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Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains

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Abstract

Genome-wide gene expression analysis using DNA microarray has a great advantage to identify the genes or specific molecular cascades involved in mental diseases, including major depression and suicide. In the present study, we conducted DNA microarray analysis of major depression using postmortem prefrontal cortices. The gene expression patterns were compared between the controls and subjects with major depression. As a result, 99 genes were listed as the differentially expressed genes in major depression, of which several genes such as *FGFR1*, *NCAM1*, and *CAMK2A* were of interest. Gene ontology analysis suggested an overrepresentation of genes implicated in the downregulation or inhibition of cell proliferation. The present results may support the hypothesis that major depression is associated with impaired cellular proliferation and plasticity. Comparison between the controls and suicide victims with major depression, bipolar disorder, or schizophrenia was also conducted in the present study. Two genes, *CAD* and *ATP1A3*, were differentially expressed in the three comparisons in the same direction. Interestingly, these two genes were also included in the differentially expressed 99 genes in major depression. It may be worth investigating the genes in relation to suicide or major depression.

Keywords: Microarray; Major depression; Suicide; Postmortem brain; *FGFR1*; *NCAM1*; *CAD*; *ATP1A3*

1. Introduction

Major depression is one of the common mental disorders, which affects approximately 10–20% of the population with a devastating outcome of suicide (Nemeroff, 1998). Several lines of studies have suggested contribution of neurobiological factors to the pathophysiology of major depression, while no specific factor has been identified and the etiology of the disease remains largely unknown. It is the case with suicide, the genetic liability to which is likely independent from that to psychiatric disorders (Mann et al., 1999).

Genome-wide gene expression analysis using DNA microarray, by which expression of thousands of genes can be monitored, has a great advantage to identify the genes or specific molecular cascades involved in the complex diseases, especially mental diseases (Mirnics et al., 2001; Bunney et al., 2003; Iwamoto and Kato, 2006). Several groups have reported DNA microarray analysis of postmortem brains obtained from patients with major depression or suicide victims. Evans et al. (2004) observed dysregulation of the fibroblast growth factor (FGF) system in subjects with major depression. The expression of *FGF1*, *FGF2*, FGF receptor 2 (*FGFR2*), *FGFR3* were downregulated in frontal cortical regions of subjects with major depression compared with bipolar disorder or controls. Choudary et al. (2005) observed dysregulation of the glutamatergic and γ-aminobutyric acid-ergic (GABAergic) signal transmission in subjects with major depression. They also observed upregulation of two $GABA_A$ receptor subunits (*$GABA_A\alpha1$* and *$GABA_A\beta3$*) specifically in suicidal subjects, diagnosed with major depression or bipolar disorder. Aston et al. (2005) compared gene expression in the temporal cortex between subjects with major depression and controls. They

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observed that the expression of 17 genes related to oligodendrocyte function significantly decreased in subjects with major depression. Sequeira et al. (2006) investigated the orbital, dorsolateral, and motor cortices derived from suicide victims with and without major depression. They observed that the expression of spermine/spermidine N^1-acetyltransferase (*SSAT*) decreased in suicide victims regardless of the diagnosis of major depression. They also investigated the limbic system in suicide victims with and without major depression and controls (Sequeira et al., 2007). Limbic expression patterns were most extensively altered in the hippocampus, where an overrepresentation of transcription and metabolism-related genes was observed in suicide victims with major depression than those without. In contrast, Sibille et al. (2004) observed no significant difference in gene expression profile of the prefrontal cortex between depressed-suicide subjects and controls.

In postmortem brain studies, sample pH and agonal state of the subjects have been extensively addressed as confounding factors (Li et al., 2004; Tomita et al., 2004; Iwamoto et al., 2005a, 2006). The subjects who had prolonged agonal states tended to have lower pH in the brain, which affect RNA integrity and gene expression profiles. The other concern is the effect of medication, because psychiatric drugs affect several lines of cellular functions. To elucidate the pathophysiology of major depression or suicide, further accumulation of gene expression analyses may be needed considering these points. In the present study, we conducted DNA microarray analysis of postmortem frontal cortices provided by the Stanley Foundation Brain Collection. Gene expression profiles were compared between controls and subjects with major depression to identify new factors associated with the disease. In addition, comparison between controls and suicide victims with major depression, bipolar disorder, or schizophrenia was also conducted for the purpose of identifying common mechanism of suicide.

2. Materials and methods

2.1. Brain samples

Samples of postmortem prefrontal cortices (Broadmann area 10) were donated by the Stanley Foundation Brain Collection. They were derived from patients with major depression, bipolar disorder, schizophrenia, and controls. Each group consisted of 15 subjects, matched for age, gender, postmortem interval (PMI), and sample pH. Detailed information of the original set of subjects was described elsewhere (Torrey et al., 2000). This study was approved by the Research Ethics Committee of RIKEN.

2.2. DNA Microarray procedure

We previously conducted DNA microarray analysis by using Affymetrix GeneChip (Affymetrix, Santa Clara, CA) (Iwamoto et al., 2004). Total RNA was extracted from 0.1 g of frozen tissues using TRIzol (Invitrogen, Groningen, Netherlands). After cleaning up using an RNeasy column (Qiagen, Hilden, Germany), the purity and integrity of total RNA was evaluated by OD measurements and denaturing agarose gel electrophoresis, respectively. DNA microarray analysis was performed according to the manufacturer's protocol (Affymetrix). Briefly, 8–10 μg of total RNA was used to synthesize cDNA. This was used to generate biotinylated cRNA. cRNA was fragmented and first applied to the Test2Chip (Affymetrix) to assess the sample quality, and then to the HU95Av2 chip (Affymetrix), which contains probe sets for about 12,000 genes. The hybridization signal on the chip was scanned using a scanner (HP GeneArray scanner, Hewlett-Packard, Palo Alto, CA), and processed by using Affymetrix microarray suite version 5.0 software package (MAS5.0). Of the 60 samples initially analyzed, 10 were not suitable for DNA microarray analysis estimated by denaturing agarose gel electrophoresis or Test2Chip analysis. We could obtain gene expression profile from 50 samples; 11 patients with major depression, 11 with bipolar disorder, 13 with schizophrenia and 15 controls. A summary of the demographic information of the subjects is shown in Table 1.

2.3. Microarray data analysis

Data analyses in bipolar disorder and schizophrenia have been reported elsewhere (Iwamoto et al., 2004, 2005b). In the present study, we focused on major depression and suicide. The gene expression data generated by microarray analysis were imported into GeneSpring GX 7.3.1 software (Agilent Technologies, Palo Alto, CA). Data of each array were normalized by dividing the median of its gene expression value. Genes called present (detected) in at least half of the samples of major depression, bipolar disorder, and controls were included in the later analysis. Differentially expressed genes between the controls and subjects with major depression were defined based on the following criteria: (i) 1.2-fold or greater change in the mean expression level, (ii) $p < 0.05$ in the two-tailed Student's *t*-test. The criterion (ii) was used in other comparisons. Consistency between findings from microarray data analysis and real-time quantitative PCR was minutely discussed in the previous study (Iwamoto et al., 2004). The effect of age, sample pH, and PMI was assessed by Spearman's correlation test.

3. Results

3.1. Gene expression changes in major depression

Of approximately 12,000 genes, 5787 genes passed the filtering procedures. The number of the differentially expressed genes that met our criteria was 99 (Table 2); 46 genes showed greater expression than in the controls, while 53 showed lower expression. Out of these 99 genes, 71 were annotated with Gene ontology (GO) terms. In GO analysis of the differentially expressed 71 genes, the most extensively overrepresented were genes implicated in the cell proliferation (Table 3).

Table 1
Summary of the demographic variables of subjects used in this study

	N	Age (years)	Gender (male:female)	PMI (h)	Medication (medicated:nonmedicated)	Cause of death (suicide:nonsuicide)
Major depression	11	46 ± 10	6M:5F	27 ± 12	9M:2NM	4S:7NS
Bipolar disorder	11	39 ± 12	8M:3F	32 ± 16	9M:2NM	8S:3NS
Schizophrenia	13	44 ± 14	8M:5F	33 ± 15	10M:3NM	4S:9NS
Controls	15	48 ± 11	9M:6F	24 ± 10	0M:15NM	0S:15NS

Table 2
Genes differentially expressed in major depression[a]

Probe set	*p* value	Fold change	Accession	Description	Confounding factors[b]
40951_at	0.0422	3.13	AL049250	KIAA0220 protein	
36423_at	0.027	1.62	W47047	P8 protein	
35367_at	0.0284	1.58	AB006780	Galectin 3	PMI
41695_at	0.00202	1.51	AB007874	Zinc finger protein 297B	
36634_at	0.0343	1.51	U72649	B-cell translocation gene 2	
40091_at	0.0332	1.45	U00115	B-cell lymphoma 6 protein	PMI
40793_s_at	0.00755	1.45	U34846	Aquaporin 4	pH
40210_at	0.0122	1.44	X75593	RAB13	
1719_at	0.00441	1.42	U61981	mutS homolog 3	
34732_at	0.0475	1.40	X65873	Kinesin family member 5B	
34091_s_at	0.0454	1.39	Z19554	Vimentin	pH, PMI
33206_at	0.0318	1.38	C18655	Suppressor of Ty 3 homolog	
37512_at	0.0451	1.38	U89281	3-Hydroxysteroid epimerase	
34820_at	0.0254	1.36	M57399	Pleiotrophin	
36059_at	0.0189	1.36	AB011540	MEGF7	PMI
539_at	0.0405	1.35	S59184	RYK receptor-like tyrosine kinase	
37201_at	0.0308	1.34	D38535	Inter-alpha (globulin) inhibitor H4	
41686_s_at	0.0384	1.34	AL042668	KIAA0752	
1603_g_at	0.0484	1.33	L33881	Protein kinase C, iota	
37294_at	0.0298	1.33	X61123	B-cell translocation protein 1	PMI
31869_at	0.0199	1.33	AB014540	KIAA0640 protein	PMI
1602_at	0.035	1.32	L33881	Protein kinase C, iota	
33407_at	0.0058	1.32	AI672098	KIAA0934 protein	
31813_at	0.0488	1.32	AB023185	Calcium/calmodulin-dependent protein kinase IIA	pH
41713_at	0.0124	1.30	U09848	Zinc finger protein	
36595_s_at	0.0218	1.28	S68805	Glycine amidinotransferase	Age, pH, PMI
40631_at	0.0374	1.26	D38305	Transducer of ERBB2, 1	pH
41809_at	0.034	1.25	AI656421	Hypothetical protein MGC4175	Age
35666_at	0.0376	1.25	U38276	Semaphorin 3F	
37622_r_at	0.0206	1.25	AF063020	PC4 and SFRS1 interacting protein 1	
2057_g_at	0.0455	1.24	M34641	Fibroblast growth factor receptor 1	PMI
2058_s_at	0.0441	1.24	M35011	Integrin, beta 5	
36875_at	0.0385	1.24	AL050018	Inhibitor of Bruton's tyrosine kinase	Age
32168_s_at	0.00317	1.24	U85267	Calcipressin 1 isoform a	
1316_at	0.0475	1.23	X55005	Thyroid hormone receptor, alpha	
40137_at	0.0391	1.23	M31724	Protein tyrosine phosphatase, non-receptor type 1	PMI
861_g_at	0.0334	1.23	U03911	mutS homolog 2	
36961_at	0.00282	1.23	AL050286	LETM1 domain containing 1	
33886_at	0.0397	1.23	AF006516	abl-interactor 1	pH
34192_at	0.0431	1.23	AB011104	KIAA0532 protein	
40844_at	0.0469	1.22	D63875	SH2 domain binding protein 1	
32784_at	0.0449	1.21	AB011108	Serine/threonine-protein kinase PRP4K	PMI
37936_at	0.0228	1.21	AI184802	PRP4/STK/WD splicing factor	
35137_at	0.0301	1.20	X69090	Myomesin 1	
38441_s_at	0.0428	1.20	X59408	CD46 antigen, complement regulatory protein	Age
39013_at	0.0467	1.20	Y11588	APG5 autophagy 5-like	
34301_r_at	0.0378	0.83	Z19574	Keratin 17	
35685_at	0.013	0.83	Z14000	Ring finger protein 1	
35262_at	0.0162	0.83	AF022229	Integrin beta 4 binding protein	
1597_at	0.0227	0.83	L13720	Growth arrest-specific 6	
32545_r_at	0.0242	0.83	L12535	ras suppressor protein 1	
37184_at	0.047	0.83	L37792	Syntaxin 1A	pH
35670_at	0.0486	0.83	M37457	Na+/K+-ATPase alpha 3 subunit	
39082_at	0.0419	0.83	Y00097	Annexin VI	
34457_at	0.0371	0.83	U76010	Solute carrier family 30 (zinc transporter), member 3	
41289_at	0.00893	0.82	AA126505	Neural cell adhesion molecule 1	pH
33450_at	0.0209	0.82	AB015906	Actin-like 6B	
35923_at	0.0496	0.82	D13305	Cholecystokinin B receptor	
38221_at	0.0338	0.82	AF100153	Connector enhancer of kinase suppressor of Ras 1	
38729_at	0.00276	0.82	M88279	FK506-binding protein 4	
40280_at	0.0402	0.82	U72508	Leucine-rich B7 protein	
40821_at	0.0128	0.82	M61832	S-adenosylhomocysteine hydrolase	
35972_at	0.0104	0.82	AA181196	Hypothetical protein FLJ11712	PMI
39274_at	0.012	0.82	X58521	Nucleoporin 62 kDa	

Table 2 (*Continued*)

Probe set	*p* value	Fold change	Accession	Description	Confounding factors[b]
40847_at	0.0323	0.82	AB018293	Microtubule associated monoxygenase, calponin and LIM domain containing 2	pH
41007_at	0.0184	0.81	AF052497	Myozenin 3	
33887_at	0.00454	0.81	D84064	Hepatocyte growth factor-regulated tyrosine kinase substrate	
37504_at	0.04	0.81	AC004893		
39885_at	0.028	0.81	W87858	Putative dimethyladenosine transferase	
40242_at	0.0286	0.80	L36529	Nuclear matrix protein p84	
32402_s_at	0.0248	0.80	Y10931	Symplekin	
34373_at	0.0498	0.80	Z97054	Upstream regulatory element binding protein 1	
38117_at	0.0166	0.80	D38555	SEC24-related protein C	pH
32031_at	0.00358	0.79	D78586	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase	
37256_at	0.0396	0.79	AI829890	HTCD37	pH
41565_at	0.00707	0.79	AF034373	Ataxin 2 related protein	
32536_at	0.00808	0.79	Z37986	Emopamil binding protein	
40191_s_at	0.0227	0.78	AI761647	RAB1A	
40619_at	0.00632	0.78	M91670	Ubiquitin-conjugating enzyme E2S	
36987_at	0.0112	0.78	M94362	Lamin B2	pH
37488_at	0.0295	0.78	L00635	Farnesyltransferase, CAAX box, beta	
877_at	0.0417	0.78	M27691	cAMP responsive element binding protein 1	
36311_at	0.0282	0.78	U40370	Phosphodiesterase 1A, calmodulin-dependent	
41199_s_at	0.0472	0.78	W27050	Splicing factor proline/glutamine rich	
32177_s_at	0.0421	0.78	AC004084	Ca2+-promoted Ras inactivator	
39855_at	0.00842	0.78	AC005787	Fzr1 protein	
1270_at	0.0253	0.77	M64788	RAP1, GTPase activating protein 1	
36779_at	0.00517	0.77	X90908	Gastrotropin	
37511_at	0.03	0.76	AB030506	B9 protein	
35992_at	0.0218	0.76	AF087036	Musculin	
1148_s_at	0.0223	0.76	HG4704-HT5146		
37274_at	0.00628	0.75	AF018631	Biotinidase precursor	
39965_at	0.00038	0.73	AI570572	Ras-related C3 botulinum toxin substrate 3	
40413_at	0.0116	0.73	U18321	Death-associated protein 3	
40808_at	0.0212	0.72	U03749	Chromogranin A precursor	
34648_at	0.0312	0.71	Z12830	Signal sequence receptor, alpha	
39011_at	0.0161	0.70	X99906	Endosulfine alpha isoform 3	
1741_s_at	0.0253	0.67	S37730	Insulin-like growth factor binding protein-2	
33230_at	0.0327	0.67	AJ131186	PRP19/PSO4 pre-mRNA processing factor 19 homolog	

[a] Genes selected based on the following criteria: (i) 1.2-fold or greater change in the mean expression level, (ii) $p < 0.05$ in the two-tailed Student's *t*-test. *p* values are from the Student's *t*-test (two-tailed), calculated using arithmetic mean.

[b] Annotated when each confounding factor significantly correlated with the gene ($p < 0.05$).

We also analyzed the genes whose expression levels were observed to be altered in subjects with major depression and/or suicide in the previous microarray studies (Evans et al., 2004; Choudary et al., 2005; Aston et al., 2005; Sequeira et al., 2006; Sequeira et al., 2007) (Table 4). However, the present gene list which were differentially expressed between the controls and subjects with major depression ($p < 0.05$ criteria) did not overlap those suggested by Evans et al. (2004), Choudary et al. (2005), Sequeira et al. (2006), or Sequeira et al. (2007). In contrast, the present gene list included 6 of 233 genes which were observed to be altered between controls and subjects with major depression in Aston et al. (2005), while 3 of 6 altered in the same direction of that observed in the previous study (*STIP1, HSA9761*, and *NMP200*).

3.2. Search for suicide-related genes

The number of the differentially expressed genes between the controls and suicide victims with major depression, bipolar disorder, and schizophrenia were 544, 348, and 480, respectively ($p < 0.05$ criteria). Three genes were included in the overlap of the three comparisons. In the three, two changed in the same direction among the three comparisons (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (*CAD*) and Na^+/K^+-ATPase alpha 3 subunit (*ATP1A3*)) (Table 5). The expression levels of these two genes did not significantly change when suicide victims were compared with non-suicidal patients in each diagnosis or all patients.

4. Discussion

In the present study, we conducted DNA microarray analysis of major depression using postmortem brains. The gene expression patterns were compared between the controls and subjects with major depression. As a result, 99 genes were listed as the differentially expressed genes in major depression (Table 2). GO analysis suggested an overrepresentation of

Table 3
Major gene categories significantly overrepresented among differentially expressed 71 genes in major depression

Category[a]	Number of genes in category (%)	Number of genes in differentially expressed 71 genes (%)	*p* value
Biological process			
GO:42127: regulation of cell proliferation	314 (4.0)	10 (14.1)	0.000482
GO:8285: negative regulation of cell proliferation	161 (2.1)	7 (9.9)	0.000595
GO:8283: cell proliferation	554 (7.1)	12 (16.9)	0.00375
GO:6886: intracellular protein transport	221 (2.8)	6 (8.5)	0.0146
GO:46903: secretion	161 (2.1)	5 (7.0)	0.015
GO:46907: intracellular transport	369 (4.7)	8 (11.3)	0.0178
GO:51649: establishment of cellular localization	375 (4.8)	8 (11.3)	0.0194
GO:51641: cellular localization	380 (4.8)	8 (11.3)	0.0208
GO:15031: protein transport	335 (4.3)	7 (9.9)	0.0312
GO:45184: establishment of protein localization	354 (4.5)	7 (9.9)	0.0403
GO:902: cellular morphogenesis	220 (2.8)	5 (7.0)	0.0485
GO:8104: protein localization	369 (4.7)	7 (9.9)	0.0485
GO:7167: enzyme linked receptor protein signaling pathway	221 (2.8)	5 (7.0)	0.0493
Cellular component			
GO:5622: intracellular	4836 (65.89)	49 (76.6)	0.0437
Molecular function			
GO: 5198: structural molecule activity	434 (5.5)	8 (11.3)	0.0387
GO: 16874: ligase activity	174 (2.2)	5 (7.0)	0.0193

[a] Gene categories were confined to those >5 probe sets belong to.

genes implicated in the downregulation or inhibition of cell proliferation (Table 3). Stress-induced reduction in neurogenesis has been proposed as a contributor to the pathophysiology of major depression (Duman et al., 1999; Czeh et al., 2001). The present results may support the hypothesis that major depression is associated with impaired cellular proliferation and plasticity.

In the list of 99 genes, *FGFR1* was upregulated in the subjects with major depression. Members of the FGF family have been implicated in the pathophysiology of depression. *FGFR1* binds both *FGF1* and *FGF2*, which are widely distributed in the human central nervous system (CNS), exerting multiple trophic actions on neurons and glia during CNS development, injury and disease (Grothe et al., 2000). In the previous *in situ* hybridization study, *FGFR1* was observed to be upregulated in hippocampus in subjects with major depression when compared with controls (Gaughran et al., 2006). In addition, expression of *FGF1* and *FGF2*

Table 4
Comparison between the present and previous micoroarray stuides

Study	Evans et al. (2004)	Choudary et al. (2005)	Aston et al. (2005)	Sequeira et al. (2006)	Sequeira et al. (2007)
Brain sample	Cohort A: 9 MD (6 suicide), 6 BP (4 suicide), 7 control Cohort B: 4 MD (1 suicide), 6 control	The same with cohort A in Evans et al. (2004)	12 MD (5 suicide), 14 control	16 MD with suicide, 18 suicide, 12 control	18 MD with suicide, 8 suicide, 13 control
Brain region	DLPFC, AnCg	DLPFC, AnCg	BA21	BA4, BA8/9, BA11	Amygdala, hippocampus, BA24, BA29
GeneChip	U133A	U95Av2	U95A	U133	U133
Number of differentially expressed genes in each study	10	16	233	26	39
Commonly included genes between the previous studies	*FGFR2* (Aston et al., 2005)	None	*FGFR2* (Evans et al., 2004); *COPA* (Sequeira et al., 2006); *PTP4A2*, *NTRK2* (Sequeira et al., 2007)	*COPA* (Aston et al., 2005); *SSAT*, *CTS*B (Sequeira et al., 2007)	*PTP4A2*, *NTRK2* (Aston et al., 2005); *SSAT*, *CTSB* (Sequeira et al., 2006)
Genes included in the gene list differentially expressed between MD and controls in the present study ($p < 0.05$)	None	None	*AL049250, ZNF36, STIP1, CCKBR, HSA9761, NMP200*	None	None

MD; major depression, BP; bipolar disorder, DLPFC; dorsolateral prefrontal cortex, AnCg; anterior cingulate cortex, BA; Broadmann area.

Table 5
Overlap of the differentially expressed genes between controls and suicide victims with major depression, bipolar disorder, and schizophrenia

Probe set	Accession	Description	MD-S vs. C		BP-S vs. C		SCZ-S vs. C	
			p value	Fold change	*p* value	Fold change	*p* value	Fold change
32031_at	D78586	Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)	0.000598	0.823	0.00459	0.798	0.00368	0.798
35670_at	M37457	Na+/K+-ATPase alpha 3 subunit (*ATP1A3*)	0.0379	0.856	0.00674	0.76	0.00528	0.643

MD-S indicates suicide victims with major depression; BP-S, suicide victims with bipolar disorder; SCZ-S, suicide victims with schizophrenia; C, controls.

were downregulated in subjects with major depression in the previous microarray study (Evans et al., 2004). Upregulation of *FGFR1* in the present study may support the dysregulation of FGF system in major depression, although no significant change in expression level of *FGF1* or *FGF2* was observed (fold changes are 1.05 and 1.01, respectively). Downregulation of neural cell adhesion molecule 1 (*NCAM1*) in the subjects with major depression was also of interest. Sequeira et al. (2007) observed downregulation of *NCAM2* in hippocampus in suicide victims with depression in comparison with controls or suicide victims without depression, while the gene did not pass the filtering procedure in the present study. NCAM is a subset of the immunoglobulin (Ig) superfamily found in the nervous systems. The protein interacts with other cell surface molecules of the Ig superfamily and appears to be necessary for specific pathfinding by axonal growth cones during development (Lane et al., 1996). Downregulation of *NCAM1* in the present result may also suggest the role of impaired cellular proliferation in the pathophysiology of major depression.

Another interest finding is upregulation of calcium/calmodulin-dependent protein kinase II-alpha (*CAMK2A*) in the subjects with major depression. CAMK2A is a subunit of CAMKII, a ubiquitous serine/threonine protein kinase that is abundant in the brain as a major constituent of the postsynaptic density (Kelly, 1991). Several studies suggested CAMK2A is implicated in the pathophysiology and pharmacology of mental disorders (Celano et al., 2003). Upregulation of aquaporin 4 (*AQP4*), the predominant water channel in the brain, was discussed elsewhere (Iwamoto et al., 2004).

Caution may be needed to interpret the results because these four genes significantly correlated with confounding factors: *FGFR1* with PMI ($p = 0.035$), and *NCAM1*, *CAMK2A*, and *AQP4* with sample pH ($p = 0.024$, 0.039, and 0.020, respectively). Although major effect of age, sample pH, or PMI was not observed in the present results (Table 2), the possibility that relatively low sample pH (mean $\pm$ S.D. = 6.2 $\pm$ 0.2 for 50 samples) affected the results might not be excluded considering sample size in the present study. The effect of medication may also be considered. Especially, the expression levels of *NCAM1* and *CAMK2A* have been observed to be affected by antidepressant treatments in animal studies (Celano et al., 2003; Varea et al., 2007). Another limitation may be that the present results were not confirmed by using other methods, although consistency between microarray data and those of real-time quantitative PCR was discussed in the previous study (Iwamoto et al., 2004).

Comparison between the controls and suicide victims with major depression, bipolar disorder, or schizophrenia was also conducted in the present study. There were two genes which belong to the three differentially expressed gene groups and whose directions were the same among the three comparisons (Table 5). One is *CAD*, encoding a trifunctional protein which is associated with the enzymatic activity of the first three enzymes in the pyrimidine biosynthesis: carbamoyl phosphate synthetase, aspartate transcarbamoylase, and dihydroorotase (Chen et al., 1989). Uracil, one of the three pyrimidines, is converted to uridine, which reportedly has antidepressant effect (Carelzon et al., 2005). A large-scale clinical trial of uridine (RG2417) for bipolar depression is underway. It has been reported that biosynthesis of pyrimidine is inhibited in the depressive state (Karkishchenko et al., 1991). The present result of downregulation of *CAD* in suicide victims might suggest that reduced uridine biosynthesis is involved in the biochemical basis of depressive state in relevance to the clinical effect of uridine. Another is *ATP1A3*, one of three isoforms of the alpha subunit of the Na^+/K^+-ATPase expressed in the nervous systems (De Carvalho Aguiar et al., 2004). Possible role of Na^+/K^+-ATPase in the pathophysiology of bipolar disorder has been implicated (El-Mallakh and Wyatt, 1995). Interestingly, these two genes, *CAD* and *ATP1A3*, were also included in the differentially expressed genes in major depression (Table 2). It may be worth investigating the genes in relation to suicide or major depression.

The present results did not replicate most of the previous microarray studies of major depression or suicide (Evans et al., 2004; Choudary et al., 2005; Aston et al., 2005; Sequeira et al., 2006; Sequeira et al., 2007) (Table 4). Several reasons may be considered for this contradiction. One is the difference of the methodology, such as subjects, brain region, or the way of comparison. For instance, brain samples used in Sequeira et al. (2006) and Sequeira et al. (2007) were obtained from suicide brain bank, in which all the samples died of suicide except for normal controls. All the patients in Sibille et al. (2004) also died of suicide. Aston et al. (2005) investigated the temporal cortex, and Sequeira et al. (2007),

the limbic system. Evans et al. (2004) and Choudary et al. (2005) compared the gene expression profile among the subjects with major depression, bipolar disorder, and controls; Sequeira et al. (2006) and Sequeira et al. (2007), among suicide victims with and without major depression and controls. Another is, maybe more important, the heterogeneity of pathophysiology of major depression or suicide. The diagnosis of major depression is based on variable sets of symptoms and subtypes have been proposed based on symptoms, such as melancholic, reactive, psychotic, and atypical depression (Nestler et al., 2002).

In conclusion, we suggested the role of genes related to impaired cellular proliferation in the pathophysiology of major depression and/or suicide. Further studies may be needed on the basis of the present results.

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