The present study consisted of two separate analyses of answers to questions of language development for the two independent samples. Therefore, the results of these respective samples may not be linked directly. This is the most significant limitation of this study. Nevertheless, the two sets of data showed two common results that have often been reported in the language development of twins. Twins tend to lag behind singletons in terms of language development and this tendency is more marked in male twins.

The age of twins in the maternal associations group varied, and not all of them had reached the stage of childhood at which language development is complete. The author used these subjects as a sample that more adequately represents the general twin population, given that the twins of the school applicants group were considered to represent more selection bias with regard to total developmental items including those of language development. The twins in the school applicants group had already completed the basic language development of childhood. Therefore, the author used this group to analyze total language development throughout childhood by means of maternal objective estimation of their children.

According to the interviews with mothers, the following advice was frequently given upon health examinations. 'Because your children are twins, language delay, compared with the development in singletons, is a natural event', or 'Your children will catch up with singletons sooner or later. Therefore, do not worry'. Surely this advice offers temporary comfort to mothers, but provides no essential solution. Mothers may feel anxious over the results of two types of comparison: comparison of twins with singletons and comparison between two their children. Concern about language development is one of the most common and most serious questions about their children that parents, especially those of twins, bring to their pediatricians or public health nurses. Although a substantial proportion of language delays in twins seemed to resolve themselves in the preschool or early school years, no epidemiologic data showing this is available in Japan. According to the present results, surely some twins are delayed, compared with singletons, in terms of language development throughout early childhood. Nevertheless, most of this delay disappears at least by 11 or 12 years of age, which the author and the co-interviewer ascertained in the medical interview with mothers of twins in the school applicants group. By a comparison within twin pairs, about 30% of DZ pairs were reported to show a clear difference in language development. In the situation of a medical examination of twins, medical staff should be aware that even within twin pairs, language development can differ considerably.

The present data was limited to normally developed twins for the data collection, and could not show when twins fully catch up with singletons or the prevalence of clinically significant language difficulties in twins. A longitudinal follow-up study of total growth and development of twins, including language development, is essential, and such a study has recently been undertaken by the authors.

In conclusion, the results of present study, using the largest dataset of Japanese twins in childhood, indicated that language development of twins in some respects differs from that of singletons, and that an estimation of language development specifically for twins is necessary for refined maternal and child health care for multiples.

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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.

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What is epigenetics?

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

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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 *et al*[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.

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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.

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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 ar'e 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

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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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特集
臨床精神薬理 8：289-296, 2005
双極性うつ病—双極性障害の新たな治療ターゲット

双極性障害の病因仮説とそれに基づく新しい治療

加藤忠史*

抄録：双極性障害の病態理解は，モノアミン-細胞内情報伝達系から，気分安定薬の神経保護作用にシフトしつつある。しかしながら，神経保護作用の分子基盤としては，GSK-3β系，イノシトール系，ヒストン脱アセチル化阻害作用，小胞体ストレス系への作用，ミトコンドリアへの作用，グルタミン酸受容体への作用など諸説あり，未だ一致した見解に至ってはいない。しかしながら，これらの中でも有力なものの一つであるミトコンドリア機能障害仮説について，既にミトコンドリア病の治療薬として開発されたtriacetyluridineの双極性障害への臨床試験が行われている。双極性障害において，病態に応じた治療が可能になる日も決して遠い未来ではないかも知れない。

臨床精神薬理 8：289-296, 2005

Key words : *bipolar disorder, lithium, mood stabilizer, valproate, pharmacotherapy*

Ⅰ．はじめに

筆者が本誌2001年2月号の特集「21世紀の精神科薬物療法」に寄稿した，「双極性障害の病因仮説と新たな治療薬の可能性」と題する一文では，主として双極性障害のモノアミン仮説および細胞内情報伝達異常仮説と，この理論に基づいた治療法について述べた[21]。その後，既に4年が経過したが，残念ながら，これらの仮説に基づいた治療薬が開発されるには至っていない。一方，抗精神病薬として開発されたolanzapineが，抗躁作用に加え，抗うつ作用，病相予防作用を持つことが報告されたことは，双極性障害の治療法における進歩であった。

Lithium，valproic acidという既存の気分安定薬の作用機序としては，神経保護作用が注目され[4,31]，今や細胞内情報伝達異常仮説以上に主流となっている。これに沿った形で，神経可塑性仮説とでも言うべき仮説が提示された[26]。しかしながら，実際に双極性障害において，どのような分子レベルの病態が神経可塑性の異常を引き起こしているか，という点は不明確であった。そんな中，我々の提案した「ミトコンドリア機能障害仮説」[20]や，Youngらが薬物の作用から提唱し[2]，我々の臨床研究[17]により支持された「小胞体ストレス反応障害仮説」は，こうした神経可塑性仮説の分子基盤を説明できる可能性のある仮説として，注目されているようである[36]。

このように，双極性障害研究は，モノアミン-細胞内情報伝達系の異常から，ミトコンドリア-小胞体ネットワークなどの障害による神経可塑性の異常とそれに対する気分安定薬の神経保護作用へと，研究の流れがシフトしつつあるのが現状である。

本稿では，これらの病態仮説とそれに基づく新

Pathophysiology of bipolar disorder and its implication for novel treatment.

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たな治療法について展望してみたい。

Ⅱ．気分安定薬の神経保護作用の発見

このような最近の双極性障害研究の流れにおけるパラダイム変換の契機は，Nonakaらによる1998年の論文[31]だと思われる。彼らは，ラットの初代培養神経細胞をlithium存在下で7日間培養すると，グルタミン酸による細胞死が起きにくくなることを見出した。この細胞死抑制作用は，NMDA受容体を介したカルシウム流入を阻害するためであった。しかしながら，既存のlithiumの作用である，イノシトールモノフォスファターゼ（IMPase）阻害作用を介したものではないことから，何らかの新規の作用機序によるものと考えられた。

その後，lithium服用により，大脳灰白質体積が増大し[28]，神経細胞のマーカーと言われるN-アセチルアスパラギン酸が増加する[29]などの作用が同定され，この作用がin vivoでも現れていると考えられるようになった。

また，その後見出されたlithiumやvalproic acidの神経細胞新生促進作用[5,12]も，こうした神経保護作用の一つと考えられる。

Ⅲ．神経保護作用のメカニズム

1．GSK-3β系

Lithiumの神経保護作用のメカニズムとして，まず考えられたのは，Nonakaらの研究に先立って発見されていた，Glucose synthase kinase 3（GSK-3β）阻害作用であった[23]。

PI 3 K（phosphatidylinositol-3 kinase）-Akt-GSK 3β系は，がん遺伝子であるv-Aktの発見を契機として同定された，細胞増殖に関わるシグナル伝達経路である。GSK 3βは，細胞死を促進し，Aktはこれを抑制するため，Aktの活性化は細胞の増殖を促す。当初はむしろlithiumの副作用である奇形発生のメカニズムに関係すると考えられていた。しかしその後，この系はNGF（神経成長因子）の細胞内シグナル伝達を担っており[8]，神経突起伸長に関与していることが判明したことや[13]，valproic acidもGSK-3β阻害作用を介して神経保護作用を持つことなどから[3]，気分安定薬の作用機序として注目されている。

2．イノシトール系

一方，気分安定薬の神経保護作用が，古典的なイノシトール仮説で説明できることを示す報告もある[45]。彼らは培養神経細胞において，lithium，valproic acid，carbamazepineの3剤が全て成長円錐の崩壊を阻止し，その面積を拡大することを示した。この作用は，lithiumの持つGSK-3β阻害作用やvalproic acidと同じヒストン脱アセチル化酵素（HDAC）阻害作用を持つ薬剤では見られないことから，これらの作用を介することは否定された。一方，この成長円錐拡大作用は，イノシトールの添加で阻害されることから，3剤ともに細胞内のイノシトールを欠乏させることを介して成長円錐を拡大させる，と結論した。その後，lithiumとvalproic acidがin vivoで細胞内イノシトール濃度を低下させることが示された[32]。Lithiumのイノシトール枯渇作用は，IMPase阻害により，イノシトールリン酸が蓄積すると共に，イノシトールが欠乏するためと考えられる。一方，valproic acidの場合は，イノシトールトランスポーター阻害作用によるものと考えられている[25]。

3．ヒストン脱アセチル化阻害作用

一方，valproic acidがヒストン脱アセチル化酵素（HDAC）阻害作用を持つことも見出された[34]。ヒストンはアセチル化により動的に制御されており，一般にアセチル化は遺伝子発現を活性化させる。従って，HDAC阻害はその領域の遺伝子の発現増加を招く。ヒストンアセチル化は，DNAのメチル化と協調しつつ，遺伝子発現のエピジェネティックな制御に関与している。

Valproic acidのHDAC阻害作用も，当初は奇形という副作用との関連が考えられたが，その後，valproic acidの神経保護作用はヒストンH3のアセチル化レベルの増加を伴うこと，HDAC阻害作用を持つtrichostatin Aも同じ作用を持つことから，valproic acidの神経保護作用はHDAC阻害作用によると提唱された[16]。

4．小胞体ストレス系への作用

Wang ら[41]は，C6グリオーマ細胞を用いて，valproic acid 3週間処置により発現量が増加する遺伝子を，Differential display（DD）法により同定した。その結果，GRP78（78-kDa glucose-regulated protein）の発現が増加することを見出し，この作用が双極性障害への作用機序と関連すると考えた。

GRP78は，小胞体ストレス反応系における重要な分子である。立体構造が整う前の作りかけの蛋白質や，立体構造に異常を来した蛋白質が小胞体に蓄積すると，蛋白質のフォールディングを行う小胞体シャペロン分子を誘導する反応（unfolded protein response，UPR）が起きる。このUPRに関わるシグナル伝達経路の鍵分子がGRP78である。GRP78は，それ自体が蛋白質の折り畳みを行うシャペロン分子であるが，普段は小胞体膜上の，ERストレス反応に関わる2つの分子，ATF 6およびIRE 1と結合している。折り畳み異常を来した蛋白質が蓄積すると，GRP78がATF 6およびIRE 1から遊離し，これがUPR開始のシグナルとなる。GRP78の解離により，ATF 6が活性化されると，ATF 6はERシャペロン遺伝子およびもう一つの鍵分子，XBP 1の転写を活性化する。転写されたXBP 1のmRNAは，やはりGRP 78の解離により活性化されたIRE 1により切断され，初めて活性型転写因子をコードするmRNAとなり，翻訳される。活性型XBP 1は，更にERシャペロンを増やすと共に，自身の転写を活性化する。

この系の神経細胞における意義はよく分かっていない。

5．ミトコンドリアへの作用

Chen ら[37]は，ラットにvalproic acidまたはlithiumを9日間あるいは4週間投与し，両者により前頭葉で共通に誘導されるmRNAをDD法により同定した。その結果，見出された遺伝子がbcl-2であった。Bcl-2はミトコンドリア外膜上に存在し，抗アポトーシス作用を持つ蛋白質である。

ミトコンドリアは，内膜と外膜により囲まれた細胞内小器官であり，呼吸鎖により形成された内膜内外のプロトンイオン勾配が，ATP産生の駆動力となっている。一方，ミトコンドリアは，アポトーシスにおいても大きな役割を果たす。各種のアポトーシス刺激により，内膜に存在するANT（アデニンヌクレオチドトランスロケーター）およびシクロフィリンD，外膜に存在する電位依存性アニオンチャネル（VDAC，別名porin）による複合体，MPT（mitochondrial permeability transition pore）が形成される。MPTが開口すると，ミトコンドリア内（マトリクス）と細胞質の間で電解質や蛋白質の透過性が高まり，膜間腔に存在していたチトクロームcの放出が，アポトーシスのシグナルとなる。Bcl-2は，MPTを抑制することで，アポトーシスに対して抑制的に働く。従って，Bcl-2の増加作用は，神経保護作用をよく説明する。

ミトコンドリアは，内膜内外のプロトンイオン勾配を利用して，カルシウムイオンを取り込む。ミトコンドリアによるカルシウム制御は，プレシナプスでは開口放出，ポストシナプスではカルシウム依存性のシナプス可塑性に，また軸索ではミトコンドリアの輸送に関与すると考えられている[27]。

最近，VDACのノックアウトマウスが恐怖条件付け，学習，およびシナプス可塑性の異常を示す[40]など，ミトコンドリアおよびミトコンドリアによるカルシウム制御が，神経可塑性において重要な役割を持つことが明らかにされている。

6．グルタミン酸受容体への作用

Manji らのグループは[9]，valproic acidとlithiumが共にAMPA型グルタミン酸受容体のトラフィッキングを抑制することを見出した。この作用はGluR 1サブユニットの，トラフィッキングに重要な役割を持つPKAサイト（GluR 1 p845）のリン酸化減少を介していた。

また，双極性うつ病に対する作用が確認されているlamotrigineは，グルタミン酸の放出を阻害する作用を持つことが知られている。

Ⅳ．双極性障害の病態

このように，気分安定薬が神経保護作用を持つこと，その分子メカニズムとして多様な説明が可能であることが，ここ数年の研究成果である。それでは，これらの薬理作用に対応する双極性障害の病態は一体何か，というのが次の疑問である。

1．GSK-3β系

Emamian ら[10]は，AKT-GSK 3β系が lithium の標的であることから，関連する精神疾患である統合失調症患者の死後脳および培養リンパ芽球で AKT 1 の蛋白量を測定し，患者でこれが低下していること，AKT 1 によりリン酸化される GSK-3β の Ser 9 のリン酸化も減少していることを見出した。更に，遺伝子関連研究で，AKT 1 の発現量低下を来すハプロタイプが統合失調症と関連し，AKT 1 のノックアウトマウスが，アンフェタミン投与時に，プレパルス抑制の異常という，統合失調症患者で見られる中間表現型を示すこと，抗精神病薬が GSK-3β の Ser 9 のリン酸化を増加させることより，この系が統合失調症の病態に重要な役割を持つと結論した。双極性障害と AKT 1 のハプロタイプも関係していることから[39]，この系が双極性障害においても重要な病態生理学的意義を持つことが示唆された。

2．イノシトール系

双極性障害におけるイノシトール系の異常については既に多くの報告があり，培養リンパ芽球における inositol monophosphatase 2 遺伝子（IMPA 2）の mRNA 減少[46]，イノシトールの低下[1]，および IMPase 活性の低下[37]，リンパ球における IMPA 2 mRNA の低下[30]，IMPA 2 多型との関連[38,47]など，双極性障害との関連を示す証拠が多数報告されており，現在も双極性障害の病態仮説として最も有力なものの一つである。

3．ヒストン脱アセチル化阻害作用

Valproic acid の神経保護作用が HDAC 阻害作用によるとしても，ヒストンアセチル化があまりにも一般的な遺伝子発現制御機構であるため，一体どの遺伝子の発現制御に影響するのか，ということが問題となる。

Costa らのグループ[40]は，Reelin に注目している。Reelin は統合失調症と双極性障害患者の死後脳で共通に低下していることが報告されており，メチオニン投与により，Reelin プロモーターの CpG アイランドのメチル化が亢進し，その遺伝子発現は減少すること，またメチオニンを投与したマウスがプレパルス抑制の異常を示すこと，valproic acid 投与は HDAC 抑制を介してメチオニンの作用に拮抗するなどから，Reelin の過剰なメチル化が統合失調症の病態に関連すると考えている。しかしこの研究では，Reelin に絞った根拠が明確ではなく，やや根拠が薄弱であると言わざるを得ない。

一方，Meaney らのグループ[43]は，動物実験により，子供の頃の養育が十分でないと，グルココルチコイド受容体（GR）遺伝子上流がメチル化され，生涯にわたり GR の発現が低下し，これがストレス脆弱性の分子基盤となる可能性を示した。HDAC 阻害剤 trichostatin A の脳室内投与が，GR 遺伝子の DNA メチル化を低下させ，ストレス耐性を改善させたことから，valproic acid の標的遺伝子が GR である可能性が示唆されるが，養育によるストレス脆弱性の形成は，むしろうつ病の病態への関与が大きいはずであり，なぜ valproic acid がうつ病に有効でないのか，という点は説明できない。

その他，valproic acid は HDAC 阻害作用を介して heat shock protein70（HSP70）を誘導し，これが作用機序に関連する，と指摘した研究もある[35]。

いずれにしても，valproic acid の作用が HDAC 阻害作用だとしても，どの遺伝子の発現制御に対する影響が重要かを明らかにしなければ，病態を明らかにしたとはいえないであろう。

4．小胞体ストレス系

我々は，一卵性双生児双極性障害不一致例の培養リンパ芽球における遺伝子発現解析で，小胞体ストレス系に重要な役割を持つ GRP78 と XBP 1

の発現が低下していたことを手がかりに，双極性障害患者の培養リンパ芽球ではERストレスに対するGRP78とXBP1の反応が低下しており，その一部はXBP1遺伝子の上流にあり，XBP1自身の結合部位を失う機能的多型（−116C/G）によるものであることを明らかにした。Valproic acidは，ATF6の発現を増加させることを介して，この多型によるERストレス反応障害を改善させた。

XBP1は，統合失調症と双極性障害に共通の連鎖部位，22q12にある。我々はこのXBP1−116多型が日本人，欧系米人で双極性障害と関連していることを報告したが[17]，その後欧米人，中国人に関して否定的な報告がなされた[7,14]。一方，中国人[6]，日本人[18]で，統合失調症との関係が報告された。この多型は，日本人と欧米人で大きな頻度の差が見られることから，人種による差が存在する可能性も考えられる。一方，我々の生理学的解析の所見は，GRP78の遺伝子多型でも説明できることから，GRP78についても検討したところ，日本人でXBP1よりも顕著な関連が見られた[19]。欧系米人では関連はなかった。これらのことから，小胞体ストレス系には複数の機能的多型があり，人種依存的に，双極性障害および統合失調症に共通の危険因子となると考えられた。

XBP1は前頭葉皮質，大脳基底核，海馬などに良く発現しており[33]，脳外傷[33]や虚血[24]で誘導される。脳におけるERストレス系およびXBP1の意義は不明であるが，メタアンフェタミン投与により線条体でGRP78が誘導されること[15]や，モノアミン枯渇が海馬XBP1の発現低下を招くこと[42]などから，XBP1−GRP78系とモノアミン神経系には何らかの機能的関連が存在する可能性が考えられる。

5．ミトコンドリア

我々は，双極性障害患者における磁気共鳴スペクトロスコピー研究で，脳エネルギー代謝異常所見を見出した。一方，McMahonらは，臨床遺伝学的解析から，双極性障害の遺伝様式がミトコンドリア遺伝子（mtDNA）の母系遺伝により説明可能であることを指摘した[20]。これらの所見に加え，ミトコンドリア病で気分障害の合併が報告されていることから，双極性障害においてミトコンドリア機能障害が存在し，ミトコンドリアのカルシウム取り込みの障害が，神経可塑性の変化を介して，双極性障害の病態に関係しているとの仮説を立てた[20]。

その後の研究で，双極性障害患者の脳内におけるmtDNA欠失増加，ミトコンドリア関連核遺伝子の双極性障害との関連，欧系米人・日本人の両方におけるmtDNA10398A多型と双極性障害の関連，この多型がミトコンドリア内カルシウム濃度を上昇させること，気分安定薬がミトコンドリア内カルシウム濃度を低下させることなどを見出した。Bcl−2の過剰発現がミトコンドリア内カルシウム濃度を低下させることから，この作用は前述のBcl−2への作用を介している可能性も考えられた。

最近，無投薬の双極性障害患者において脳内乳酸が増加していること，双極性障害患者の死後脳におけるミトコンドリア関連遺伝子の発現低下，ANT1変異によるCPEOで罹患者全員が双極性障害を併発していた家系の報告など，双極性障害におけるミトコンドリア機能障害を示す所見が，他のグループからも次々と報告されている[22]。

6．グルタミン酸受容体への作用

双極性障害患者死後脳の海馬では，NMDA受容体は減少していたが，AMPA結合密度は不変であったという。一方，側坐核では，AMPA受容体の低下が示唆されている。我々の死後脳研究でも，双極性障害患者でKA型グルタミン酸受容体（GRIK1），代謝型グルタミン酸受容体（GRM1）の低下が見られた。また，遺伝子関連研究でも，NMDA受容体2Aサブユニット（GRIN2A）の機能的CAリピート多型との関連，NMDA受容体の修飾因子であるD−serinの代謝に関係するG72との関連などが報告されている。これらの遺伝子との関連は，統合失調症と共通であり，グルタミン酸仮説は元来統合失調症で提唱されたものであることから，両疾患に共通の病態を反映する可能性がある[22]。

V．治療薬開発へ向けて

このように多くの仮説が乱立する中，治療薬開発の試みが進んでいるとは言い難いが，前述の仮説の中でも，比較的示唆的なデーターの多いミトコンドリア機能障害仮説に基づいた，双極性うつ病に対する治療法の臨床試験が行われている。

Repligen 社は，triacetyluridine（RG2133）というウリジンのプロドラッグを，ミトコンドリア病に対する治療薬として開発した。これは，ミトコンドリア DNA を失ったローゼロ細胞が，ウリジンとピルビン酸の補充で生存できるようになることから発想されたと思われる。この薬は既にミトコンドリア病に対して，FDA が認可している。一方，この薬の双極性障害およびうつ病に対するフェーズ1の臨床試験が，ハーバード大学 McLean 病院で19名の患者に対して行われた。その結果，躁状態を引き起こすことなく，双極性うつ病に対して奏効したという。2004年中には，双極性障害を対象にしたこの薬の第2相試験を開始するとのことである。

その他，臨床試験は行われていないが，ミトコンドリア Na^+/Ca^{2+} 交換体阻害作用が知られており，既にてんかん等の治療薬として広く用いられている clonazepam，ミトコンドリア K^+ チャネル阻害作用を介して，ミトコンドリア内 Ca^{2+} 濃度上昇を抑制する作用を持つ diazoxide（シェリングプラウ社の抗高血圧剤で，未承認ながら高インスリン血症による低血糖症に対して個人輸入等の形で用いられているという）などの双極性障害への応用可能性が期待される。

その他，GSK-3β 阻害薬がうつ病の動物モデルに対して有効との報告がある[11]。

V．おわりに

多彩な仮説が同時に並行して存在し，五里霧中のような双極性障害研究であるが，薬理という視点から見ると，意外に多くの所見が蓄積し，いずれもがある一定の方向を向いているようにも見える。

これらの仮説のうち，どれかが重要でどれかは重要でないのか，あるいは患者によって異常が異なるのかなど，不明な点は多く残っている。我々の検討では，mtDNA10398A 多型によるミトコンドリア内カルシウム濃度の上昇には lithium，valproic acid の両方が奏効し，XBP1-116G 多型による ER ストレス反応の障害は，lithium では改善せず，valproic acid のみが改善した。

臨床的な lithium 反応と比較すると，mtDNA 10398A 型は lithium 反応が良いことと，XBP-116 G 多型は lithium 反応が悪いことと関係しており，ミトコンドリア機能障害が lithium 反応性の病態を，ER ストレス反応障害が lithium 抵抗性，valproic acid 反応性の病態を反映している可能性が考えられる。

双極性障害患者に，病態に応じたテーラーメード医療が可能になる日も，決して遠すぎる未来ではないかも知れない。

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