**, 255–259.
38. Barrientos, A., Marin, C., Miro, O., Casademont, J., Gomez, M., Nunes, V., Tolosa, E., Urbano-Marquez, A. and Cardellach, F. (1998) Biochemical and molecular effects of chronic haloperidol administration on brain and muscle mitochondria of rats. *J. Neurosci. Res.*, **53**, 475–481.
39. Whatley, S.A., Curti, D., Das Gupta, F., Ferrier, I.N., Jones, S., Taylor, C. and Marchbanks, R.M. (1998) Superoxide, neuroleptics and the ubiquinone and cytochrome b5 reductases in brain and lymphocytes from normals and schizophrenic patients. *Mol. Psychiatry*, **3**, 227–237.
40. Balijepalli, S., Kenchappa, R.S., Boyd, M.R. and Ravindranath, V. (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. *Neurochem. Int.*, **38**, 425–435.
41. Modica-Napolitano, J.S., Lagace, C.J., Brennan, W.A. and Aprille, J.R. (2003) Differential effects of typical and atypical neuroleptics on mitochondrial function *in vitro*. *Arch. Pharm. Res.*, **26**, 951–959.
42. Washizuka, S., Kakiuchi, C., Mori, K., Kunugi, H., Tajima, O., Akiyama, T., Nanko, S. and Kato, T. (2003) Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder. *Am. J. Med. Genet.*, **120B**, 72–78.
43. Irizarry, R.A., Hobbs, B., Collin, F., Beazer-Barclay, Y.D., Antonellis, K.J., Scherf, U. and Speed, T.P. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, **4**, 249–264.
44. Liu, G., Loraine, A.E., Shigeta, R., Cline, M., Cheng, J., Valmeekam, V., Sun, S., Kulp, D. and Siani-Rose, M.A. (2003) NetAffx: Affymetrix probesets and annotations. *Nucleic Acids Res.*, **31**, 82–86.
45. Storey, J.D. and Tibshirani, R. (2003) Statistical significance for genomewide studies. *Proc. Natl Acad. Sci. USA*, **100**, 9440–9445.
46. Tusher, V.G., Tibshirani, R. and Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl Acad. Sci. USA*, **98**, 5116–5121.
47. Efron, B. and Tibshirani, R.J. (1993) *An Introduction to the Bootstrap.* Chapman and Hall, London.
48. Iwamoto, K., Bundo, M., Yamamoto, M., Ozawa, H., Saito, T. and Kato, T. (2004) Decreased expression of NEFH and PCP4/PEP19 in the prefrontal cortex of alcoholics. *Neurosci. Res.*, **49**, 379–385.