TABLE I. NEO PI-R Scores by Genotypes of the -116C/G Polymorphism of the *XBP1* Gene in 195 Healthy Japanese Volunteers (162 Females and 33 Males)

Genotype	C/C	C/G	G/G	F value (df = 2)
All subjects (n = 195)	n = 27	n = 93	n = 75	
Neuroticism	102.5 (20.0)	104.0 (19.9)	97.2 (18.9)	2.57*
Extraversion	94.6 (10.3)	99.1 (14.5)	98.9 (14.2)	1.15
Openness	108.6 (16.5)	110.2 (13.1)	109.1 (13.1)	0.19
Agreeableness	109.6 (13.7)	113.3 (12.4)	117.6 (12.9)	4.61***
Conscientiousness	100.3 (12.8)	100.8 (16.8)	104.2 (17.5)	1.10
Females (n = 162)	n = 23	n = 79	n = 60	
Neuroticism	106.7 (18.6)	105.2 (19.2)	97.4 (19.3)	3.50**
Extraversion	94.5 (10.6)	97.8 (14.8)	99.1 (13.8)	0.91
Openness	110.3 (16.2)	111.0 (15.0)	109.6 (13.7)	0.04
Agreeableness	110.1 (14.0)	110.1 (14.0)	113.7 (13.1)	3.67**
Conscientiousness	97.8 (11.5)	100.6 (16.9)	104.5 (16.9)	1.74

Mean (SD).
*$P < 0.1$.
**$P < 0.05$.
***$P = 0.01$ (uncorrected).

RESULTS

As summarized in Table I, the NEO PI-R score of agreeableness showed a trend for an association with the genotype of the -116C/G of the *XBP1* gene in all subjects ($F = 4.61$, $df = 2$, $P = 0.01$). The association was also observed in females ($F = 3.67$, $df = 2$, $P < 0.03$). In addition, the score of neuroticism was associated with the genotype in females ($F = 3.50$, $df = 2$, $P < 0.04$). A tendency of the association with neuroticism was also observed in all subjects ($F = 2.57$, $df = 2$, $P < 0.1$). Subjects with the G allele, especially those with the G-G genotype, tended to show higher agreeableness and lower neuroticism.

When the subscales of the agreeableness and the neuroticism were studied, straightforwardness ($F = 3.22$, $df = 2$, $P = 0.04$) and altruism ($F = 5.43$, $df = 2$, $P = 0.005$) of the agreeableness and anger-hostility ($F = 5.34$, $df = 2$, $P < 0.006$) and depression ($F = 4.53$, $df = 2$, $P < 0.02$) of the neuroticism were associated with the genotype in all subjects. The straightforwardness, altruism, anger-hostility, and depression are associated with the genotype also in females ($F = 2.89$, 3.87, 5.50, and 4.39, $df = 2$, $P = 0.06$, 0.02, 0.005, and 0.01).

DISCUSSION

The present study investigated a role of the -116C/G polymorphism of the *XBP1* gene in the development of personality. Personality traits were evaluated using the NEO PI-R, in healthy Japanese volunteers, primarily consisting of female medical staff. As a result, a statistically significant association was observed between the polymorphism and the NEO PI-R score of agreeableness ($P < 0.01$). Subjects with the G allele of the polymorphism tended to have higher score of agreeableness than those without the G allele.

The G allele of the polymorphism was in the recent study significantly associated with a risk for bipolar disorder [Kakiuchi et al., 2003]. Subjects with the G allele had a 4.6-fold increased risk for the disorder compared with those without the allele in their study. Higher agreeableness, which was found in subjects with the G allele in the present study, may be related with an old idea of the Kretchmer's cycloid temperament, which is characterized by warmth and altruism [Kretchmer, 1955]. Clinical impressions might be that this temperament could be observed in a portion of subjects with bipolar disorder. However, statistical investigations have suggested no specific personality for an association with bipolar disorder [Carpenter et al., 1999]. Therefore, the association between agreeableness and the XBP1 polymorphism should be interpreted with caution in light of their relationship with bipolar disorder.

The present study in addition observed a trend of an association between the *XBP1* gene and the NEO PI-R score of neuroticism. This should be interpreted with caution also. The G allele, which is the risk allele for bipolar disorder in Kakiuchi et al. [2003], appeared to be associated with lower score of neuroticism in the present study, especially in females. Neuroticism has been in general observed to be higher in subjects with mood disorder and anxiety disorder and to affect symptoms of the patients [Carpenter et al., 1999; Jain et al., 1999; Lozano and Johnson, 2001; Bienvenu and Stein, 2003].

It may be acknowledged that the observed association could be related with traits of psychiatric disorders in a portion of the subjects with family history of major psychosis were excluded from the study according to the information obtained at the brief interview and from a questionnaire. However, the information may not be thorough and those with family history of minor mental disorders could be included in the study.

The mechanism how the XBP1 polymorphism affects the development of personality remains to be explored, given the limited data on its role in the brain. It is postulated however that XBP1 could be associated with neuroplastic change in the brain. XBP1 may have a role in "tuning" the cellular function associated with ER stress. In lymphocytes, ER stress due to antigen exposure induces, via increased immunogloblin production, XBP1, which finally differentiates the B cells into plasma cells [Iwakoshi et al., 2003]. XBP1 is expressed in the frontal cortex, basal ganglia, and hippocampus in mice [Paschen et al., 2004], and induced by brain trauma [Paschen et al., 2004] or ischemia [Kumar et al., 2003]. GRP78, the downstream gene of XBP1 and a ER chaperone, is induced by metamphetamine [Jayanthi et al., 2004]. These suggest that XBP1 could be associated with neuroplastic change and therefore with neuropsychiatric functions of the brain, although the role of the ER stress response and XBP1 in the brain remains to be further elucidated.

In conclusion, the present study observed a statistical trend of an association between the -116C/G polymorphism of the *XBP1* gene and the personality score of agreeableness and neuroticism. Although the results are interesting, their interpretation should be with caution in light of the previous observation on the role of the gene in bipolar disorder [Kakiuchi

et al., 2003]. Further studies are required to confirm the role of gene in the development of personality, and also of bipolar disorder.

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Lithium response and −116C/G polymorphism of *XBP1* in Japanese patients with bipolar disorder

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Three mood stabilizers, lithium, valproate and carbamazepine, are known to be effective for a subset of patients with bipolar disorder, but the mechanism(s) of action for these three drugs is controversial (Gray et al., 2003). The response to treatment varies among individuals, but to date, no biological marker for predicting treatment response has been identified (Ikeda and Kato, 2003). Several groups have been pursuing a genetic marker that can be used to predict treatment response to lithium. Several polymorphisms of the genes such as serotonin transporter (Del Zompo et al., 1999; Serretti et al. 2001), tryptophan hydroxylase (Serretti et al., 1999) and inositol polyphosphate 1-phosphatase (Steen et al., 1998), have been suggested to relate to the lithium response, but these findings are still controversial. Thus, clinicians select mood stabilizers only empirically.

We recently identified a functional single-nucleotide polymorphism, −116C/G in the promoter region of the *XBP1* gene, which alters the endoplasmic reticulum stress response. The functional impairment caused by this substitution can be rescued by treatment with valproate (Kakiuchi et al., 2003). Since lithium and carbamazepine did not improve the impairment caused by the −116G polymorphism in vitro, we hypothesized that lithium, the first-choice drug for bipolar disorder, is not effective for patients with a −116G/G genotype. To test this hypothesis, we investigated the possible association between the treatment response to lithium and *XBP1* genotype.

The subjects were 56 patients with bipolar disorder [aged 46.8 ± 13.4 yr (mean ± S.D.), 38 females and 18 males, 38 with bipolar I disorder and 18 with bipolar II disorder]. Their serum lithium concentrations were maintained at therapeutic concentrations (0.4–1.3 mM). When their serum concentration was less than 0.3 mM, it was considered as non-compliance. All subjects were Japanese and gave written informed consent to participate. The ethics committees of participating institutes approved this study. Consensus diagnosis by at least two senior psychiatrists according to the DSM-IV criteria (American Psychiatric Association) was made for each patient using an unstructured interview and by scrutinizing medical records. Response to maintenance lithium treatment was retrospectively assessed by reviewing the chart records. During maintenance treatment, use of certain additional drugs was allowed, but the addition of antidepressants or antipsychotics was regarded as a relapse.

The subjects were classified into three groups with regard to lithium response; these groups were as previously defined (Kato et al., 2000). In brief, lithium responders (responders) had no relapse during maintenance lithium treatment. Lithium partial responders (partial responders) showed decreased frequency or severity of relapse during maintenance lithium treatment compared to before the initiation of lithium treatment. Lithium non-responders (non-responders) had relapses during lithium treatment. Patients were excluded when their compliance was poor. Patients diagnosed as 'rapid cyclers' were regarded as non-responders. These clinical assessments were performed blind to *XBP1* genotype, which was determined by PCR direct sequencing (Kakiuchi et al., 2004).

Among 56 cases, 10 patients were judged as responders, 24 patients as partial responders, and 22 patients as non-responders. There was no significant difference in the duration of observation period among the three groups [responders, 30.1 ± 35.3 (mean ± S.D.) months; partial responders, 74.5 ± 86.0 months; and non-responders, 43.1 ± 47.0 months; $p = 0.13$, d.f. = 2, one-way ANOVA], nor between the responders and the combined group of partial and non-responders ($p = 0.21$, χ^2 test). Patients with the −116G/G genotype showed a significantly smaller proportion of responders, compared to −116C/C or −116C/G patients ($p = 0.049$, χ^2 test; Table 1).

Our results suggest that lithium may not be effective for bipolar patients with the −116G/G genotype in the promoter region of *XBP1*. This finding seems to be in accordance with our in-vitro data showing that only

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Table 1. Relationship between *XBP1* (−116) genotype and lithium response in bipolar disorder

Genotype	Responders	Partial responders (PR)	Non-responders (NR)
C/C	1	1	0
C/G	7	9	11
G/G	2	14	11
	Responders	PR and NR	
C/C or C/G	8	21	
G/G	2	25*	

* $p^* < 0.05$ by χ^2 test.

valproate could improve the functional impairment caused by the G/G genotype (Kakiuchi et al., 2003). However, the association between *XBP1* genotype and lithium response was not significant after Bonferroni correction of all possible comparisons. In addition, we did not observe a significant difference between genotypes when the combined group of partial responders and responders was compared with non-responders. Thus, it cannot be ruled out that this difference was a statistical false-positive, which resulted from the small sample size. A study using a larger number of subjects should be performed.

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Statement of Interest

The authors declare competing financial interests. RIKEN has a pending patent on the results of this paper.

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REVIEW ARTICLE

Genetic or epigenetic difference causing discordance between monozygotic twins as a clue to molecular basis of mental disorders

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Classical twin research focused on differentiating genetic factors from environmental factors by comparing the concordance rate between monozygotic (MZ) and dizygotic twins. On the other hand, recent twin research tries to identify genetic or epigenetic differences between MZ twins discordant for mental disorders. There are a number of reports of MZ twins discordant for genetic disorders caused by genetic or epigenetic differences of known pathogenic genes. In the case of mental disorder research, for which the causative gene has not been established yet, we are trying to identify the 'pathogenic gene' by comprehensive analysis of genetic or epigenetic difference between discordant MZ twins. To date, no compelling evidence suggesting such difference between MZ twins has been reported. However, if the genetic or epigenetic difference responsible for the discordant phenotype is found, it will have impact on the biology of mental disorder, in which few conclusive molecular genetic evidences have been obtained.

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Introduction

Evidence proving the etiological roles of genetic factors in schizophrenia and bipolar disorder was derived mainly from twin studies. While the monozygotic (MZ) twins have identical genotypes, dizygotic (DZ) twins share only 50% of their genotypes on average. Thus, higher concordance rate in MZ twins compared with DZ twins is a hallmark of the role of genetic factors in a disease. Using this classical approach, the concordance rate in MZ twins in schizophrenia and bipolar disorder was found to be significantly higher than that in DZ twins. However, in spite that many candidate loci and candidate genes were proposed and analyzed, these findings are not yet conclusive. *DISC1* (disrupted in schizophrenia 1), cloned from a break point of balanced chromosomal translocation linked with mental disorders in a large pedigree, may be only one exception. In this situation, an alternative or complementary approach to study the molecular basis of mental disorders has been pursued.

Since the MZ twins provide a valuable opportunity of studying the role of genetic factors, methodology used for the twin study has been continuously evolving. If we refer the classical twin research noted above as 'first-generation twin research', second-generation twin research may be the study to identify environmental risk factors causing discordance, or to identify endophenotypes associated with the disease. The former strategy was used, for example, to identify the role of birth complications in the etiology of schizophrenia.[1,2] Using the latter approach, decreased hippocampal volume in schizophrenia[3] was established as an intermediate phenotype.[4] These studies were based on an assumption that there is no difference of genomes between MZ twins.

The first study in which the presence of genetic or epigenetic difference was pre-assumed in MZ twins was reported by Polymeropoulos *et al.*[5] Since then, several groups also have tried to identify genetic or epigenetic difference between MZ twins.[6–10] These studies seem to have induced a paradigm shift to the third-generation twin research, from focusing on the higher concordance rate in MZ twins, to looking for the genetic or epigenetic difference between MZ discordant twins.[11]

In this review, the theoretical concept of such study to search for genetic or epigenetic difference between MZ discordant twins is explained and its application to schizophrenia and bipolar disorder is summarized.

What is epigenetics?

Epigenetics is defined as the study of mitotically or meiotically heritable variations in gene function that

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cannot be explained by changes in DNA sequence.[12] For such mechanisms, methylation of the cytosine residue in the DNA molecule, and acetylation, methylation, and other modifications of histones have been well described. These modifications stably affect gene expression through alteration of DNA–protein interaction. These events refer to the inheritance from a parental cell to daughter cells. With regard to the inheritance from parents to the offspring, DNA methylation status is once totally reprogrammed at the fertilization. However, the DNA methylation can be conserved throughout the process of fertilization in some special cases.[13,14]

As evidenced by the study of clone animals, a different phenotype can be produced from the genomes having completely the same DNA sequences. The phenotypic difference between cloned animals and donor should have arisen from epigenetic differences. Similarly, we can postulate that epigenetic difference is responsible for the discordance of phenotypes between MZ twins.

Tsujita *et al*[6] showed that electrophoresis patterns of the genomic DNA digested with a methylation-sensitive restriction enzyme were different between MZ twins discordant for schizophrenia. This finding raised a possibility that phenotypic discordance between MZ twins may be caused by some epigenetic difference rather than 'genetic' difference, that is difference of DNA sequence.[15] This does not always preclude a possible effect of environmental factors in discordant phenotypes of MZ twins, since environmental factors may affect the DNA methylation status.[16–18]

Cause of discordance between MZ twins

A number of case reports have revealed that phenotypic discordance between MZ twins can be arisen from several kinds of genetic or epigenetic differences, which are inter-correlated each other.[19] For example, expansion of triplet repeat can alter DNA methylation status, and reduced DNA methylation of transposon can cause transposition and finally causes disruption of a gene. Thus, dichotomy of 'genetic' and 'epigenetic' is difficult, and only a tentative classification is given below.

Genetic

Point mutation To our knowledge, there are only two cases of MZ twins, in which a point mutation causative for the discordance of a disease was identified. One case is the MZ twin pair discordant for Darier's disease. Darier's disease is an autosomal dominantly inherited dermatological disease caused by mutations in the *ATP2A2*, encoding endoplasmic reticulum Ca^{2+}-ATPase.[20] In this pair of MZ twins discordant for Darier's disease, a point mutation of *ATP2A2* was identified. This mutation, G23E, was not found in the healthy co-twin and their parents, suggesting that it was a *de novo* mutation. In the other report, a mutation, Glu92X, in interferon regulatory factor 6 (*IRF6*) was identified in a MZ twin having Van der Woude syndrome characterized by the cleft lip and palate with lip pits, whose healthy co-twin did not have this disease.[21] This mutation was not found in healthy co-twins and their parents. They reported this finding as one of the evidences to prove the causative role of *IRF6* in Van der Woude syndrome.

This kind of genetic difference may happen at or after the twinning. In these reports, however, it cannot be ruled out that both twins had the mosaic mutation whose percentage is different.

Chromosomal Abnormality Chromosomal abnormality is sometimes seen as mosaicism. Since the percentage and the tissue distribution may differ between the MZ twins, mosaicism of chromosomal abnormality can cause discordance between MZ twins. Machin[19] extensively reviewed the MZ twin cases with discordant phenotype caused by chromosomal abnormalities. They reviewed 16 pairs of MZ twins discordant for Turner syndrome. In addition, other discordant MZ twins, such as trisomy 21 and trisomy 13, were also reported. After that, a number of cases of MZ twins having discordant phenotypes arisen from *de novo* mosaic chromosomal abnormalities were reported. These include Turner syndrome,[22] skin pigmentation,[23] minor anomalies,[24] and sex phenotypes.[25,26]

Since there are a number of case reports of MZ discordant twins caused by mosaicism of chromosomal abnormality, it may be a frequent cause of discordance in MZ twins. However, it might be biased by methodology. While discordance of DNA sequence is difficult to identify, the methods to detect chromosomal abnormality are well established and can be tested in clinical settings. This might be the reason of higher number of case reports on discordance caused by chromosomal abnormality.

Phenotypic discordance is known in the MZ twins with chromosome 22q11 deletion. This could be explained by epigenetic mechanism,[27] rather than difference in chromosomal abnormality.

Mitochondrial DNA (mtDNA) heteroplasmy

In the mitochondrial encephalomyopathies, mutated mtDNA usually coexists with wild-type mtDNA. This phenomenon is referred to as heteroplasmy. Clinical phenotype alters with the ratio and tissue distribution of the mutation. *De novo* heteroplasmic 11778 mutation of mtDNA reportedly caused the discordance of Leber's disease in MZ twins.[28] An MZ twin pair discordant for chronic progressive external ophthalmoplegia was also reported. In this pair, both twins had a small amount of 4115 base pair deletion in muscles, but the affected twin had much higher amount of deletion.[29] Discordant phenotypes due to uneven amount of heteroplasmic mutations were also found in DZ twins with myopathy, encephalopathy,

lactic acidosis, and stroke-like episodes (MELAS)[30] and myoclonic epilepsy with ragged-red fibers (MERRF).[31] However, effects of nuclear genes could not be ruled out in these DZ twin cases.

Triplet repeat Triplet repeat expansion is known to cause inherited disorders, such as Huntington's disease.[32] Triplet repeat expands during meiosis due to slippage of the DNA polymerase. This causes expansion of the repeat from generation to generation, which is known as the molecular basis of anticipation. On the other hand, triplet repeat also expands during somatic cell mitosis, which causes mosaicism of the length of the repeat. Thus, length of the triplet repeat may be different between MZ twins.

Phenotypic discordance of fragile X syndrome caused by the difference of length of CGG repeat in the 5′-UTR of the *FMR-1* gene was reported in a male MZ twin pair.[33] Repeat expansion of *FMR-1* causes methylation of the CpG island, and results in the inactivation of this gene.

Epigenetic

X-chromosome inactivation While males have only one X chromosome, females have two X chromosomes. To avoid the unbalance of expression levels of the genes on the X chromosome between sexes, one of two X chromosomes in females is inactivated in humans. In females, the X chromosome of paternal origin is once inactivated during embryogenesis. After being once reactivated, one of the X chromosomes is randomly inactivated. Since this phenomenon randomly occurs during the development, it causes heterogeneity of X chromosome inactivation among tissues. Due to the mosaicism of X chromosome inactivation, X-linked genetic diseases are sometimes discordant between female MZ twins.[19] Discordant phenotypes were reported in X-linked mental retardation, Duchenne type muscle dystrophy,[34,35] red–green color blindness, Hunter disease,[19] and Fabry's disease.[36] For example, in female MZ twins discordant for fragile X syndrome, the length of CGG repeat did not differ, but its methylation status was different. The normal allele of FMR-1 was methylated and inactivated in the affected twin, while mutant allele was methylated and inactivated in healthy twin.[37]

Loss of imprinting Genomic imprinting is a phenomenon in which one of two alleles, from maternal or paternal origin, is inactivated by DNA methylation. Methylated and inactivated allele is referred to as 'imprinted'. Many of the imprinted genes are related to the cell growth, and loss of imprinting is known to be one of the causes of cancer.

Beckwith–Wiedemann syndrome (BWS) is a congenital disorder characterized by hyperplasia of organs and tumor susceptibility, caused by several different genetic or epigenetic mechanisms such as chromosomal abnormalities, point mutations, and loss of imprinting of the genes on 11p15, the most studied imprinted chromosomal region. Since the phenotype is not so severe, it does not always cause clinical problems.

Among female MZ twins, prevalence of BWS is higher than expected, and they are mostly discordant. *KCNQ1* (voltage-gated potassium channel 1) in the imprinted region on 11p15 is regulated by an antisense RNA gene, *KCNQ1OT1* (*KCNQ1*-overlapping transcript 1), which is also regulated by imprinting. Among five pairs of MZ twins discordant for BWS, lack of DNA methylation of *KCNQ1OT1* in fibroblasts was observed only in the affected twins.[38] Thus, loss of imprinting is the cause of BWS in these cases. A similar finding, discordant DNA methylation, was also seen in lymphocytes. However, it is of note that loss of imprinting was observed in both twins in some of the MZ twin pairs. This could be due to the blood transfusion *in utero*. Since lymphocytes with loss of imprinting have enhanced growth, these cells may be selectively amplified in the healthy co-twin.

It has been postulated that loss of imprinting itself caused twinning. This can explain the higher rate of twins in BWS.

Recently, it was reported that children born by *in vitro* fertilization (IVF) is more frequently seen in BWS (4%, 6/149) compared with general population (less than 1.2%).[39] All were sporadic cases with loss of imprinting of differentially methylated region (KvDMR1) within the *KCNQ1*.[40] In the other study, four of 37 cases with BWS was born by IVF, while IVF was identified as the method of conception in only one of 148 matched controls.[41] Angelman syndrome and retinoblastoma are also reportedly associated with IVF.[42] These findings suggest that IVF is a risk factor of abnormality in DNA methylation.

Mobile elements Approximately 40% of the human genome is comprised of retroelements such as retrotransposon, transposon, and endogenous retrovirus. While retrotransposon transposes after transcribed into RNA, transposon transposes as DNA. In the case of endogenous retroviruses, expressed RNA is reverse transcribed and assembled into the genome by reverse transcriptase. Since transposition of transposon was first discovered in corn, transposition of transposon or retrotransposon is known to be active in plants.

Kazazian *et al*[43] first described a disease caused by the transposition of mobile element in humans. They found the insertion of the mobile element, LINE1 (L1), into the factor VIII gene, in two of 240 unrelated patients with hemophilia A. *De novo* insertion of Alu into an intron of *NF1* causing neurofibromatosis was also reported.[44] Such mechanism could explain the discordance between MZ twins in some cases. Since transposition of transposon is regulated by DNA methylation, abnormal DNA methylation of transposon may be associated with activity of transposition. Thus, transposon is related to both epigenetic and genetic mechanisms.

A possible role of human endogenous retrovirus (HERV) is also suggested in schizophrenia. Karlsson *et al*[45] examined the expression levels of endogenous retroviruses in cerebral spinal fluid (CSF) in patients with schizophrenia and found that their expression is higher in the CSF of schizophrenic patients. HERV-W-related RNA was detected in plasma of patients with schizophrenia.[46]

Retroviruses Retrovirus infection, that can be integrated into genome, could also explain the discordance between twins.[47,48]

Relevance of epigenetics in mental disorders
The field of epigenetics is too broad to be covered by this short review on discordant twins. Epigenetic regulation of gene expression is used in a wide variety of biological functions, such as tissue-specific gene expression, differentiation of cells, epigenetic memory, suppression of retroelements, and genomic imprinting. If epigenetics is related to mental disorders, all of them may be relevant. Although tissue-specific gene regulation and epigenetic memory need to be studied in the brain, it is difficult to obtain brain samples from discordant twins. Practically, we can obtain only blood cells or other non-neuronal cells from discordant twins. Abnormalities of imprinting might be detected in non-neuronal cells, according to the case of BWS.[38] Thus, we mainly focused on imprinting.

Bipolar disorder Petronis proposed that epigenetic mechanisms might be relevant to the pathophysiology of bipolar disorder based on several lines of evidence such as the relatively high degree of discordance in MZ twins, characteristic age at onset, parent-of-origin effects (POE), and fluctuation of the disease course.[49] Especially, POE in the transmission of bipolar disorder suggests the role of genomic imprinting. In bipolar disorder, several reports suggested the involvement of POE.[50,51] POE refers to the phenomenon that the sex of the parent transmitting the disease affects the severity or age at onset of the offspring. These include higher number of affected mothers compared with affected fathers, higher prevalence rate of the disorder among maternal relatives compared with paternal relatives, and lower age at onset in the proband with affected father compared with those with affected mother, and higher number of maternally inherited pedigrees compared with paternally inherited pedigrees.[50,51] However, some of these findings were not replicated.[52] On the other hand, linkage of bipolar disorder with chromosome 18 (18q22 and 18p11) was observed only in the paternal transmission,[53] which was replicated in several studies.[54] POE was also reported in other chromosomes such as 6q,[55] 13q12, and 1q41.[56] POE is seen in the diseases caused by the imprinted genes. Thus, imprinted genes on these chromosomes are suggested to have a role in the etiology of bipolar disorder.

It is also suggested that epigenetics may be relevant to bipolar disorder, since some drugs can affect the DNA methylation. Among the mood stabilizers, valproate is known to be a histone deacetylase (HDAC) inhibitor. Histone acetylation is coupled with DNA methylation and plays a role in the epigenetic regulation of gene expression. If inhibition of HDAC by valproate is related to its efficacy, it may suggest that such epigenetic gene regulation may be relevant to bipolar disorder.[57] On the other hand, S-adenosyl methionine (SAM) is known to be effective for bipolar depression.[58] SAM supplies methyl residue in DNA methylation reaction, and was reported to enhance DNA methylation *in vitro*.[59] The effect of SAM on bipolar disorder may also be mediated by alteration of DNA methylation status. However, such evidence is too circumstantial to prove the role of epigenetic factors in this disorder.

Schizophrenia Gottesman and Bertelsen[60] examined the risk of schizophrenia in the offspring of MZ twins discordant for schizophrenia. The risks in the offspring of twins did not differ, suggesting that the cause of discordance is not heritable, but rather environmental or epigenetic. The possible role of epigenetics has been postulated in pathophysiology of schizophrenia from several clinical features such as inheritance pattern, discordance of MZ twins, and fluctuating course.[61]

One of the findings suggestive of POE, higher number of affected mothers compared with affected fathers, is also seen in schizophrenia. In paternally transmitted cases, anticipation, lower age at onset in offspring compared with the parent, was more prominent than maternally transmitted cases.[62,63] However, this finding is not replicated in other studies and might be due to selection bias.[64] Twin is one of the risk factors for schizophrenia.[65] This has been regarded as reflecting birth complications such as anoxia. However, this could also be explained by epigenetic abnormality as discussed above.

Costa and colleagues[66] have been studying the epigenetic animal model of schizophrenia. They applied methionine to the mice and found that reelin is hypermethylated in these mice and these mice showed altered behavior resembling schizophrenia.

Search for epigenetic discordance between MZ twins
To date, a number of studies have been performed to reveal genetic or epigenetic difference between MZ twins discordant for mental disorders.

Schizophrenia
DNA sequence: To date, the difference of DNA sequence between the MZ twins discordant for schizophrenia has been searched for. No difference of genotypes was found between twins, by genotyping 94 microsatellite markers in five pairs of MZ twins discordant for schizophrenia.[5] No difference of DNA sequence between discordant MZ twins was found, by random amplification of polymorphic DNA

(RAPD) method using 10 retroviral related primers as well as eight random primers,[67] or genomic representational difference analysis (RDA) using six different enzyme digest representations.[10] No difference of repeat length was found between discordant MZ twins in the CAG repeat in atrophin-1, the causative gene for dentatorubral-pallidoluysian atrophy (DRPLA).[68] The CAG/GAA repeat was not expanded in discordant MZ twins using the repeat expansion detection (RED) method.[7]

More recently, Nguyen *et al*[8] separated the DNA fragment obtained from the sequences surrounding the CAG repeat using the high-performance liquid chromatography-based method, targeted genomic differential display (TGDD), and reported that the difference of peaks was larger between the discordant twins compared with the concordant twins. However, the locus causative for this difference has not been identified yet.

DNA methylation: Deb-Rinker *et al*[47,48] analyzed the lymphocytes obtained from MZ twins discordant for schizophrenia using RDA and found a new retrovirus, which was expressed only in the affected twin. They named this as schizophrenia-related retrovirus-1 (SZRV-1) and SZRV-2. Loss of DNA methylation of SZRV-2 was detected in this patient with schizophrenia. It has not been established whether or not SZRV-2 is a cause of schizophrenia in this case, or in general.

Tsujita *et al*[6] used the restriction landmark genome scanning (RLGS) method[69] to screen the difference of genomes between MZ twins discordant for schizophrenia.[6] In this method, genomic DNA was digested with a methylation-sensitive restriction enzyme, *Not*I, the fragments were analyzed by two-dimensional electrophoresis, and the pattern seen in the gel was compared between twins. They identified two spots showing different intensities between twins, suggesting genetic or epigenetic difference. The results obtained by RLGS might reflect the difference of DNA sequence, but more likely reflected difference of DNA methylation.

Petronis *et al*[12] have been studying the possible role of epigenetic factors in mental disorders. They analyzed the DNA samples obtained from two pairs of twins discordant or concordant for schizophrenia and examined the DNA methylation status of the upstream region of the dopamine D2 receptor.[9] They examined the difference of the DNA methylation status between twins, which was named 'epigenetic distance'. They reported that epigenetic difference was larger between discordant twins compared with concordant twins.

McDonald *et al*[10] used genomic RDA to identify discordance of DNA sequence or DNA methylation between twins discordant for schizophrenia. For two of six enzyme digest representations, methylation-sensitive enzyme, *Hpa*II, was used. They identified an apparent difference of one gene when two enzymes, *Hpa*II and *Mbo*I, were used for digestion. However, this DNA sequence was derived from bacterial genomic fragment of *Pseudomonas aeruginosa*, suggesting possible contamination. They concluded that there is no genetic or epigenetic difference between MZ twins discordant for schizophrenia.

Gene expression: Using the DD method, Friedhoff *et al*[70] cloned a new gene of unknown function from lymphocytes of MZ twins discordant for schizophrenia. The expression level of this gene, *oksc12b*, was lower in affected twin compared with healthy co-twin. However, the expression level of this gene in the brains of patients with schizophrenia did not differ from controls, suggesting no pathophysiological significance.[71]

Summary of the findings: In summary, difference of DNA sequence has been searched for in MZ twins discordant for schizophrenia, but most of the studies did not support the genetic difference. The results in the difference in DNA methylation seem a little more promising. However, no conclusive evidence has been obtained yet. Gene expression difference was also searched for, but any effort to reveal genetic or epigenetic difference has not been taken yet.

Bipolar disorder Compared with schizophrenia, few molecular genetic studies of discordant twins have been published in bipolar disorder, possibly because MZ twins completely discordant for bipolar disorder are quite rare. Although the summary of published studies reported that the concordance rate of bipolar disorder in MZ twins is approximately 70%, this largely depends on the definition of concordance. For example, in the study by Bertelsen *et al*,[72] 46 of 69 MZ twins were completely concordant. However, 14 of other 23 were partly concordant, that is, the others had some mental disorder or had committed suicide. Thus, MZ twins completely discordant for bipolar disorder are rarely seen.

Gene expression: In an attempt to identify the genetic or epigenetic difference between twins, the authors examined two pairs of MZ twins discordant for bipolar disorder and a pair of healthy twins.[73] Intracellular calcium response was different between the lymphoblastoid cells obtained from discordant twins. By DNA microarray analysis, two genes, *XBP1* and *GRP78* (*HSPA5*), both of which have pivotal roles in endoplasmic reticulum (ER) stress signaling, were commonly downregulated in affected twins.[73] *XBP1* is located at 22q12, the common linkage locus for bipolar disorder and schizophrenia. *GRP78* is regulated by *XBP1* and induced by valproate.

Based on this finding in twins, we further examined the role of this pathway in pathophysiology of bipolar disorder by case–control studies. Response of *XBP1* and *GRP78* to ER stress was attenuated in bipolar disorder. This difference was partly explained by the functional polymorphism in the promoter of *XBP1*, named −116C/G. The functional disturbance caused by −116G was improved not by lithium but by valproate. The genotype was associated with treatment response to lithium in Japanese bipolar patients.[74] This polymorphism was associated with

bipolar disorder in Japanese case–control samples. Although it was also associated in Caucasian trios obtained from NIMH in the first report, it was not replicated in an extended NIMH trio samples, triads from Bulgaria and the UK, as well as case–control samples from various European populations.[75] A case–control study in Chinese also did not support the association.[76]

On the other hand, it was recently reported that this polymorphism was associated with schizophrenia in Japanese[77] and Chinese.[76] The other gene in this cascade, *GRP78*, was also associated with bipolar disorder, suggesting that there are more than one functional polymorphisms affecting ER stress response.[78]

DNA sequence and DNA methylation: In these MZ discordant twins, the genotype was the same between the twins; one pair had C/G and the other pair had G/G. Thus, the cause of discordance was not due to this polymorphism. No other difference of genomic DNA sequence could be identified in the *XBP1* gene. We also quantified the copy number of *XBP1* using quantitative genomic PCR, but there was no difference. We further analyzed the DNA methylation status of the CpG island of *XBP1*. However, the CpG island was not methylated in both affected twins and their healthy co-twins. Thus, the discordance of endophenotype, reduction of *XBP1* expression, was not caused by genetic or epigenetic difference of *XBP1* itself.

Although we selected the genes commonly altered in both of these twins, the primary discordance may not be caused by these genes. Especially, it is of note that one of the discordant twins is also discordant for several somatic diseases, including ossification of the posterior longitudinal ligament (OPLL).[79] Thus, there might be discordance in some gene upstream to the molecular cascade of bipolar disorder and that of OPLL. We are now searching for the epigenetic discordance causing altered gene expression in these discordant twins.

Methodological considerations and future strategies

Subjects To search for the genetic or epigenetic difference between discordant twins, identification of a suitable twin pair would be the most important point. Firstly, if one of the twins had onset of the disease just several years before, they may not be truly discordant, since the other twin may have disease onset soon. Thus, the ages of the subjects should be 10 or 20 years after the age at onset of the affected twin. Secondly, in the case with marked environmental insult such as infection, perinatal complication, or head trauma, such cases may not be suitable for the search of genetic or epigenetic difference. Thirdly, phenotypic discordance should be complete. In the case of mental disorders, phenotype definition is not a dichotomy of 'disease' and 'healthy'. If the index case had schizophrenia and the other twin had schizoid personality disorder, they are incompletely discordant, and the possibility of genetic or epigenetic difference may be smaller than completely discordant cases. Fourthly, discordance of comorbid genetically determined somatic disease or intermediate phenotype might also be a hallmark of genetic or epigenetic difference.

Tissue The ideal source for epigenetic analysis would be the brain tissue, but it is practically impossible in the study of discordant twins. Peripheral blood cells are usually used for this kind of study.

In the case of peripheral blood cell, effects of medication are difficult to control, since the affected twin is usually medicated with various psychotropic drugs, some of which can affect histone acetylation and DNA methylation. In addition, the subpopulation of white blood cells, such as granulocytes, B lymphocyte, or T lymphocyte, can be altered by mental status, hormones, or medication. In lymphocytes, difference of genome rearrangement status between cells can obscure other more important genetic difference.

Using lymphoblastoid cell lines, some of the above-noted problems, such as effects of drugs and cellular heterogeneity can be minimized. However, Epstein–Barr virus that is used for transformation may alter the DNA methylation status of some genes.[80] Transposon may become active during cell culture, especially when the DNA methylation inhibitor, 5-aza-deoxycitidine (5-aza-dC), is applied.[81] In both cases, the possible effects of blood transfusion *in utero* might obscure the difference between twins.[38]

Most of the above-noted problems can be overcome by using fibroblasts, which can also be cultured and stored. Although it is somewhat more invasive to perform skin biopsy than drawing blood, it does not cause pain and scar at all, when adequately performed.[82]

Methodology To identify the epigenetic discordance between twins, several methodologies could be used. Among these methods, RLGS is the most established method, although it is laborious. Although it had been difficult to identify the gene with DNA methylation difference responsible for the spot detected, the recent development of *in silico* RLGS has made it easier.[83] Microarray-based comprehensive analysis of CpG island would be an ideal method to screen the DNA methylation difference between discordant twins.[84]

We are currently searching for the DNA methylation difference using two different strategies. One is methylation-sensitive representative differential analysis (MS-RDA).[85] In this method, the genomic region with different methylation status between two genomes can be selectively amplified using methylation-sensitive restriction enzymes. We also applied 5-aza-dC to lymphoblastoid cells obtained from discordant twins to unmethylate all DNAs in these cells. Before and after the 5-aza-dC treatment, gene expression

patterns were examined by DNA microarray. Although this method has an apparent disadvantage of the difficulty of excluding false positives caused by secondary effect of drug treatment, this method has been successfully applied to the identification of hypermethylated CpG islands in cancer cells.[86] We checked the DNA methylation status of the candidate genes that upregulated after the 5-aza-dC treatment only in one of the twins, and found the differences in methylation status between MZ twins, although their pathophysiological significances remain elusive (Iwamoto *et al*, in preparation).

Conclusion

Search for genetic or epigenetic difference between MZ twins discordant for mental disorders might be a promising strategy to identify the genes responsible for mental disorders. Once the responsible mutation or epimutation was found in the affected twin, this information would become a clue to study the pathophysiology of mental disorders. Further studies are warranted to identify genetic or epigenetic difference between MZ twins responsible for discordant phenotypes.

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GENETICS OF BIPOLAR DISORDER

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CONTENTS

Summary

Many linkage loci and candidate genes have been reported in molecular genetic studies of bipolar disorder. However, none of these findings have been consistently replicated. Meta-analyses of linkage studies have also reported conflicting results. Among recently reported candidate genes, BDNF, G72, AKT1, GRIN2A, XBP1, GRK3, HTR4, IMPA2 and GABRA1 may have some importance. Study of the possible roles of epigenetics or analysis of genetic diseases, in which bipolar disorder is one of phenotypes, may also be promising. In addition to monoaminergic and intracellular signaling pathways, recent studies have revealed possible roles for mitochondrial dysfunction, for glutamatergic dysfunction and for the endoplasmic reticulum stress pathway.

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Introduction

Bipolar disorder, also known as manic-depressive illness, is characterized by recurrent manic and depressive episodes, with a lifetime prevalence of approximately 0.8% (1). The patient's social life is

severely disturbed by behavioral problems during manic episodes, and individuals with this disorder also have a high mortality rate due to suicide. Three mood stabilizers, lithium, valproate and carbamazepine, are known to be effective for the prophylactic treatment of this disorder.

The role of genetic factors in the pathophysiology of bipolar disorder has been established based on twin, adoption and family studies. Twin studies revealed a higher concordance rate in monozygotic twins compared with dizygotic twins. This clearly indicates that genetic factors contribute to the onset of bipolar disorder (1). Moreover, the much higher concordance rate in monozygotic twins as compared to dizygotic twins suggests that multiple genetic factors are involved. Two adoption studies also showed a higher prevalence rate of mood disorders in biological parents compared with nurturing parents (2, 3). Family studies also supported a role for genetic factors in this disorder.

Linkage studies

The main strategies used to explore the genetics of bipolar disorder have gradually evolved from parametric linkage study, association study of candidate genes using case–control analysis, family-based association studies, nonparametric linkage analysis and haplotype analysis of candidate genes. However, the history of the molecular genetics of bipolar disorder is just like a roller coaster, characterized by two extremes, optimism and pessimism (4).

Although the paper published in *Nature* (5) reporting the linkage of bipolar disorder with chromosome 11 caused optimism, decreased evidence of linkage after disease onset in several family members caused pessimism that linkage studies of bipolar disorder are not reproducible (6). This event, caused by the phenocopy phenomenon, was the first setback in the molecular genetics of bipolar disorder.

However, after nonparametric linkage analysis became popular, relatively consistent results were obtained. This facilitated the use of linkage analysis again, and resulted in optimism.

To date, more than 22 genome-wide linkage analyses have been reported, but no locus has been replicated in any linkage studies. However, several loci were replicated in two or more linkage studies. The meta-analysis of linkage studies showed the evidence for linkage with 13q and 22q (7), while the other meta-analysis using raw data of the linkage studies reported the weak evidence of linkage with 9p21-p13.3, 10p11.22-q22.1, 1p32.1-p22.1, 19q13.2-qter, 9p13.3-q21.32, 17p13-q22, 18p11.23-q12.1, 18pter-p11.23 and 14q23.2-q32.11 (8). However, there was no evidence for linkage with chromosomes 13 and 22 in the latter study. The reason for this inconsistency is unknown.

Association studies

Genes participating in monoaminergic neurotransmission were good targets for earlier candidate gene studies (9). However, the results of these studies showed inconsistent results. Such inconsistency can be interpreted as reflecting type I error due to the small number of subjects or to genetic heterogeneity. Thus, family-based association analysis began to be used in larger populations in which genetic heterogeneity should be taken into account.

Among the polymorphisms of monoamine-related genes, some were found to cause functional alteration and to be associated with bipolar disorder in two or more studies. These include monoamine oxidase A (MAO-A) (10–12), serotonin transporter (HTT) (5HTT) (12–15) and serotonin 2C receptor (HTR2C) (14, 16). Catecol-*O*-methytransferase (COMT) is also included in such genes, although association was found only for rapid-cycling bipolar disorder (17, 18). However, the odds ratio was not large and the effects of publication bias cannot be totally ruled out.

Among recently reported candidate genes, association with brain-derived neurotrophic factor seems to be the most promising. Association with the Val66Met polymorphism of brain-derived neurotrophic factor was simultaneously discovered by a comprehensive association analysis of 90 single-nucleotide polymorphisms (SNPs) from 76 genes (19) and another candidate gene analysis (20). After that, this polymorphism was found to affect activity-dependent secretion of brain-derived neurotrophic factor and to alter hippocampal function and memory. However, association of brain-derived neurotrophic factor with bipolar disorder was not replicated in three additional studies in Japanese (21, 22) and Chinese (23).

Accompanied by the progress of rapid and high-throughput genotyping methods, haplotype analysis of many SNPs using mainly intron SNPs has become popular. This strategy was found to be productive in schizophrenia research; however, it has

the disadvantage of being unable to identify the functional polymorphisms responsible for the association.

G72 was initially found to be associated with schizophrenia, but later found to be associated with bipolar disorder (24). The association of G72 with bipolar disorder has been replicated in several subsequent studies (25–27). There is another gene encoding antisense RNA, G30, in this region. G72 protein was suggested to interact with D–amino acid oxidase and to be related to metabolism of D-serin, a modulator of the NMDA receptor. However, no functional polymorphism has been identified in this gene.

Other genes that showed association with bipolar disorder in at least two ethnicities, but for which no functional polymorphism was identified, include inositol monophosphatase (IMPA2) (28, 29), GABA receptor α1 subunit (30, 31) and serotonin 4 receptor (32).

As high-throughput genotyping methods continue to advance, whole-genome association analysis will become increasingly popular.

Mitochondrial DNA

When considered together, altered energy metabolism in the brain (33), a higher number of maternally transmitted pedigrees compared with paternally transmitted pedigrees (34) and comorbidity of mood disorders with mitochondrial disorders (35) suggest that mitochondrial DNA (mtDNA) might have pathophysiological significance in bipolar disorder. We therefore hypothesized that altered calcium signaling due to mitochondrial DNA mutations/polymorphisms may play an important role in bipolar disorder (35).

Kirk *et al.* (36) sequenced whole mitochondrial genomes in 25 bipolar disorder probands with maternally transmitted pedigrees. Although they found no specific mtDNA polymorphisms associated with bipolar disorder, they found fewer closely related haplogroups in bipolar disorder, suggesting that some unknown mtDNA polymorphism affects vulnerability to this disorder. McMahon *et al.* (37) sequenced whole mitochondrial genomes in nine probands with maternally transmitted pedigrees. Among the four polymorphisms nominally associated with bipolar disorder, mtDNA 10398G>A was the only one altering amino acid sequence. We also screened mtDNA polymorphisms in 43 bipolar patients and found that the mtDNA 10398G>A polymorphism was associated with bipolar disorder (38). This polymorphism was observed to alter intracellular calcium signaling (39).

Triplet repeat expansion

In bipolar disorder pedigrees, a phenomenon called "anticipation," *i.e.*, age at onset is lower in lower generations, has been observed (40). This phenomenon could be explained by trinucleotide repeat expansion. Using the repeat expansion detection method, expanded trinucleotide repeats of two genes, SEF2-1B at 18q21.1 and ERDA1 at 17q21.3, were identified in bipolar patients (41). Of these, SEF2-1B was reported to be expanded in bipolar disorder; however, subsequent studies did not support this association (42–44).

An approach from DNA microarray

Niculescu *et al.* (45) identified G-protein–coupled receptor kinase 3 as the gene upregulated after amphetamine administration using DNA microarray. They subsequently found that this gene was associated with bipolar disorder (46). However, no functional polymorphism has yet been identified.

Using pooled RNA samples from 10 bipolar patients and 10 controls, postmortem brain cDNA microarray analysis revealed a decrease in TGF-β1 in brains from bipolar patients (47). A study using serial analysis of gene expression (SAGE) reported an increase in NFκB (48). The downregulation of neuropeptide Y in the postmortem brains of schizophrenics identified by DNA micoroarray was also found in bipolar disorder (49).

We performed comprehensive DNA micoroarray analysis of the 50 postmortem brain samples, including 11 patients with bipolar disorder, obtained from Stanley Foundation Brain Bank (50). These results suggested that downregulated genes included receptors, channels and transporters, while upregulated genes included chaperones and stress proteins. Differentially expressed genes included a number of genes related to Ca^{2+} signaling, such as GRIK1, HTR2C, CACNA1A, GRM1 and LIM. Among the genes altered in the postmortem brains, differential expression was confirmed in lymphoblastoid cells for LIM and HSPF1. These findings suggest that this differential expression may reflect a genetic association (51). LIM encodes an adaptor protein linking N-type Ca^{2+} channel and protein kinase C (PKC). On the other hand, HSPF1 is a

molecular chaperone transporting proteins from mitochondria. This was increased both in postmortem brains and lymphoblastoid cells, which might reflect an upregulation secondary to mitochondrial dysfunction.

DNA microarray analysis of lymphoblastoid cells from lithium-responsive bipolar disorder patients identified that the α_{1B}-adrenergic receptor was upregulated in patients, but it decreased after lithium treatment (52).

We performed gene expression analysis using lymphoblastoid cells from two pairs of monozygotic twins discordant for bipolar disorder and found that two genes in the endoplasmic reticulum stress response pathway, XBP1 and HSPA5 (GRP78), were commonly downregulated (53). Case–control analysis revealed decreased endoplasmic reticulum stress response in lymphoblastoid cells from bipolar disorder patients. We found a polymorphism in the promoter region of XBP1, -116C>G, losing a binding site of XBP1 protein. This polymorphism altered the endoplasmic reticulum stress response and was associated with bipolar disorder in Japanese and NIMH pedigrees. Reduced endoplasmic reticulum stress response in bipolar disorder patients could be explained at least partly by this polymorphism. The impaired endoplasmic reticulum stress response due to this polymorphism was improved not by lithium but by valproate. These findings suggest that XBP1 polymorphism is a risk factor for bipolar disorder and could become a marker for treatment response. However, Cichon *et al.* (54) examined this polymorphism in a larger number of families and case–control samples and found that the -116 polymorphism of XBP1 was not associated with bipolar disorder. Another group also reported lack of association of this polymorphism with bipolar disorder (55). Thus, association of this polymorphism with bipolar disorder is not currently supported by clinical studies. We found that polymorphisms of HSPA5 (GRP78) were also significantly associated with bipolar disorder in Japanese but not in NIMH pedigrees (56). This finding suggests that there might be several functional polymorphisms in the endoplasmic reticulum stress response pathway which might confound the association analysis.

Response to maintenance lithium treatment was associated with the XBP1 polymorphism in one study (53). If this finding is replicated, this polymorphism could be used as a marker for treatment response. The locus of XBP1, 22q, is a common linkage locus to bipolar disorder and schizophrenia. Association of this polymorphism with schizophrenia was reported from two groups, including ours (57, 58).

Recently, Konradi *et al.* (59) performed gene expression analysis in the postmortem brains of bipolar disorder patients and found overall downregulation of mitochondria-related genes, which is compatible with our mitochondrial dysfunction hypothesis (35). However, gene expression of mitochondria-related genes could be affected by agonal factors (60) and needs further confirmation.

Overall downregulation of oligodendrocyte-related genes, initially reported in schizophrenia, has also been observed in bipolar disorder (61).

Nakatani *et al.* (62) performed comprehensive gene expression analysis of the hippocampus and cerebral cortex in an animal model of depression involving learned helplessness in rats. They found downregulation of serotonin 2A and IP3 receptors in the cerebral cortex, which may be relevant to the biology of bipolar disorder. Among the differentially expressed genes, they found downregulation of LIMKI, which has a LIM domain similar to LIM. The only gene altered in both of these two brain regions was NDUFV2, a gene encoding a subunit of mitochondrial complex I. NDUFV2 is located on 18p11, one of the replicated linkage loci. We found that the haplotype containing a functional polymorphism (-602A>G) that alters promoter activity was associated with bipolar disorder in Japanese and NIMH pedigrees (63, 64). Recently, NDUFV2 was found to be downregulated in the postmortem brains of patients with bipolar disorder (65). Taken together, these findings suggest that NDUFV2 plays an important role in the pathophysiology of bipolar disorder.

Relationship with schizophrenia

The possibility that bipolar disorder shares genetic vulnerability with schizophrenia has been suggested. Linkage analyses have suggested linkage of these two disorders with common loci such as 1q, 10q, 13q11, 18p11 and 22q11 (66). A report of the linkage of psychotic bipolar disorder with 13q and 22q also supports this finding (67). However, an extensive meta-analysis of linkage studies of bipolar disorder and schizophrenia has not shown commonly linked loci (68).

Association studies have also shown that several genes are associated with both of these two

disorders, *i.e.*, G72 (24), GRIN2A (69), AKT1 (70), IMPA2 (28, 29) and XBP1 (53). GRIN2A encodes the 2A subunit of the NMDA receptor. Association of bipolar disorder with GRIN1 (NMDA subunit 1) has also been reported (71). It is noteworthy that these glutamate-related genes are associated not only with schizophrenia but also with bipolar disorder. Recent findings suggest glutamatergic dysfunction in bipolar disorder. For example, mood stabilizers inhibit trafficking of AMPA receptors (72), and non-NMDA glutamate receptors GRIK1 and GRM1 were downregulated in the postmortem brains of bipolar disorder patients (50). This evidence suggests a possible role for alterations in glutamatergic neurotransmission in bipolar disorder.

A twin study reported on monozygotic twins, one of which had schizophrenia and the other bipolar disorder (73). Studies of neuroimaging, psychophysiology and neuropsychology have also showed common findings in these two disorders, *e.g.*, ventricular enlargement, increased dopamine D2 receptor binding potential, decreased D1 receptor binding potential, and decreased volume of the temporal pole and anterior cingulate (61). These results suggest that bipolar disorder and schizophrenia share a common molecular pathology.

Epigenetics

Since the results of genetic association studies are not consistent across studies, it has been postulated that epigenetic factors may also contribute to the disease (74). One of the reasons that we can postulate a role for epigenetics in bipolar disorder is the possible influence of parent-of-origin effect in this disorder. Parent-of-origin effect refers to the phenomenon in which the gender of the transmitting parent affects the onset and severity of the disease in offspring (75). The influence of parent-of-origin effect was reported in the linkage analysis of chromosome 18 in bipolar disorder (76). This phenomenon is observed when genomic imprinting is involved. Genomic imprinting is a phenomenon in which a maternally or paternally transmitted allele is inactivated by DNA methylation, and hemiallelic expression is therefore observed. Many imprinted genes are related to development, and some are related to behavior in animals (77). Thus, imprinted genes on chromosome 18 might be good targets for further study.

Pharmacological evidence also supports the hypothesis that DNA methylation is involved in the pathophysiology of bipolar disorder (78). Valproate is a histone deacetylase inhibitor and thus affects DNA methylation, while S-adenosyl methionine, which supplies methyl residue in DNA methylation reaction, is effective for bipolar depression.

Overall, there are relatively few studies on the epigenetics of bipolar disorder.

Bipolar disorder accompanied by genetic disease

Chronic progressive external ophthalmoplegia is an adult-onset, relatively mild mitochondrial myopathy caused by multiple deletions of mtDNA. It has been reported that autosomal dominantly inherited chronic progressive external ophthalmoplegia sometimes accompanies depression (79). Over the past several years, three autosomal genes responsible for chronic progressive external ophthalmoplegia have been identified. Among them, a mutation of adenine nucleotide translocator 1 (ANT1) was associated with a phenotype characterized by bipolar disorder and chronic progressive external ophthalmoplegia in all affected family members (80). Comorbidity of depression or bipolar disorder was reported in the families with mutations in the two other genes, polymerase gamma (81) and Twinkle (79, 82).

Wolfram disease is an autosomal recessive disorder characterized by many somatic symptoms such as hearing loss and diabetes. Accumulation of multiple deletions of mtDNA in the brain is reported in this disease (83). Patients with Wolfram disease frequently have bipolar disorder or depression (84). In addition, a higher frequency of depression and suicide was reported in nonaffected carrier of Wolfram disease mutations (85). These findings suggest that accumulation of multiple deletions of mtDNA may be one of the causes of bipolar disorder. Furthermore, we found that the 4977bp deletion of mtDNA was increased in lymphocytes (86) and in postmortem brains (87) in some cases. This finding further supports a role for mtDNA deletions in the etiology of bipolar disorder.

Another genetic disease linked with bipolar disorder is Darier's disease. Since the responsible gene, ATP2A2, encodes endoplasmic reticulum Ca^{2+}-ATPase, the relationship between bipolar disorder and ATP2A2 mutation has drawn attention (88).

Relationship with chromosomal abnormalities

Cases of bipolar disorder with chromosomal abnormalities suggest a possible relationship be-

tween bipolar disorder and several chromosomal regions: 11q21-25 (balanced translocation), 15q11-13 (deletion), 21q22 (trisomy), Xq27-28 (fragile X), 1q31-32 (fragile site) and 22q11-13 (deletion) (89).

DISC1 was cloned from the breakpoint of the balanced chromosomal translocation, (1;11)(q42.1;q14.3), which was linked with depression, schizophrenia and bipolar disorder (90). Recently, a role for DISC1 in neurite extension and localization to mitochondria has been reported (91). DISC1 may be related to the common pathophysiology of schizophrenia and bipolar disorder.

A family linkage of the balanced translocation t(9;11)(p24;q23) with mood disorders (five family members with bipolar disorder and one with recurrent major depression) was also reported (92). In this family, DIBD1, a gene cloned from the breakpoint 11q23, encoded mannosyltransferase, participating in *N*-glycosylation. DIBD1 expressed in the brain and a missense mutation, V289I, altering well-conserved amino acid, was identified. Although this was not associated with bipolar disorder, a weak linkage with an intron polymorphism was seen. This interesting gene needs further study.

Conclusion

As described above, the molecular genetic study of bipolar disorder has accelerated in recent years. DNA microarray and high-throughput genotyping methods have facilitated such study. In addition, the number of researchers investigating mental disorders has increased considerably, further promoting the possibility of advancement in this field. Hopefully, the current optimism concerning the future of the molecular genetic study of bipolar disorder will not end as a daydream.

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