笠井　清登

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資　　料

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Up-Regulation of *ADM* and *SEPX1* in the Lymphoblastoid Cells of Patients in Monozygotic Twins Discordant for Schizophrenia

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The contribution of genetic factors to schizophrenia is well established and recent studies have indicated several strong candidate genes. However, the pathophysiology of schizophrenia has not been totally elucidated yet. To date, studies of monozygotic twins discordant for schizophrenia have provided insight into the pathophysiology of this illness; this type of study can exclude inter-individual variability and confounding factors such as effects of drugs. In this study we used DNA microarray analysis to examine the mRNA expression patterns in the lymphoblastoid (LB) cells derived from two pairs of monozygotic twins discordant for schizophrenia. From five independent replicates for each pair of twins, we selected five genes, which included *adrenomedullin (ADM)* and *selenoprotein X1 (SEPX1)*, as significantly changed in both twins with schizophrenia. Interestingly, *ADM* was previously reported to be up-regulated in both the LB cells and plasma of schizophrenic patients, and *SEPX1* was included in the list of genes up-regulated in the peripheral blood cells of schizophrenia patients by microarray analysis. Then, we performed a genetic association study of schizophrenia in the Japanese population and examined the copy number variations, but observed no association. These findings suggest the possible role of *ADM* and *SEPX1* as biomarkers of schizophrenia. The results also support the usefulness of gene expression analysis in LB cells of monozygotic twins discordant for an illness. © 2007 Wiley-Liss, Inc.

KEY WORDS: **adrenomedullin; selenoprotein; DNA microarray; gene expression; genetic association study**

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INTRODUCTION

Genetic factors in schizophrenia have been shown by family, twin, and adoption studies. A higher concordance rate of schizophrenia in monozygotic twins (41–79%) compared with that in dizygotic twins (0–14%) especially supports the contribution of genetic factors in schizophrenia [Shih et al., 2004]. As the risk genes for schizophrenia, a balanced translocation disrupting disrupted schizophrenia-1 (*DISC1*) [Millar et al., 2000] and a chromosomal deletion at 22q11 [Bassett and Chow, 1999] are well established. As common variants associated with schizophrenia, dystrobrevin-binding protein 1 (*DTNBP1*) [Straub et al., 2002] and neuregulin 1 (*NRG1*) [Stefansson et al., 2002], which were identified from linkage analysis, were reported. However, the association of DTNBP1 haplotype with schizophrenia is not consistent among studies [Mutsuddi et al., 2006]. Further studies to identify the molecular pathology of this illness are needed.

In addition to the traditional genetic approaches, an additional strategy to identify the genetic basis of endophenotypes of schizophrenia is becoming popular. In this approach, endophenotypes, measurable biological variables associated with genetic risk of schizophrenia, are first identified; then their genetic basis is studied [Braff et al., 2007]. Many established endophenotypes, such as eye tracking abnormality [Holzman et al., 1977], ventricular enlargement [Reveley et al.,

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1982], reduced hippocampal volume [Suddath et al., 1990], hypofrontality [Berman et al., 1992], and neuropsychological measures [Goldberg et al., 1995], were validated by the study of monozygotic twins discordant for schizophrenia.

In an attempt to identify molecular endophenotypes, biochemical differences in blood between the monozygotic twins discordant for schizophrenia have been investigated. These studies showed some differences between twins: plasma haptoglobin levels [Vander Putten et al., 1996], DNA methylation status [Tsujita et al., 1998; Petronis et al., 2003], soluble interleukin-2 receptors (SIL-2Rs) [Rapaport et al., 1993], mRNA expression level of a certain transcript [Friedhoff et al., 1995], retrovirus [Deb-Rinker et al., 1999], catecholamine levels [Walker et al., 2002], DNA stability [Nguyen et al., 2003], and lipid metabolism [Tsang et al., 2006]. On the other hand, no difference was found for viral nucleic acids [Sierra-Honigmann et al., 1995], platelet monoamine oxidase activity [Reveley et al., 1983], and genomic sequences [Polymeropoulos et al., 1993; Vincent et al., 1998; McDonald et al., 2003]. If a robust difference between discordant twins is well validated, such a finding will become a clue to identify the cause of this difficult illness [Kato et al., 2005a].

To identify the genes differentially expressed between the twins, one may use peripheral blood cells. However, this method is hampered by the fact that most of the patients are under treatment with drugs such as antipsychotics, which potentially affect the gene expression patterns. One possible method to avoid these confounding factors is to use the lymphoblastoid (LB) cells. Gene expression patterns in LB cells can be assessed with minimum inter-individual variability [Cheung et al., 2003], and the effect of drugs may be avoided or reduced by culturing the cells.

We previously performed DNA microarray analysis and examined the mRNA expression pattern using LB cells of monozygotic twins discordant for bipolar disorder. On the basis of our findings, we suggested the possible contribution of the endoplasmic reticulum stress response pathway to the pathophysiology of the illness [Kakiuchi et al., 2003]. Recently, Matigian et al. [2007] also performed DNA microarray analysis in three pairs of monozygotic twins discordant for bipolar disorder and found that genes related to the WNT signaling pathway were altered in patients. Several other groups have also applied the similar strategy to other illnesses such as autism and rheumatoid arthritis [Haas et al., 2006; Hu et al., 2006].

In this study, we used DNA microarray analysis to examine the mRNA expression pattern in the cells of two pairs of monozygotic twins discordant for schizophrenia. Because one of the problems in this strategy is lack of statistical analysis due to small sample size, we performed five independent experiments for each pair of twins. The expression of five genes commonly was shown to be altered in both of the twins, and two genes survived after the exclusion of three immunoglobulin-related genes. Interestingly, both of the final genes, *adrenomedullin* (*ADM*) and *selenoprotein X1* (*SEPX1*), had been reported to be up-regulated in the cells or plasma of schizophrenic patients. We further tried to identify the genetic basis of up-regulation of *ADM* and *SEPX1* levels in schizophrenia by a case-control association analysis of schizophrenia in the Japanese population. Because copy number variation (CNV) was reported to exist around these loci, CNV was also examined.

MATERIALS AND METHODS

Subjects

For the DNA microarray analysis, two pairs of monozygotic twins discordant for schizophrenia (SZ twins) were recruited. The SZ twins A were 54-year-old males, and SZ twins B were 24-year-old females, who were previously reported elsewhere [Kunugi et al., 2003].

The SZ twins A were diagnosed by the consensus of two senior psychiatrists after independent unstructured interviews. Their family history was obtained from interviews of the twins. They had two healthy sisters, and their parents did not have major mental disorders. The affected twin of this pair (SZ-twin-A1) graduated from a university and worked as an office worker for 2 years. At age 25, he developed disorganized behavior and thought, accompanied by excitation. He also had non-systematic delusion of persecution and auditory hallucination. He was hospitalized in a psychiatric ward for 3 months. After the first episode, he was admitted to psychiatric hospitals 13 times. He began to develop negative symptoms and changed jobs several times because of interpersonal problems. He married at age 32, but divorced 1 year later. After that, he could not continue to work and lived alone, supported by social welfare. His diagnosis according to the International Classification of Diseases, Revision 10 (ICD-10) was schizophrenia, disorganized type. He was also diagnosed to have diabetes mellitus. He had been treated with 150 mg of clocapramine hydrochloride, a typical antipsychotic, and 3 mg of trihexyphenidyl hydrochloride, as an antiparkinsonian drug. It is not known whether his diabetes is a side effect of these drugs. His co-twin had been working at a company for 30 years and had been married. He was not diagnosed to have any major mental disorders or personality disorders. He did not have diabetes.

The proband of SZ twins B was diagnosed by the consensus of two senior psychiatrists after independent unstructured interviews. The diagnosis of the proband according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association) was schizophrenia. Her co-twin was interviewed with the use of the schedule for affective disorders and schizophrenia (SADS), which revealed no current or past history of affective disorders or psychotic disorders. Their mother was interviewed and found to be healthy. Their father was also healthy, according to the available information. The symptoms of the proband is minutely described elsewhere [Kunugi et al., 2003]. In brief, the proband's symptoms began around the age of 15, with delusion of persecution. After that, she developed auditory hallucination and negative symptoms.

For the case-control association study, the genomic DNA derived from peripheral blood cells of 223 patients with schizophrenia (45.7 ± 14.9 years old, 129 males and 94 females) and 364 controls (50.4 ± 12.5 years old, 184 males and 180 females) in the Japanese population were analyzed. They were diagnosed according to the DSM-IV criteria. Controls were selected from students, nurses, office workers, and doctors in participating institutes, and their friends. A senior psychiatrist interviewed the controls and found no major mental disorders. Only a subset of the controls were interviewed with the use of a structured interview, the mini-international neuropsychiatric interview (M.I.N.I.) [Sheehan[Q3], 1998 #61]. In the Japanese population, no significant population stratification has been repeatedly reported in several studies [Kakiuchi et al., 2003; Arinami et al., 2005; Shimizu et al., 2006].

For the quantitative genomic polymerase chain reaction (PCR), we used genomic DNA derived from LB cells of the two pairs of discordant SZ twins, 46 Japanese unrelated schizophrenia patients (38.6 ± 14.6 years old, 18 males and 28 females), and 11 controls (56.3 ± 11.0 years old, 8 males and 3 females), and 13 schizophrenia patients (55.0 ± 9.9 years old, 9 males and 4 females) obtained from NIMH Genetics Initiative Pedigrees. Written informed consent was obtained from all subjects. The ethics committees of the Brain Science

Institute (RIKEN) and participating institutes approved the study.

Cell Culture

The lymphocytes derived from peripheral blood were transformed by Epstein-Barr (EB) virus and cultured with the use of standard techniques as described before [Kato et al., 2002]. For mRNA quantification by DNA microarray analysis, we extracted the RNA from frozen cells, and thawed and recultured the cells. The culture of the cells and mRNA extraction were performed independently five times for each pair of twins.

DNA Microarray

DNA microarray experiments were performed as described previously with the use of an Affymetrix HU133A chip (Affymetrix, Santa Clara, CA) [Kakiuchi et al., 2006]. We used 5 μg of total RNA for reverse-transcription into cDNA, and biotin-labeled cRNA was synthesized from the cDNA. After testing the integrity of the samples by the Test2Chip (Affymetrix), fragmented cRNA was applied to the HU133A chip. The hybridization signal on the chip was scanned and subjected to image analysis (Affymetrix).

Analysis of DNA Microarray Data

The microarray raw data were processed by MAS5.0 (Affymetrix) and robust multiarray average (RMA) methods [Irizarry et al., 2003], and analyzed with the use of GeneSpring software (SiliconGenetics, Redwood, CA). Data were normalized by the median value. Genes expressed differently in each pair of twins were selected by the following criteria: (1) the genes were called as present in all samples (five samples of affected twin and five samples of control co-twin); (2) both the parametric test and the non-parametric test showed a significant difference ($P < 0.05$) between the five cultures in a patient and five cultures in the co-twin by both normalization methods (MAS5.0 and RMA). Then, the genes commonly changed to the same trend in both SZ twins A and SZ twins B according to these four statistical comparisons: MAS5 and RMA, parametric and non-parametric.

Genetic Association Studies

We selected five SNPs (rs7944706, rs6484148, rs6484147, rs4597056, rs726102) for *ADM* according to the linkage disequilibrium (LD) map database on SNPbrowserTM (Applied Biosystems, Foster City, CA). Although a previous report in the Japanese population hypothesized a possible role of dinucleotide repeat in the 4 kb downstream of *ADM* in the pathophysiology of hypertension, this microsatellite marker was not associated with plasma ADM concentration [Ishimitsu et al., 2001]; thus, this marker was not selected for the analysis. We selected three SNPs (rs9928312, rs9934331, rs1003904) for *SEPX1*, because their TaqMan probes were commercially available and they are polymorphic in Japanese according to the LD map database on HapMap projects accessed with the SNPbrowserTM software. We performed genotyping by TaqMan probes and ABI7900HT according to the protocol recommended by the manufacturer (Applied Biosystems). Assessment of LD patterns by the standardized disequilibrium coefficient (D′) and squared correlation coefficient (r^2), and analysis of haplotypic distribution, and frequencies were performed with the use of the COCAPHASE programs (http://portal.litbio.org/Registered/Option/unphased.html). Global significance was calculated by the random permutation test (10,000 times).

Quantification of Genome Copy Number

The copy number of *ADM* and *SEPX1* was analyzed by the real-time PCR method with the use of SYBR/GREEN dye (Applied Biosystems) as described elsewhere [Kato et al., 2005b]. *MLC1* (megalencephalic leukoencephalopathy with subcortical cysts gene 1) was used as a single copy control gene and the copy number of *ADM* was calculated as a relative ratio to *MLC1*. A minimum of three probes for *ADM* was used. For quality control, a gene on the X chromosome [phosphofructo-2-kinase (*PF2K*)] was also examined by SYBR/GREEN dye, and separation between males and females was confirmed. Sequences of primers and probes for these analyses will be provided upon request.

RESULTS

Microarray Analysis in the Cells of Monozygotic Twins Discordant for Schizophrenia

By the criteria described above, five genes were identified (Table I). Among the up-regulated genes in schizophrenia, two genes (GenBank accession nos. L06101 and Z00008) were immunoglobulin-related genes, and *CD200* (GenBank accession no. AF063591) was also a member of the immunoglobulin superfamily (OMIM 155970). This result possibly reflects transformation of a subset of B-cells by the EB virus rather than a difference in disease state. Surprisingly, both of the finally listed genes [*ADM* (GenBank accession no. NM_001124) and *SEPX1* (GenBank accession no. NM_016332)] have been reported to be altered in schizophrenia. The mRNA expression of *ADM* was reported to be up-regulated in the LB cells derived from schizophrenia patients, and the plasma ADM level was significantly higher in schizophrenic patients than in controls [Zoroglu et al., 2002; Huang et al., 2004; Yilmaz et al., 2007]. *SEPX1* was included in the list of genes up-regulated in the peripheral blood cells of schizophrenia by microarray analysis [Glatt et al., 2005]. Interestingly, the expressions of both genes were up-regulated in all the studies, which was the same trend shown in this study. These results suggested that *ADM* and *SEPX1* were strong candidate genes for schizophrenia.

Association Analysis of *ADM* and *SEPX1* in Schizophrenia

If up-regulation of *ADM* and *SEPX1* is a risk factor for schizophrenia, genetic variations of these genes may contribute to the illness. Thus, we also performed association analysis of *ADM* and *SEPX1* in schizophrenia in the Japanese population. We examined the genotype of five SNPs for *ADM* and three SNPs for *SEPX1*. LD patterns for *ADM* and *SEPX1*, as measured by D′ and r^2, are shown in Figure 1. No significant association was observed in single SNPs (Table II) and haplotypes (Table III) for *ADM*, and in single SNPs for *SEPX1* (Table II).

Quantification of Genome Copy Number

In addition to sequence variations, CNVs may also contribute to the up-regulation of *ADM* and *SEPX1*. Indeed, CNVs were reported for the loci of both genes [*ADM* (RP11-79E12) and *SEPX1* (RP11-451K7 and Variation_5329), http://projects.tcag.ca/variation/]. The CNV may cause altered mRNA expression and may confound the results of association analysis. Thus, we quantified the copy number of *ADM* and *SEPX1* genes by the real-time PCR method in two pairs of discordant SZ twins, 46 Japanese unrelated schizophrenia patients, and 11 controls, and from genetic information for 13 schizophrenia patients obtained from NIMH Genetics Initiative Pedigrees. However, we observed no loss or gain of the genome in the tested loci (data not shown).

TABLE I. The Result of DNA Microarray Analysis in the Lymphoblastoid Cells of Monozygotic Twins Discordant for Schizophrenia

Probe ID	Genbank	Symbol	SZ twin1						SZ twin2					
			MAS			RMA			MAS			RMA		
			FC	P(P)	P(non-P)	FC	P(P)	P(non-P)	FC	P(P)	P(non-P)	FC	P(P)	P(non-P)
Up-regulation														
211641_x_at	L06101		1.428	0.042	0.028	1.273	0.022	0.009	1.380	0.030	0.028	1.277	0.011	0.016
217977_at	NM_016332	SEPX1	1.183	0.015	0.047	1.175	0.012	0.016	1.124	0.044	0.028	1.129	0.011	0.028
202912_at	NM_001124	ADM	1.622	0.011	0.028	1.564	0.005	0.009	1.966	0.002	0.009	1.922	0.004	0.009
216517_at	Z00008		1.684	0.022	0.028	1.683	0.038	0.028	2.076	0.000	0.009	1.839	0.001	0.009
Down-regulation														
209583_s_at	AF063591	CD200	0.755	0.016	0.009	0.750	0.019	0.009	0.603	0.003	0.016	0.704	0.005	0.016

FC, fold change; P(P/non-P), P-value calculated by parametric/non-parametric test using GeneSpring software.

DISCUSSION

In this study, we demonstrated that mRNA expressions of *ADM* and *SEPX1* were up-regulated in the LB cells of the two patients with schizophrenia compared with their healthy co-twins. This observation is consistent with the previous reports examined in unrelated patients and controls. Genetic association studies of *ADM* and *SEPX1* for schizophrenia in the Japanese population, however, did not support the association of SNPs in these genes with schizophrenia. Further, we did not observe CNVs in these genes.

ADM is a potent vasodilator peptide consisting of 52 amino acids (OMIM103275), which was initially identified from pheochromocytoma [Kitamura et al., 1993]. ADM is synthesized by many tissues including the central nervous system and is known to bind to calcitonin receptor-like receptor. The reported roles of ADM are variable, such as dilation of blood vessels and increase in urine output. *ADM* is also abundantly expressed in the central nervous system, especially in the thalamus, hypothalamus, and pituitary gland, and it regulates neuroendocrine response to stress [Taylor and Samson, 2004]. Intracerebroventricular administration of ADM is known to affect water intake and salt appetite. A probably reactive increase of ADM in plasma is reported in some diseases such as heart failure, renal diseases, septic shock, and diabetes mellitus [Beltowski and Jamroz, 2004]. This increased level in plasma was first reported in patients with schizophrenia [Zoroglu et al., 2002]. This observation might reflect reactive up-regulation associated with some somatic condition associated with schizophrenia. However, elevated mRNA levels also were reported in LB cells of schizophrenia patients [Huang et al., 2004], which suggested that increase of ADM is intrinsic rather than reactive. In this study, *ADM* mRNA level was increased in the affected co-twins. Thus, intrinsic increase of ADM may be related to the pathophysiology of schizophrenia.

SEPX1 is one of the selenoproteins, which includes selenocystein, and is abundant in liver, leucocytes, and pancreas (OMIM 606216). The function of SEPX1 has not been clarified; however, interestingly, selenium-binding protein1 (*SELENBP1*), which also binds to selenium, was demonstrated to be up-regulated in both the brain and the peripheral blood leukocytes in patients with schizophrenia, and was suggested to be a candidate biomarker of schizophrenia [Glatt et al., 2005]. In the list of genes up-regulated in peripheral blood cells in this report, *SEPX1* was also included. In the present study, *SEPX1* mRNA level was also increased in the affected co-twins. Thus, the up-regulation of *SEPX1* may play a role in the pathophysiology of schizophrenia. Geographical analysis showed that low selenium in soil and food might be associated with schizophrenia [Brown, 1994]. At deficiency selenium is preferentially retained in the brain compared with other organs, and several studies have shown that selenium deficiency is associated with mood [Benton, 2002]. A possible role of selenium transport has been proposed in schizophrenia [Berry, 1993]. Thus, the roles of selenium metabolism in pathophysiology of schizophrenia may merit further study.

Although linkage with schizophrenia and presence of CNVs around the ADM and SEPX loci [Yamada et al., 2004; Moon et al., 2006; Redon et al., 2006] prompted us to perform an association study, no association was found. This result suggests that up-regulation of *ADM* and *SEPX1* might be a phenomenon secondary to schizophrenia. However, in the association study, we studied only 223 schizophrenic patients and 364 control subjects. The number of the subjects and the number of SNPs examined are not large enough to totally exclude a possible association between schizophrenia and the SNPs of *SEPX1* and *ADM*. In addition, the result should be treated with caution, because there was a significant difference in gender between patients with schizophrenia and controls ($P < 0.05$).

TABLE II. The Result[Q4] of Case-Control Studies in Japanese Population

		Genotype			HWE	*P* -value	Allele		*P* -value
					ADM				
rs7944706		A/A	A/G	G/G			A	G	
	CT	50	176	138	0.606		276	452	
	SZ	35	102	86	0.604	0.748	172	274	0.823
rs6484148		C/C	C/T	T/T			C	T	
	CT	43	166	155	0.887		252	476	
	SZ	25	84	114	0.121	0.117	134	312	0.106
rs6484147		C/C	C/T	T/T			C	T	
	CT	43	166	155	0.887		252	476	
	SZ	25	84	114	0.121	0.117	134	312	0.106
rs4597056		C/C	C/T	T/T			C	T	
	CT	157	163	44	0.865		477	251	
	SZ	114	84	25	0.121	0.160	312	134	0.116
rs726102		A/A	A/G	G/G			A	G	
	CT	42	165	157	0.892		249	479	
	SZ	25	84	114	0.121	0.147	134	312	0.140
					SEPX1				
rs9928312		A/A	A/G	G/G			A	G	
	CT	45	175	144	0.464		265	463	
	SZ	27	102	94	0.934	0.820	156	290	0.622
rs9934331		C/C	C/G	G/G			C	G	
	CT	103	176	85	0.559		382	346	
	SZ	61	109	53	0.752	0.969	231	215	0.821
rs1003904		A/A	A/G	G/G			A	G	
	CT	158	150	56	0.044		466	262	
	SZ	78	105	40	0.653	0.129	261	185	0.060

CT, control; SZ, schizophrenia; HWE, Hardy–Weiberg equilibrium.
P values are calculated by Fisher's exact test.

With regard to endophenotypes of schizophrenia, mainly psychophysiological, neurocognitive, and neuroimaging findings have been proposed [Gottesman and Gould, 2003]. Relatively few studies focused on blood analysis in schizophrenia. Altered mRNA levels in LB cells were reported for *ADM* [Huang et al., 2004] and *PDLIM5* [Iwamoto et al., 2004]. Alterations in peripheral blood leukocytes mRNA were reported for *SELENBP1* and other candidate genes [Glatt et al., 2005], mitochondria-related transcripts [Whatley et al., 1998; Mehler-Wex et al., 2006], dopamine receptors [Ilani et al., 2001; Kwak et al., 2001; Zvara et al., 2005; Boneberg et al., 2006], alpha 7-nicotinic acetylcholine receptor subunit (*CHRNA7*) [Perl et al., 2006], and transforming growth factor beta receptor II (*TGFBR2*) [Numata et al., 2007]. Although none of these candidate mRNA markers in blood cells has been established, it is promising that two genes detected in this study have already been reported in the literature. *ADM* and *SEPX1* are a promising target of further research of biomarkers of schizophrenia.

After our previous report of gene expression analysis in monozygotic twins discordant for bipolar disorder [Kakiuchi et al., 2003], we used the same approach in LB cells [Haas et al., 2006; Hu et al., 2006; Matigian et al., 2007] or different tissues [Zhou et al., 2005; Cutting and Snowden, 2006; Sarkijarvi et al., 2006]. The present results that two previously reported genes were identified in the twins supported the validity of this methodology. It has been difficult to apply statistical analysis to a limited number of twin samples. Thus, in this study, we performed five independent experiments for each pair of twins. Although it is difficult to prove the validity of this method, it is possible that this extensive analysis enabled the successful selection of these two genes.

In this study, the two pairs of twins discordant for schizophrenia did not have other family history. Thus, the dysregulation of genes in the affected twin is not due to a heritable factor such as a genetic polymorphism, but rather to some environmental or epigenetic effect. Thus, lack of association of the two genes with schizophrenia may be reasonable.

Although we focused on *ADM* and *SEPX1* in this study, the change in *CD200* might also be potentially interesting, because several studies reported that the immune system in schizophrenics may be involved in its susceptibility [Nawa and Takei, 2006]. Moreover, *CD200* has a unique expression pattern that is expressed on B-cells and neurons [Wright et al., 2001].

TABLE III. Haplotype Analysis of ADM in Japanese SZ Samples

Haplotype	SZ	CT	χ^2	*P*-value	Global *P*-value
ADM					
A-T-T-C-G	168 (0.380)	270 (0.381)	0.00184	0.965	
G-C-C-T-A	132 (0.298)	238 (0.336)	1.76	0.184	
G-T-T-C-G	142 (0.321)	200 (0.282)	1.94	0.162	0.345

SZ, schizophrenia; CT, control.
Global *P*-value was calculated by a random permutation test (10,000 times) with the use of COCAPHASE program.
Only haplotypes that were verified at least once were analyzed.

A

ADM — D' (upper triangle), r2 (lower triangle)

	rs7944706	rs6484148	rs6484147	rs4597056	rs726102
rs7944706		1	1	1	1
rs6484148	0.3172		1	1	1
rs6484147	0.3172	1		1	1
rs4597056	0.3093	1	1		1
rs726102	0.3114	1	1	1	

B

SEPX1 — D' (upper triangle), r2 (lower triangle)

	rs9928312	rs9934331	rs1003904
rs9928312		1	0.4592
rs9934331	0.6233		1
rs1003904	0.2071	0.5721	

Fig. 1. Intermarker linkage disequilibrium pattern for *ADM* (**A**) and *SEPX1* (**B**). The standardized disequilibrium coefficient (D') and squared correlation coefficient (r^2) calculated by the COCAPHASE program are shown for Japanese control samples.

CD200 is expressed in developing neuronal cell bodies and axons [Morris and Beech, 1987]. Thus, *CD200* may be a promising target for further study.

In conclusion, we demonstrated the possible pathological contribution of *ADM* and *SEPX1* to schizophrenia and the usefulness of LB cells of monozygotic twins discordant for schizophrenia.

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In Review

Comprehensive Gene Expression Analysis in Bipolar Disorder

Tadafumi Kato, MD, PhD[1], Chihiro Kakiuchi, MD, PhD[2], Kazuya Iwamoto, PhD[3]

Objective: To review recent findings by DNA microarray in bipolar disorder (BD).

Method: A literature search was performed.

Results: Comprehensive gene expression analysis in the brain, peripheral blood cells, and olfactory neuroepithelium would be a promising strategy for the research of BD. To date, alterations in glutamate receptors (GR), mitochondria-related genes, chaperone genes, oligodendrocyte genes, and markers of gamma amino butyric acidergic (GABAergic) neurons in postmortem brains are replicated by several different strategies. However, alterations in mitochondria-related genes are associated with agonal factors, sample pH, and effects of drugs. Analysis of blood cells showed altered endoplasmic reticulum stress pathway and other molecular cascades. Analysis of olfactory epithelium showed altered expression of genes associated with apoptosis.

Conclusions: These findings warrant that comprehensive gene expression analysis by DNA microarray will be useful to identify the molecular cascades responsible for BD.

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Clinical Implications

- Gene expression analysis in postmortem brains, blood cells, and olfactory neuroepithelium will be useful to develop biomarkers for BD.
- Alterations in GRs, mitochondria-related genes, chaperone genes, oligodendrocyte genes, and GABAergic markers are reported in postmortem brains.
- Alterations in endoplasmic reticulum stress and apoptosis pathways were suggested by the analysis of blood cells and neuroepithelium.

Limitations

- Many findings have not yet been well replicated.
- These findings need to be verified in larger samples.
- Alterations in mitochondria-related genes in postmortem brains are associated with agonal factors, sample pH, and effects of drugs.

Key Words: *bipolar disorder, glutamate receptors, mitochondria, chaperones, oligodendrocyte, GABA, brain, lymphoblastoid cells*

In spite of extensive studies, pathophysiology of BD is still enigmatic. The role of genetic factors is well established from twin studies, but the causative genes or genetic risk factors have not been well established.[1] The other well-studied area of biological research is neuroimaging. Those studies suggested that the volume of several brain areas, such as anterior cingulate, amygdala, and hippocampus, are altered in BD.[2] However, the results are not always consistent with each other. The most established finding is that there is increased incidence of subcortical hyperintensity detected by MRI, although such a finding is not specific to BD because this is frequently seen in healthy, elderly people. The third important area of research would be postmortem brain studies.[3] Before the SMRI[4] started its activity to provide brain bank samples to anyone with an adequate research plan, the number of postmortem brain studies of BD patients was quite small. Since SMRI started to provide the samples, the number of studies dramatically increased and the postmortem brain study became a hot research area. Another area of study involved obtaining peripheral blood samples. One replicated finding obtained from such studies is altered basal or agonist-stimulated calcium levels in blood cells derived from patients with BD.[5] In addition to those studies involving patients' samples, studies focusing on the pharmacology of mood stabilizers are also widely performed. To date, a number of hypotheses to account for the action mechanisms of mood stabilizers have been suggested and are still controversial.

Abbreviations used in this article

BD	bipolar disorder
ER	endoplasmic reticulum
GABA	gamma amino butyric acid
GABAergic	gamma amino butyric acidergic
GAD	glutamic acid decarboxylase
GR	glutamate receptor
GRIK1	glutamate receptor kainate 1
HSPF1	heat shock protein 40
LARS2	mitochondrial leucyl-tRNA synthetase
MRI	magnetic resonance imaging
NPY	neuropeptide Y
PCR	polymerase chain reaction
PKC	protein kinase C
RT-PCR	reverse transcribed quantative polymerase chain reaction
SMRI	Stanley Medical Research Institute
SNP	single nucleotide polymorphism
SST	somatostatin
TGF-1β1	transforming growth factor-beta 1

In this situation, the recent development of comprehensive gene expression analysis using DNA microarray is expected to be a powerful tool to identify molecular pathophysiology of BD (see Figure 1). Since Bezchlibnyk et al[6] first used DNA microarray for the study of BD, this technology has been applied to postmortem brains, peripheral leukocytes, transformed lymphoblastoid cells, and biopsied olfactory neuroepithelium derived from patients with BD.

In this review, studies focusing on molecular cascades related to pathophysiologic mechanisms of BD are summarized (see Table 1). After the postmortem brain studies are summarized by subcategorizing the findings into several sections, studies of peripheral blood cells and neuroepitherium are introduced. Finally, limitations and future directions are presented. Detailed methodological issues regarding the platforms used for the DNA microarray analysis and the method of data analysis were discussed elsewhere.[7]

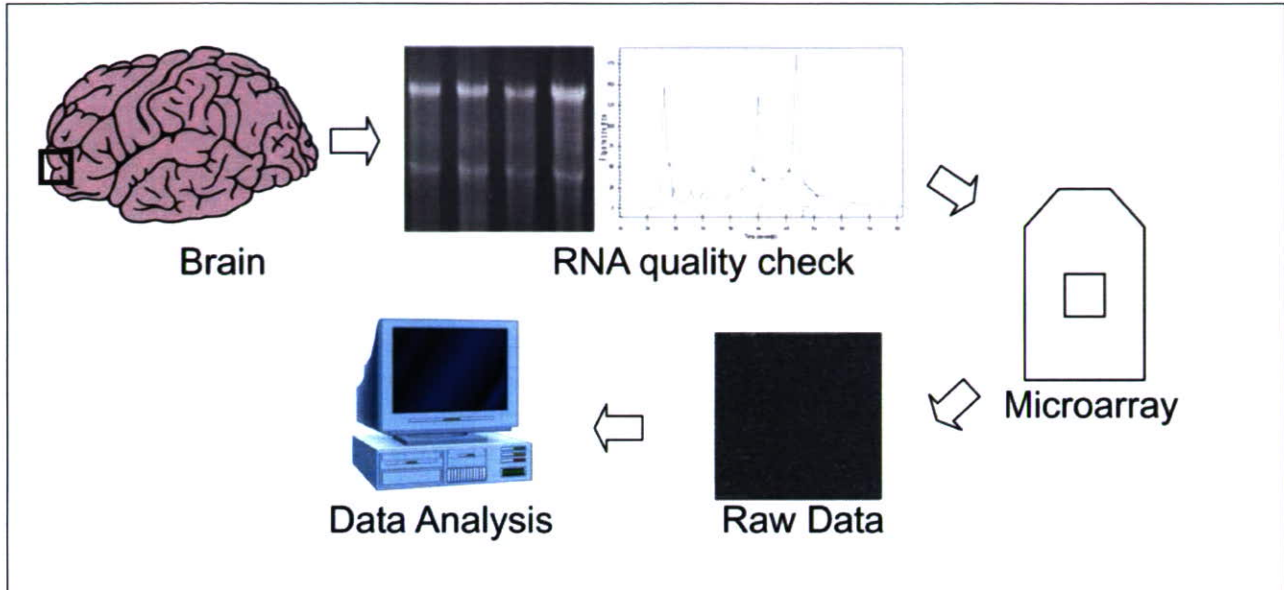
Postmortem Brain Studies

Initial Studies

Sun and colleagues[8] used the serial analysis of gene expression method to identify the differentially expressed genes in the postmortem brains of a patient with BD in comparison with an age- and sex-matched control subject. They found upregulation of serotonin transporter and NF-κB2 genes and confirmed these findings in a case–control study in the brains of 19 patients with BD and 15 controls. The upregulation of serotonin transporter mRNA in BD may be compatible with a recent finding showing increase of serotonin transporter binding potential in vivo measured by positron emission tomography.[9] A recent study showed increased levels of NF-κB protein in lymphocytes obtained from 15 patients with bipolar depression, compared with 25 controls,[10] which may be in accordance with the initial finding in postmortem brains.

Bezchlibnyk et al[6] used a cDNA microarray carrying about 1200 genes to search for genes relevant to biochemical process of BD. They mixed the RNA extracted from postmortem brain samples obtained from 10 patients with BD or 10 controls obtained from Stanley Foundation Neuropathology Consortium and analyzed by a set of cDNA microarray. They reported differential expression of 24 genes, and focused on 5 candidate genes. They validated the finding of decreased TGF-1β1 by analyzing individual samples by RT-PCR method. The results showed statistically significant decrease of TGF-1β1 expression. TGF-1β1 is a multifunctional cytokine involved in various functions such as cell growth and differentiation, and thus a possible role in BD was suggested. Reduced TGF-1β1 level was also reported in plasma of 70 patients with mania, compared with 96 controls.[11] It was rather higher in patients with schizophrenia,[12] and higher

Figure 1 The methodology of DNA microarray analysis. RNA is extracted from brain samples. The quality of RNA is checked by gel electrophoresis and agilent bioanalyzer. RNA samples are reverse-transcribed using oligo dT primer. From the cDNA, biotynated cRNAs are produced by in vitro transcription. The cRNA sample is used for GeneChip analysis (Affymetrix). The raw data are analyzed after normalization is applied.



scores of Hamilton Depression Rating Scale were associated with lower levels.[13] These findings support the initial finding and suggest a possible role of TGF-1β1 as a biomarker of BD. However, owing to its multiple functions, its specificity and clinical significance as a biomarker may be challenged.

Expression of PDLIM5

Iwamoto et al[14] performed comprehensive gene expression analysis of the postmortem brains obtained from the same brain resource as Bezchlibnyk et al, but used one oligonucleotide microarray per sample. They found altered expression of 53 genes. These included downregulation of neurotransmission-related genes such as calcium channel P/Q type alpha 1A, serotonin 2C receptor, metabotropic GR (GRM1), ionotropic GRIK1, and increased stress response-related genes. Among the differentially expressed genes found in the postmortem brains, altered expression of HSPF1 and LIM (PDLIM5) was also seen in lymphoblastoid cells derived from patients with BD. HSPF1 was increased both in the brain and lymphoblastoid cells, while PDLIM5 was increased in the brain but decreased in the lymphoblastoid cells. PDLIM5 encodes an adaptor protein linking PKC and N-type calcium channel. Since alterations of both calcium signalling and PKC activity have been reported in BD, they further pursued the role of this gene in BD. Decreased expression of PDLIM5 in lymphoblastoid cells was further verified in an extended sample of bipolar I disorder, as well as those with bipolar II disorder and schizophrenia.[15] Genetic association analysis showed association with BD, while the association with schizophrenia is not consistent in 2 studies.[16,17] More recently, reduced expression of PDLIM5 was found to be related to depressive state.[18] These combined findings suggest the role of PDLIM5 expression may be a biomarker for mental disorders.

Chaperones and Stress Response Genes

Jurata et al[19] also performed comprehensive gene expression analysis in the postmortem brains of patients with BD and controls. They used 2 platforms, oligonucleotide microarray (Affymetrix Hu133A) and cDNA microarray (Agilent Human 1), to compare the efficiency of these platforms to detect differentially expressed genes. First, they used parietal cortex samples of 1 bipolar patient and 1 control. They subsequently examined 8 prefrontal cortex samples each of patients with BD and controls. They concluded that sensitivity to detect the differential expression was higher in oligonucleotide array than in cDNA microarray. They reported 47 genes showing differential expression using both platforms. There was no overlap with the findings by Bezchlibnyk et al.[6] However, upregulation of genes of chaperones or stress response proteins, such as GRP94 (gp96) and interferon-induced transmembrane protein 2, is similar to the finding by Iwamoto et al,[14] who reported upregulation of several chaperone or stress response genes such as HSPF1 and

Table 1 Summary of major findings

Findings	Postmortem brain	Peripheral blood/neuroepithelium
NF-κB	BP↑[8]	BP↑(protein)[10]
TGF-1β1	BP↑[6]	Mania↓[11]
		Depression↓[13]
		Schizophrenia↑[12]
PDLIM5	BP↑[14,17]	BPI, BPII↓[14,15]
		Depression↓[18]
Chaperones	BP↑[14,19]	HSPF1, BPI, BPII↓[14,15]
	Depression↑[20]	
Mitochondria-related genes	BP↓[14,21,25,26]	BPI[32]
NDUFV2	BP↑[28,29]	BP↓[31,32]
LARS2	BP↑[27]	—
Ubiquitin pathway	BP↓[34]	—
GABA neuron markers	BP, NPY↓[53]	—
	BP, SST, GAD1↓[21]	
	BP, SST↑[29]	
GABA and glutamate receptors	BP[41]	—
	BP↓GRIK1[14,41]	
Oligodendrocyte genes	BP↓[43]	
ER stress pathway	—	BP↓[56]
APOBEC3B, etc	—	BP[61]
MAX	—	BP↑[62]
Apoptosis-related genes	—	BP↑[63]

interferon induced transmembrane protein 3. Upregulation of HSPF1 was also found in lymphoblastoid cell lines in patients with both bipolar I[14] and bipolar II disorders.[15] Increased expression of endoplasmic reticulum chaperons such as GRP78, GRP94, and calreticulin is also reported in patients with major depression who died by suicide.[20]

Mitochondria-Related Genes

Konradi et al[21] performed gene expression analysis in the hippocampal samples of 9 patients with BD and 10 controls, as well as 8 patients with schizophrenia, obtained from Harvard Brain Bank, using oligonucleotide microarray. They found that mitochondria-related genes were globally downregulated in BD. They found that genes related to mitochondrial oxidative phosphorylation or ubiquitin proteasome pathway. They discussed that the effect of drugs cannot explain this finding because patients with schizophrenia taking similar drugs did not show such marked global downregulation of mitochondria-related genes. They interpreted that this finding reflects mitochondrial dysfunction in BD.

Global downregulation of mitochondria-related genes had also been observed in postmortem brains samples with low pH or agonal factors such as prolonged hypoxia and ischemia.[22,23] Indeed, sample pH was significantly lower in BD group in the study of Konradi et al.[21] Thus the finding in BD may be explained by those factors. Iwamoto et al[24] performed DNA microarray analysis in the Stanley Microarray Collection samples. Whereas the global downregulation of mitochondria-related genes in BD reported by Konradi et al[21] was replicated, lower sample pH was associated with downregulation of mitochondria-related genes. In addition, several classes of psychotropic drugs, such as antipsychotics, valproate, and antidepressants, were found to be associated with reduced expression of mitochondria-related genes. In

drug-free patients, mitochondria-related genes were instead upregulated.

Vawter and colleagues[25] as well as Sun et al[26] also replicated the global downregulation of mitochondria-related genes in BD. Vawter et al[25] minutely analyzed the interaction between disease and confounding factors to observe the gene expression patterns in postmortem brains. Whereas they classified the genes affected by disease and (or) agonal-pH factors, they were prudent enough to avoid proposing any particular genes as specific to BD. They also found that copy number of mtDNA tended to be increased in the patients with BD. Sun et al[26] replicated the finding of global downregulation of mitochondria-related genes and effects of pH, but proposed that reduced pH itself might be a reflection of mitochondrial dysfunction in BD.

Munakata et al[27] reanalyzed the previously reported DNA microarray data focusing on mitochondrial genes. They found that LARS2 was upregulated in BD. Because a well characterized mitochondrial DNA 3243 A>G mutation on mitochondrial leucyl tRNA is reported to impair the amioacylation of tRNA catalyzed by LARS2, they hypothesized that this finding might be compensatory upregulation by the presence of the mtDNA 3243 mutation. They verified that LARS2 was indeed upregulated in the cybrids carrying 3243G mutation and, using the protein nucleic acid-clamped PCR method, they searched for this mutation in the postmortem brains. They found that 3 patients, 2 with BD and 1 with schizophrenia, showed relatively high level of 3243 mutation, not only in the brain but also in the liver. This suggested that accumulation of mtDNA mutation in the brain might play a part in the pathophysiology of BD.

While many mitochondria-related genes were reported to be downregulated, possibly reflecting low sample pH, increased expression of NDUFV2 encoding 24kDa subunit of complex I in parietotemporal or frontal cortices was reported by 2 groups.[28,29] This is compatible with upregulation of NDUFV2 in the rat depression model of learned helplessness.[30] On the other hand, reduced NDUFV2 level was found in lymphoblastoid cells of BD patients.[31] Reduced NDUFV2 level was correlated with downregulation of other mitochondria-related genes in lymphoblastoid cells.[32] Although this was initially attributed to promoter polymorphisms,[31,33] the genetic association was not replicated in extended samples.[29]

Ubiquitin Pathway

Ryan et al[34] examined the prefrontal and orbitofrontal cortices samples from the Stanley Microarray Collection and performed comprehensive gene expression analysis. No significant alteration was found in the prefrontal cortex, while ubiquitin pathway is dowregulated and G-protein coupled receptors were upregulated in orbitofrontal cortex.

GABA Neuron Markers

In the data by Konradi et al,[21] downregulation of GAD67 and SST, both of which are markers of GABAergic neurons, was also reported. Downregulation of SST and GAD67 was replicated in our analysis (Iwamoto et al, unpublished finding). Somatostatin concentrations in cerebrospinal fluid in patients with BD showed conflicting results: decrease,[35] no change,[36] or increase.[37] These findings may be in accordance with reduced GAD67 positive GABAergic neurons in BD[38,39] or decreased GABA concentration measured by in vivo proton magnetic resonance spectroscopy.[40] Conversely, Nakatani et al[29] used the samples from an Australian brain bank to perform gene expression analysis and found the differential expression of 8 genes (RAP1GA1, SST, HLA-DRA, KATNB1, PURA, NDUFV2, STAR, and PAFAH1B3), including the upregulation of SST. Although the observed change of SST was in the opposite direction to previous studies, their subsequent genetic association study showed the association between SNP markers of SST and BD.

GABA and Glutamate Receptors

Choudary et al[41] reported that 2 GABA receptor genes (GABRA5 and GABBR1) were upregulated in the dorsolateral prefrontal cortex. Dysfunction of subpopulation of GABAergic interneurons and compensatory upregulation of GABAergic receptors on pyramidal neurons has been suggested as a pathophysiological mechanism of schizophrenia.[42] These observations may reflect the pathophysiology shared by these 2 mental disorders.

Three GR genes (GRIA1, GRIA3, and GRM3) were also shown to be upregulated in the dorsolateral prefrontal cortex and (or) anterior cingulate cortex. On the other hand, downregulation of GRIK1 encoding kainate type GR found in this study is in accordance with other reports.[14,29]

Comparison With Schizophrenia

Tkachev et al[43] performed gene expression analysis in the postmortem brains of patients with schizophrenia and found global downregulation of oligodendrocyte-related genes such as myelin-associated glycoprotein and myelin oligodendrocyte glycoprotein. Similar findings were reported by the other investigators in various brain regions.[44–50] They used RT-PCR and found that downregulation of oligodendrocyte-related genes was also seen in BD. We did not detect such downregulation in the initial sample set; however, we could replicate it in the second sample set (Iwamoto et al, unpublished finding).

While Konradi and colleagues[21] reported global dowregulation of mitochondrial genes in BD but not in schizophrenia, Alter et al[51] dissected hippocampal pyramidal

neurons and found that mitochondria-related genes were downregulated in schizophrenia but not in BD.

Downregulation of the GABAergic neuron marker genes and (or) loss of GABAergic interneurons, were also reported in schizophrenia.[52] In one initial study, Kuromitsu et al[53] used oligonucleotide microarray for gene expression analysis of pooled RNA samples from patients with schizophrenia and controls. They found that NPY was downregulated in the brains of patients with schizophrenia. Downregulation of NPY in the prefrontal cortex was also confirmed in BD by RT-PCR. NPY is one of several peptides expressed in GABAergic interneurons. This finding is compatible with a previously reported finding.[54]

These findings suggest that downregulation of GABAergic interneuron-related genes, mitochondria-related genes, and oligodendrocyte-related genes may be common to schizophrenia and BD, although there are some inconsistencies. However, it is also possible that these apparent alterations might reflect the effects of confounding factors such as agonal-pH factors, medication, and cause of death.

When all confounding factors are controlled, the number of samples and the power of analysis decreases. Thus it is a matter of debate about how these factors should be controlled.[7]

Public Database

As described above, SMRI played a significant role in the postmortem brain studies of BD. After this brain bank started to distribute the brain samples to researchers, the number of papers on postmortem brain studies in BD markedly increased. Although intellectual property rights belonged to the SMRI when these brain bank samples were used, these data were not used for commercial purpose but were made open to the public.[55] This database will further facilitate the research of BD. However, the list of differentially expressed genes in the database tend to be different from the original report, reflecting the different methods for data analysis, exclusion of confounding factors, and so on. This might potentially cause confusion among the researchers who are not familiar with the DNA microarray studies using postmortem brains.

Summary of Findings

As written above, altered expression of chaperone and stress response genes, mitochondria-related genes, GABAergic neuron markers such as SST and GAD1, GRIK1 encoding kinate type GR, and oligodendrocyte-related genes were replicated in more than one study. Several expression changes detected in postmortem brains, such as NF-κB, TGF-1β1, PDLIM5, HSPF1, and NDUFV2, were also detected in peripheral blood cells and thus might be used as biomarkers for BD.

Peripheral Blood Cells and Olfactory Neuroepithelium

Monozygotic Twins Discordant for BD

Kakiuchi et al[56] used oligonucleotide microarray for the analysis of lymphoblastoid cell lines derived from 2 pairs of monozygotic twins discordant for BD and 1 pair of control twins. Among the genes commonly downregulated in these 2 pairs of twins, XBP1 and HSPA5 (GRP78) were related to ER stress response, and they further focused on this pathway. ER stress response was attenuated in the lymphoblastoid cells derived from patients with BD in a small case–control study. They identified a SNP, −116 C/G, which impairs XBP1-induced XBP1 expression, owing to loss of XBP1 binding site on its promoter. This SNP was associated with BD in the original report, but subsequent studies did not support this association.[57,58] More recently, So and colleagues[59] also reported that XBP1 induction by ER stress was attenuated in the lymphoblastoid cells derived from patients with BD, but this was not solely explained by XBP1 polymorphism. By searching for the target genes of XBP1 in neuroblastoma cells using DNA microarray, Kakiuchi et al[60] found that WFS1, the causative gene for Wolfram syndrome, is induced by XBP1 through ER stress response element on its promoter. Wolfram syndrome is known to comorbid with mood disorders. This finding supports the role of XBP1-WFS1 pathway in BD.

Search of Peripheral Blood for Biomarkers

Tsuang et al[61] analyzed the gene expression patterns in peripheral blood leukocytes derived from patients with BD and reported that APOBEC3B, ADSS, ATM, CLC, CTBP1, DATF1, CXCL1, and S100A9 would become biomarkers to distinguish the patients from those with schizophrenia or controls. Middleton et al[62] also performed gene expression analysis of peripheral blood leukocytes. By comparing the patients with their healthy sib pairs, upregulation of myc-associated factor X was found. For other 248 genes altered in BD, they discussed the significance of the finding in relation to the genetic linkage loci, and suggested that chromosome 6q showed both linkage and gene expression alteration signals.

Olfactory Neuroepithelium

Considering the difficulties in managing confounding factors in the study of postmortem brain studies, use of olfactory neuroepithelium would be a promising alternative, in spite of its invasiveness. McCurdy et al[63] performed gene expression analysis in biopsied olfactory epithelium derived from patients with BD. They found that cultures from BD patients showed high ratio of cell death, and alteration of apoptosis-related genes were detected by their original oligonucleotide microarray. Genes associated with intracellular inositol signalling were also altered.

Summary of Findings

In summary, endoplasmic reticulum stress pathway was implicated in the pathophysiology of BD based on the findings of discordant twins. Several genes found by gene expression analysis of peripheral blood cells were proposed as biomarkers of BD. A study in olfactory neuroepithelium showed enhanced apoptotic pathway in BD.

Discussion

As described above, data of gene expression analysis in postmortem brains cannot be free from artifacts, owing to premortem or antemortem changes. Such changes may be linked with diagnosis, because the cause of death is different, and pathophysiology of the illness itself may affect these processes. In addition, effect of drugs always confounds the results. Due to these difficulties, the findings obtained by the gene expression analysis using postmortem brains are not conclusive on their own.

Conversely, the analysis of peripheral blood cells also has problems because it is not the target organ of mental disorder and many important genes expressed in the brain are not expressed in peripheral blood cells. Thus only abnormalities of basic metabolism could be assessed in peripheral blood cells. In addition, effects of drugs are difficult to exclude in clinical settings. It is also difficult to distinguish the effect of trait-dependent alteration from state-dependent alteration. Use of lymphoblastoid cell lines would be one way to control these problems.

Use of olfactory neuroepithelium biopsy samples may be a promising alternative. However, because of its invasiveness, such research has been done in only limited institutions.

The only way to obtain conclusive results would be to verify the findings obtained in postmortem brains by multiple approaches, such as analysis of peripheral blood, genetics, animal models, and so on. When the data obtained from these different approaches are compared, methodological consideration is essential because each research strategy has characteristic artifacts, which should be carefully controlled.

In spite of the methodological problems, several findings, such as alterations in GRs, PDLIM5, mitochondrial dysfunction, impaired endoplasmic reticulum stress response, and alterations in GABAergic neurons, are validated by several different strategies. Before concluding that these pathways are important for the pathophysiology of BD, we should verify that these findings are caused by disease process rather than effects of confounding factors using animal models. To clarify the causal relation, behavioural consequence of animal models having abnormalities in these pathways should be examined. If the roles of these cascades are confirmed by those studies, these findings should be tested as peripheral biomarkers. Further studies would clarify the etiology of BD and provide clinically useful biomarkers.

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Résumé : Une analyse exhaustive de l'expression génétique dans le trouble bipolaire

Objectif : Examiner les résultats récents d'un jeu ordonné de microéchantillons d'ADN dans le trouble bipolaire (TB).

Méthode : Une recherche de la documentation a été exécutée.

Résultats : Une analyse exhaustive de l'expression génétique dans le cerveau, des globules de sang périphérique, et du neuroépithéliome olfactif serait une stratégie prometteuse pour la recherche sur le TB. Jusqu'ici, les modifications des récepteurs de glutamate (RG), des gènes liés aux mitochondries, des gènes chaperons, des gènes oligodendrocytes, et des marqueurs des neurones d'acide gamma-aminobutyrique (GABAergiques) dans les cerveaux postmortem sont reproduites par plusieurs stratégies différentes. Cependant, les modifications des gènes liés aux mitochondries sont associées avec des facteurs agonaux, un échantillon de pH, et les effets des médicaments. L'analyse des globules sanguins a montré une modification de la voie de stress du réticulum endoplasmique et d'autres cascades moléculaires. L'analyse de l'épithéliome olfactif a montré une expression modifiée des gènes associés à l'apoptose.

Conclusions : Ces résultats garantissent que l'analyse exhaustive de l'expression génétique par jeu ordonné de microéchantillons d'ADN sera utile pour identifier les cascades moléculaires responsables du TB.

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SPECIAL FEATURE

Frontiers in Epigenetics Medicine

Epigenetics in mood disorders

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Abstract Depression develops as an interaction between stress and an individual's vulnerability to stress. The effect of early life stress and a gene–environment interaction may play a role in the development of stress vulnerability as a risk factor for depression. The epigenetic regulation of the promoter of the glucocorticoid receptor gene has been suggested as a molecular basis of such stress vulnerability. It has also been suggested that antidepressive treatment, such as antidepressant medication and electroconvulsive therapy, may be mediated by histone modification on the promoter of the brain-derived neurotrophic factor gene. Clinical genetic studies in bipolar disorder suggest the role of genomic imprinting, although no direct molecular evidence of this has been reported. The role of DNA methylation in mood regulation is indicated by the antimanic effect of valproate, a histone deacetylase inhibitor, and the antidepressive effect of *S*-adenosyl methionine, a methyl donor in DNA methylation. Studies of postmortem brains of patients have implicated altered DNA methylation of the promoter region of membrane-bound catechol-*O*-methyltransferase in bipolar disorder. An altered DNA methylation status of PPIEL (peptidylprolyl isomerase E-like) was found in a pair of monozygotic twins discordant for bipolar disorder. Hypomethylation of PPIEL was also found in patients with bipolar II disorder in a case control analysis. These fragmentary findings suggest the possible role of epigenetics in mood disorders. Further studies of epigenetics in mood disorders are warranted.

Keywords Biopolar disorder · DNA methylation · Environment · Epigenetics · Mood disorders · Stress vulnerability

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Introduction

It is clear that both genes and the environment confer risk for mood disorders. A relative recent development in the field of biological psychiatry has been the focus on attempts to understand functional outcomes of the additive and combinatorial effects of genes and the environment at the molecular level [1]. As such, the interplay between a relatively fixed genome and an often variable environment involves epigenetic factors.

Epigenetic changes are long-lasting modifications in gene function that do not involve changes in gene sequences. Recent evidence suggests that these changes may occur in both dividing and nondividing cells [2–5] and may be transmitted intergenerationally [6, 7]. Epigenetic mechanisms involve modifications of the functional unit of the genome, the nucleosome, which is composed primarily of an octamer of pairs of H2A, H2B, H3, and H4 histones, around which is wrapped a 147-bp segment of DNA [8]. This configuration allows for the regulation of transcription through the control of access to the gene. Although many epigenetic modifications influence gene regulation, in the context of molecular psychiatric analysis, the most prevalently studied modifications to date are DNA methylation of CpG dinucleotides and acetylation and methylation at the N-terminal tails of histones. DNA is methylated by the transfer of a methyl group from *S*-adenosyl methionine

PROOF