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 cotwin. 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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Acta Med. Nagasaki 50: 17－22

Nagasaki Schizophrenia Study: Influence of the Duration of Untreated Psychosis on Long-Term Outcome

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To elucidate the association between the duration of untreated psychosis and long-term outcome in schizophrenic patients, we followed up a cohort of first-episode schizophrenic patients in Nagasaki. The present study was conducted in the framework of the World Health Organization Collaborative Study on Determinants of Severe Mental Disorders, which our research group participated in as The Nagasaki World Health Organization Collaborating Center for Research and Training in Mental Health. The cohort was established during the period from 1979 to 1980 and consisted of 107 patients initially diagnosed as schizophrenia according to ICD-9. The subjects of the present study were 97 members of the cohort (54 males and 43 females) in whom we could measure the duration of untreated psychosis. The 97 study subjects were followed up for 15 years since they were enrolled in the cohort and were checked their mental conditions at 1-, 2-, 5-, 10- and 15-year follow-up points. The triplet of the 1st, 2nd and 3rd quartiles of the duration of untreated psychosis in them was (1, 4, 12) months (the mean was 9.9 months). During the whole period of 15-year follow-up, 5 patients died, 40 were lost for follow-up and 52 remained contacted at 15-year follow-up point; out of these 52 patients, 22 were contacted through the whole period. During the first 10 years of the follow-up, the patients diagnosed to have completely been remitted at follow-up point showed a significantly or marginally shorter duration of untreated psychosis as compared to those diagnosed not to have completely remitted; the triplet of the 1st, 2nd and 3rd quartiles of the duration of untreated psychosis in those diagnosed to have completely remitted at 1-year follow-up point was (1, 3, 4) months, while that in those diagnosed not to have completely remitted at the same follow-up point was (2, 6, 12) months and the difference was significant (p=0.036, Wilcoxon rank-sum test). Similarly, the triplets of the 1st, 2nd and 3rd quartiles of the duration of untreated psychosis in the two groups diagnosed with and without complete remission at 2-, 5- and 10-year follow-up points were (1, 3, 4) and (2, 6, 17) months (p=0.021), (1, 3, 8) and (1, 6, 17) months (p=0.149), and (1, 2, 3) and (3, 6, 12) months (p=0.008), respectively. However, no difference was observed between the two groups at 15-year follow-up point; (1, 4, 12) and (1, 4, 9) months (p=0.828). The results of the present study indicate that the duration of untreated psychosis will probably influence on the outcome of schizophrenia at least 10 years.

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Keywords: Duration of untreated psychosis; Schizophrenia; Long-term outcome; Predictors; First-episode psychosis

Introduction

Many studies on first-episode schizophrenic patients have shown that the duration of untreated psychosis (DUP) and psychiatric admission often last months or years. There is evidence that schizophrenic patients with a longer DUP show poorer response to acute treatment, thus resulting in worse short-term outcome, compared to those with a shorter DUP.[1-5] However, only a few studies have dealt with the possible association between DUP and long-term outcome.[6-9] Moreover, most of previous studies were retrospective and/or used less restrictive concepts for schizophrenia than the diagnostic criteria in ICD-9/10, thus limiting their conclusions.

Compared to other countries, patients in Japan have longer hospital stays. Other characteristics of the Japanese system are a large

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number of psychiatric beds per unit of population, of which more than 90% are in private hospitals, and the strong stigma associated with mental disorders. In Japan, there have been a few reports on the relationship between DUP and outcome of schizophrenia patient.[10,11] The present paper reports the relàtionship between DUP and long-term outcome of first-episode schizophrenic patients in Japan.

The patients included in this study were recruited in a clearly-defined catchment area. The patients were diagnosed with schizophrenia according to the ICD-9 criteria and were followed from first contact to 1, 2, 5, 10, and 15 years later.

Methods and Subjects

Background

The present study was based on the World Health Organization (WHO) Collaborative Study on Determinants of Severe Mental Disorders (DOSMeD Study), which our research group participated in as The Nagasaki World Health Organization Collaborating Center for Research and Training in Mental Health.[12,13] The DOSMeD study was commenced in 1979 and is still continuing. WHO gave the DOSMeD Study the status of the second stage of the International Pilot Study of Schizophrenia (IPSS).[14] The IPSS, which was commenced in 1965, established the basic epidemiological research methodology on the study of mental disease. In addition, it elucidated the clinical and psychosocial character of schizophrenia and cleared up differences in outcomes of schizophrenia in participating countries. The main goals of the DOSMeD Study were to confirm the IPSS data and to calculate the incidence rates of schizophrenia in participating countries.

Subjects

Prior to the DOSMeD Study, we conducted a preliminary study under the protocol of the DOSMeD Study in October and November 1978 to determine the pattern that patients residing in Nagasaki city consult doctors for mental disorders.[12] We confirmed from the preliminary study that patients residing in Nagasaki city would probably be found completely if we could obtain the cooperation of the following 30 psychiatric care organizations in Nagasaki city and its vicinity: 18 private mental hospitals, 1 mental hospital of Nagasaki prefecture, 5 private psychiatric clinics, 4 psychiatry departments of public general hospitals and 2 health care centers.

Medical doctors at the Department of Nueropsychiatry of Nagasaki University Hospital (called researchers for short) called the 30 cooperating facilities by telephone at around 11 a.m. every day for 2 years, from January 1, 1979 to December 31, 1980, and asked about new cases that could be included in this study. Table 1 shows the adoption criteria for this study. Patients who had an organic brain disorder or who were dependent on alcohol or drugs were excluded. A researcher visited the facility, which made a positive response, to explain the patient and family the purpose and methods of this study, and if they agreed to participate in this study, the structured interviews were conducted for patients by using the assessment schedules. After the consultation period, we conducted a leakage study to find cases not then reported from the facilities. We also enrolled patients who did not meet the adoption criteria at first contact but did so later. The procedure used for selecting new schizophrenic patients, reported in detail elsewhere,[12] was epidemiologically accurate enough for evaluating the incidence rate of schizophrenia in Nagasaki city.

Table 1. Adoption criteria for the World Health Organization Collaborative Study on Determinants of Severe Mental Disorders (DOSMeD Study)[13]

Patients had one of the following symptoms (a) or (b).

(a) During the past one year the patient had at least one of the following.

1. Hallucinations or pseudo hallucinations in any mordality
2. Delusions
3. Marked thought and speech disorder (e.g., incoherence, irrelevance, thought blocking, neologisms, and incomprehensibility of speech)
4. Marked psychomotor disorder (e.g., negativism, mutism or stupor; catatonic excitement; constrained attitudes or unnatural postures maintained for long periods)
5. Emergence or marked exacerbation of bizarre and grossly inappropriate behavior (e.g., talking or giggling to self, acts incomprehensible to others, loss of social constraints, etc.)

(b) During the past one year, patients had presented a definite change of personality and behavior for at least one of the following.

1. Marked reduction or loss of interest, initiative and drive, leading to serious deterioration of the performance of usual activities and tasks
2. Emergence or marked exacerbation of social withdrawal
3. Severe excitement, purposeless destructiveness or aggression
4. Episodic or persistent state of overwhelming fear or severe anxiety
5. Gross and persistent self-neglect

The DOSMeD Study in Nagasaki enrolled a total of 107 patients who were initially diagnosed as schizophrenia according to ICD-9. The subjects of the present study were 97 members of the Nagasaki DOSMeD Study cohort in whom we could measure the duration of untreated psychosis (DUP).

Study instruments

Table 2 shows the instruments that were used at the commencement of the DOSMeD Study. All interviews were done by psychiatrists who had been trained in the use of these instruments. In this study we measured DUP from the Psychiatric and Personal History Schedule (PPHS, WHO 1978)[13] in Table 2. The PPHS contains over 200 items for assessment, such as personal history, history of present illness and family illness, as well as social and economic factors. The PPHS also determines the time of onset of schizophrenia, judged by the researcher who interviewed the patient. We defined the DUP as the period in months between the onset of the illness and the time of initial visit at a medical facility.

Table 2. Instruments used in the World Health Organization Collaborative Study on Determinants of Severe Mental Disorders (DOSMeD Study) at first assessment

Instruments	Contents
PPHS: Psychiatric and Personal History Schedule (WHO, 1978)[13]	Family history, life history, etc.
DPS: Diagnostic and Prognostic Schedule (WHO, 1978)[13]	Previous illness, present illness, diagnosis, outcome, etc.
PSE-9: Present State Examination 9th edition (Wing et al., 1974)[15]	Psychotic symptoms in the last month
LES: Life Event Schedule (WHO, 1978)[13]	Life events, etc.
KAS: Katz Adjustment Schedule (Katz et al., 1963)[16]	Adjustments for home and society

Definition and classification of outcomes

In this study, remission was defined as no symptoms or signs of a psychotic episode for at least 4 weeks. Complete remission was defined as the state of no symptoms and maintenance of the natural character that existed before the illness. Incomplete remission was defined as not being psychotic but having (1) residual symptoms, (2) non-psychotic symptoms or (3) a character change. We then classified the courses of disease into 5 categories shown in Figure 1 according to the PPHS: complete remission with no relapse, complete remission with relapse, incomplete remission with no relapse, incomplete remission with relapse and continuous psychotic illness; we called the group of the first two categories "good outcome" and the other group of the remaining categories "poor outcome."

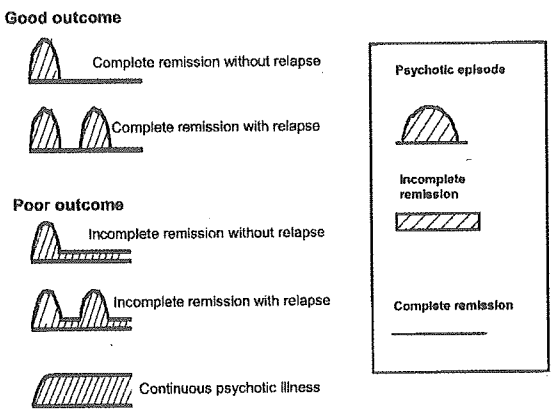

Figure 1. Classification of the course of illness. We call the group of the first two categories, i.e., complete remission without relapse and complete remission without relapse, "good outcome" and the other group of the remaining categories "poor outcome."

Statistical analysis

We used the items for the course of the disease in the PPHS to assess the outcome of the disease at 1-, 2-, 5- and 10-year follow-up points; in 1984-1985 and 1990-1991, Nagasaki University carried out 5- and 10-year follow-up studies on the course and the outcome for the present subjects. The outcome of the disease at 15-year follow-up point was assessed using the schedule of the WHO International Study of Schizophrenia (ISoS), which was initiated by WHO in 1994-1995 as the 15-year follow-up of the DOSMeD.[17,18]

We analyzed the relationship between DUP and the outcome of the disease at 1-, 2-, 5-, 10- and 15-year follow-up points by comparing the distribution of DUP between the two groups of the patients with and without complete remission at respective follow-up points; we used Wilcoxon rank-sum test for comparison. PROC NPAR1WAY and PROC UNIVARIATE of SAS system were used for the necessary calculation.

Results

Out of 97 patients in the present study, 54 (55.7%) were males and 43 (44.3%) were females. The age at onset of the disease varied from 15 to 53 years, and the triplet of the 1st, 2nd and 3rd quartiles was (19, 23, 29) years with the mean (standard deviation) of 24.8 (7.82) years. Eight (8.2%) patients were living alone and 89 (91.8%) were living with their families. Seventy-five (77.3%) were single, 18 (18.6%) were married and 4 (4.1%) were divorced.

The duration of untreated psychosis (DUP) of patients varied from 1 to 132 months and the triplet of the 1st, 2nd and 3rd quartiles was (1, 4, 12) months with the mean (standard deviation) of 9.9 (17.1) months. The DUP did not differ significantly between males and females (p=0.443) as shown in Figure 2.

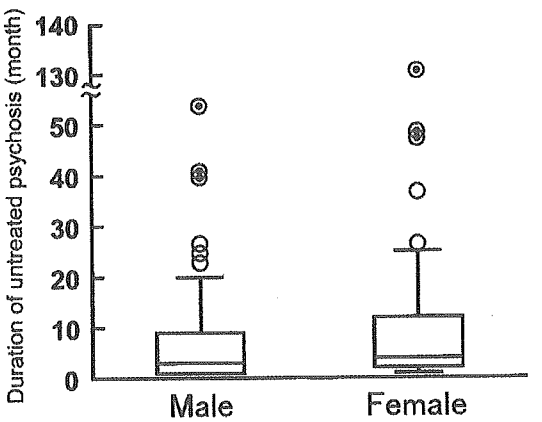

Figure 2. Box-and-whisker plots of the duration of untreated psychosis (month) by gender. The bottom and top ends of the box and the bar inside the box correspond to the 1st, 3rd and 2nd quartiles of the sample (or 25th, 75th and 50th sample percentiles), respectively. The open circle and the double circle with black dot represent extreme values called "outside" and "far out," respectively.

The behavior of patients in participation in the study was very complicated as shown in Figure 3. The proportion of participating patients decreased steadily at 1-, 2- and 5-year follow-up points (75.3%, 67.0% and 51.5%, respectively) then remained stable at 10- and 15-year follow-up points (53.6% and 53.6%, respectively).

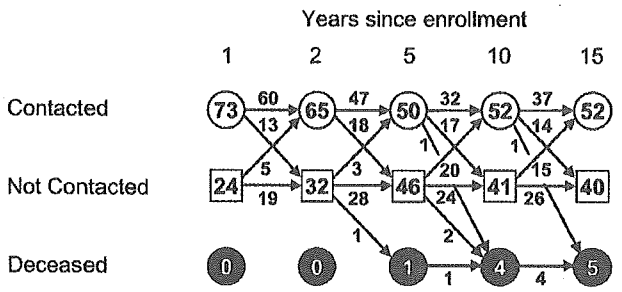

Figure 3. Dynamics of patients' participation in the study through the follow-up. The numerals in the open circle, box and closed circle denote the number of patients who were contacted, not contacted and deceased at respective follow-up points. The numerals on the line connecting two nodes (open circle, box or closed circle) denote the number of patients who moved from one node to the other node.

The outcomes of patients contacted at respective follow-up points showed a complicated pattern as well (Figure 4). The proportion of patients with complete remission gradually decreased while that of those with incomplete remission or with continuous psychotic illness gradually increased; however, the change was not large except for the first two years. The breakdown of patients shown in Figure 4 at respective follow-up points was as follows: the number of patients with complete remission without relapse (with relapse in parentheses) was 15 (16), 6 (20), 6 (11), 3 (14) and 4 (13) at 1-, 2-, 5-, 10- and 15-year follow-up points, respectively; the number of patients with incomplete remission without relapse (with relapse in parentheses) was 14 (2), 14 (5), 9 (18), 3 (22) and 0 (17) at 1-, 2-, 5-, 10- and 15-year follow-up points, respectively; and the number of patients with continuous psychotic illness was 26, 20, 6, 10 and 18 at 1-, 2-, 5-, 10- and 15-year follow-up points, respectively.

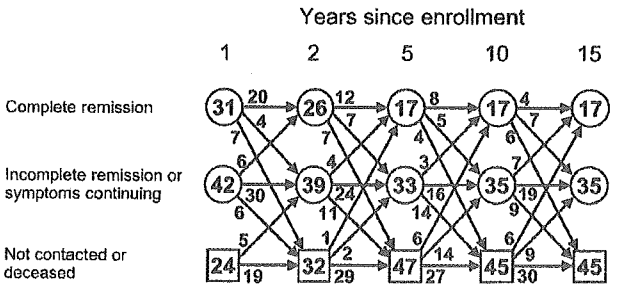

Figure 4. Dynamics of the outcomes in the patients through the follow-up. The numerals in the open circle, box and closed circle denote the number of patients who were contacted, not contacted and deceased at respective times of the follow-up. The numerals on the line connecting two nodes (open circle, box or closed circle) denote the number of patients who moved from one node to another node.

Figure 5 compares the distribution of DUP in patients with complete remission (good outcome group) and that in those with incomplete remission or with continuous psychotic illness (poor outcome group) classified by the outcome observed at respective follow-up points. During the first 10 years of the follow-up, the patients with good outcome at each follow-up point showed a significantly or marginally shorter DUP as compared to those with poor outcome; the triplet of the 1st, 2nd and 3rd quartiles of the DUP in those with good outcome at 1-year follow-up point was (1, 3, 4) months, while that in those with poor outcome at 1-year follow-up point was (2, 6, 12) months and the difference was significant (p =0.036). Similarly, the triplets of the 1st, 2nd and 3rd quartiles of the DUP in the two groups with good and poor outcomes at 2-, 5- and 10-year follow-up points were (1, 3, 4) and (2, 6, 17) months (p=0.021); (1, 3, 8) and (1, 6, 17) months (p=0.149); and (1, 2, 3) and (3, 6, 12) months (p=0.008). However, no difference was observed between the two groups at 15-year follow-up point; (1, 4, 12) and (1, 4, 9) months (p=0.828).

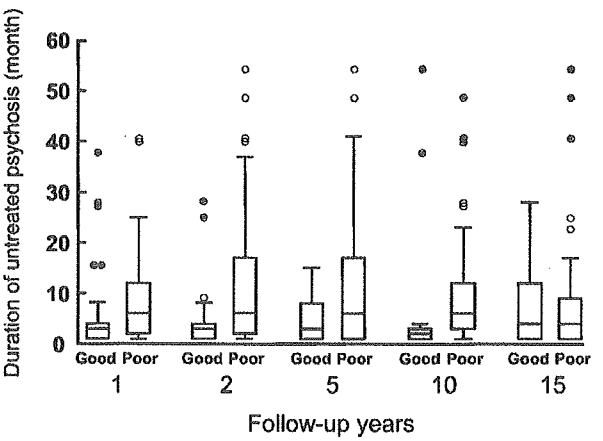

Figure 5. Box-and-whisker plots of the duration of untreated psychosis (month) by outcome at the time of the follow-up. Good: complete remission without relapse or complete remission with relapse; Poor: incomplete remission without relapse, incomplete remission with relapse or symptoms continuing. See Figure 2 for the details of the plots.

Discussion

The duration of untreated psychosis in the patients of the present study varied from 1 to 132 months with the median of 4 months and is compatible with that reported by other studies[3,4,10,11,19-23] (Table 3).

Norman and Malla[24] stated in their review of DUP, "On balance, it seemed fair to say that there was evidence suggesting a relationship between DUP and the initial response to treatment, although the robustness of such findings and their independence from all potential confounding variables yet to be established." In another follow-up study, Malla et al.[23] examined the relationship between DUP and several other predictors and 1-year outcome, and they confirmed the independent role of DUP in remission and positive symptoms at 1-year follow-up point, thus providing support for

Table 3. The duration of untreated psychosis reported by other studies and the present study[13]

Study	Number of patients	Median (month)	Mean (month)
Lobel et al. (1992)[4]	70	9.75	13.0
Beiser et al. (1993)[19]	72	2	14
Larsen et al. (1996)[20]	43	6.5	28.5
McGrorry et al. (1996)[3]	200	7.5	56.3
Browne et al. (2000)[21]	53	6.5	22.7
Thomas et al. (2000)[22]	52	6.5	14.8
Malla et al. (2002)[23]	88	—	11.2
Kobayashi (2002)[10]	62	1	8.7
Yamazawa et al. (2004)[11]	83	—	13.7
Present study	97	4	9.9

[a]The duration of untreated psychosis was measured in week in the first 7 studies and we converted those measurements in month by equating 4 weeks with 1 month.

Table 4. Status of patients at each follow-up point by duration of untreated psychosis

Follow-up point	Status[a]	Duration of untreated psychosis (month)[b]			
		1–3 (48)	4–6 (16)	7–12 (13)	13– (20)
1-year	Good	20 (41.7)[c]	5 (31.3)	1 (7.7)	5 (25.0)
	Poor	17 (35.4)	7 (43.8)	9 (69.2)	9 (45.0)
	Missing	11 (22.9)	4 (25.0)	3 (23.1)	6 (30.0)
2-year	Good	16 (33.3)	6 (37.5)	2 (15.4)	2 (10.0)
	Poor	16 (33.3)	4 (25.0)	8 (61.5)	11 (55.0)
	Missing	16 (33.3)	6 (37.5)	3 (23.1)	7 (35.0)
5-year	Good	10 (20.8)	2 (12.5)	3 (23.1)	2 (10.0)
	Poor	13 (27.1)	6 (37.5)	4 (30.8)	10 (50.0)
	Missing	25 (52.1)	8 (50.0)	6 (46.2)	8 (40.0)
10-year	Good	13 (27.1)	2 (12.5)	0 (0.0)	2 (10.0)
	Poor	11 (22.9)	9 (56.3)	7 (53.8)	8 (40.0)
	Missing	24 (50.0)	5 (31.3)	6 (46.2)	10 (50.0)
15-year	Good	8 (16.7)	3 (18.8)	2 (15.4)	4 (20.0)
	Poor	15 (31.3)	7 (43.8)	7 (53.8)	6 (30.0)
	Missing	25 (52.1)	6 (37.5)	4 (30.8)	10 (50.0)

[a]Good: complete remission without relapse or complete remission with relapse;
Poor: incomplete remission without relapse, incomplete remission with relapse or symptoms continuing;
Missing: not contacted or deceased.
[b]Number of patients in parentheses.
[c]Number of patients (percentage).

early intervention. In the present study, a longer DUP was significantly associated with a poor outcome at 1- and 2-year follow-up points. This result was in line with other prospective studies. These results suggested that DUP could be used as a predictor of the short-term outcome of schizophrenia. Malla et al.[23] also suggested the importance of early detection and intervention for schizophrenia.

Regarding the long-term outcome, Norman and Malla[24] reported that there have been few investigations of the relationship of DUP to long-term outcomes, such as negative symptoms and cognitive functioning; nor were possible confounders of DUP investigated widely. However, the findings of Bottlender et al.,[25] who later investigated DUP and the outcomes of 58 patients at 15-year follow-up point, combined with those reported by previous studies, strongly suggested that the DUP was not only associated with short-term but also long-term outcome in schizophrenia.

In the present study, the DUP was lower in patients with good outcome than in those with poor outcome within 10 years from the commencement of follow-up; the difference was significant at 1-, 2- and 10-year follow-up points (p=0.036, p=0.021 and p=0.008, respectively) but it was not significant at 5-year follow-up point (p=0.149). The insignificant difference in the DUP between the two groups at 5-year follow-up point was mainly due to the fact that a significant proportion of patients with good outcome and relatively short DUP at 2-year follow-up point were not contacted at 5-year follow-up point (Table 4). These results suggest that the DUP could be used as a predictor of the outcome of schizophrenia at least up to 10 years. In contrast to the study by Bottlender et al.,[25] the present study showed no difference in DUP between the patients with good and poor outcomes at 15-year follow-up point.

Although we followed up the cohort of schizophrenic patients, main analysis in the present study was of the repetition of cross-sectional analyses. The reason we used such analysis was: (1) the patients' behavior in participation was very complicated in that some patients not contacted at one follow-up point were contacted at another follow-up point and vice versa; (2) no information was available for patients' mental condition at the time of not contacted; (3) the outcome alternated in some patients between good one and poor one through the follow-up. Although we could not perform formal analysis, which should be most appropriate, we tried to examine the temporal changes in the outcome of the cohort members by DUP (Table 4).

The results (including Table 4) of the present study suggest that the DUP could probably influence the course of illness in schizophrenic patients for about 10 years at most, and thereafter the influence of other factors such as medication and other treatments, social support, support from the family and life style may be greater.

The present study ultimately underscores the importance of early detection of first-episode schizophrenic patients and early intervention in them; early detection is inevitable for immediate reduction in unnecessary suffering and early intervention will increase the likelihood to improve the long-term outcome. Early detection and intervention programs represent an extremely important innovation in the treatment of schizophrenia. Unfortunately, we were unable to carry out a 20-year follow-up, but we are now preparing to perform a 25-year follow-up on these patients.

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Review Article

Signal Transduction and Mood Disorders

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Several theories regarding pathophysiology of mood disorders (depression or bipolar disorders) and the mechanisms of therapeutic agents (antidepressnts or mood stabilizer) have been proposed. Inhibition of monoamine reuptake into nerve endings by antidepressants is one of the cornerstones of the monoamine hypothesis on depression. Many studies have focused on alterations in levels of monoamines and their receptors. More recent studies have been extended to examination of the post-receptor intracellular targets. These include several classes of the guanosine triphosphate-binding proteins that couple receptors and effectors, adenylate cyclase and the inositol phosphate second messenger system. This review summarizes studies on signal transduction and neural plasticity in terms of mood disorders.

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Introduction

Guanine nucleotides binding proteins (G proteins) have been considered as modulators that proliferate signal information from receptors to effectors. G proteins related to membrane signaling have a hetrotrimetric structure (alpha, beta and gamma) and the alpha subunit has a GTP binding site involved in GTPase activity hydrolyzing GTP to GDP. Hence, the G proteins involve a distinctive family of protein molecules implicated in the apparatus of switching signal transduction on and off in the cell.[1] Bipolar disorders are characterized by two episodes of the manic and depressive states, and patients are in general considered to be in remission during the period between the two episodes. Therefore, it is plausible that some difficulty exists in emotional adjustment involving the switching mechanisms which rush or shrink neurotransmission in the brain. Taken together above, one might assume that there is a close link between G protein as the protein molecule in the on-off signal switching and bipolar disorders. Since the amine hypothesis on bipolar disorders was presented, many hypotheses on the etiology of mood disorders have been argued along with the complexity of relations among plural neural transmission systems, which contrast with the increase or decrease in solitary neurotransmission. One of the neurological bases of signal interaction is that different receptors share coupling with the same G protein. Then the signal input from each receptor occurs interactively via the G protein (crosstalk). It is also known that receptor sensitivity is diminished when the release of neurotransmitter is prolonged. In contrast, receptor sensitivity will be raised when the release of neurotransmitters is reduced. Thus, G protein has an important role in regulating receptor affinity to neurotransmitters to keep the temper stable in our social circumstances.

Alterations of biological hypotheses on mood disorders

Table 1 outlines changes in the biological hypotheses on mood disorders. More than 40 years ago, it was established that numerous antidepressants have the capability to inhibit the reuptake of monoamines or monoamine oxidase (MAO). Based on this evidence, the monoamine deficiency theory in depression was proposed. However, there is some disagreement in this theory as below.

(1) Not all antidepressants have the ability to inhibit the reuptake of monoamine or MAO activity.
(2) Not all monoamine reuptake inhibitors have antidepressive effects.
(3) Experiences with antidepressant therapy have revealed that, at clinical doses, it generally takes 2 to 3 weeks for a clinical response to take place, while reuptake inhibition and MAO inhibition can emerge instantly (within minutes) after a single dose of active compounds.

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Table 1. History of biological hypothesis on mood disorders

Period	Hypothesis	Mechanism of therapeutics
1960s	Monoamine deficiency	Monoamine reuptake inhibition
1970s	Receptor supersensitivity NA-ACH imbalance	ß, 5HT2 receptor downregulation Muscarinic receptor inhibition
1980s	GABA dysfunction Dopamine dysfunction Second messenger dysregulation	$GABA_B$ receptor upregulation Dopamine receptor stimulation Second messenger effects
1990s	G protein function imbalance	Modulate G protein function
2000s	Neural plasticity Stem cell disorder	Upregulation of camp-CREB-BDNF Neurogenesis

In the late 1970s, many researchers paid attention on postsynaptic adaptation to chronic treatment with antidepressants. Downregulation of ß-adrenergic receptors or 5HT2A receptors is observed in many kinds of antidepressant administration for 1-2 weeks. In addition, ß-adrenergic agonist-stimulated cAMP production in rat brain slices decreases as well. These observations parallel the lag time for therapeutic advantage seen in the clinical treatment of antidepressants. Downregulation is believed to happen via the neuron's homeostatic mechanisms, in which prolonged exposure increases monoamine in the synaptic gap by reuptake inhibition or MAO inhibition of the antidepressant action. Conversely, second generation antidepressants, which do not inhibit monoamine reuptake or catabolism, also induce receptor downregulation with chronic administration. In consideration of this receptor downregulation, the receptor supersensitivty hypothesis in depression has been presented. This hypothesis appears to diametrically contradict the original monoamine hypotheses. If the receptor supersensitivty hypothesis is correct, specific blockers of these receptors might be clinically effective. However, few data support this hypothesis. Moreover, it is reported that selective serotonin inhibitors do not produce the receptor downregulation. Therefore, it seems unlikely that a specific neurotransmission system is just enhanced or reduced by the action of antidepressants. Furthermore, various neurochemical agents have been shown to act as potent antidepressants. These include selective serotonin uptake inhibitors, GABAergic agents, ß-adrenergic agonists, phosphodiesterase inhibitors, inositol (second messenger precursor), forskolin analogs (second messenger activators) and omega 3 fatty acids. Tricyclic antidepressants also act as neurotransmitter receptor-effector systems which include dopamine, Ach, GABA and certain peptides.

In addition, the mechanisms of action of antidepressants and the pathology of mood disorders involve receptor-G protein-effector function because G protein is a key element in the interaction of different neurotransmitters and play a critical role in neurotransmission (amplification and deamplication). More recent hypothesis will be fully described later.

Antidepressants and G proteins

General desensitization phenomena caused by agonist exposure involve a two-step reaction, i.e. functional uncoupling of the receptor and G protein in the early stage and receptor internalization in the late stage. However, no one has established that the mechanism of antidepressant-induced desensitization is the same as that of receptor-agonist stimulation. In contradiction to the development and recovery of ß receptor desensitization induced by agonist exposure, antidepressant-induced desensitization is a slow phenomenon. Furthermore, Dibner and Molinoff[2] reported that ß receptors in brain slices treated with desipramine caused further ß receptor downregulation in *in vitro* exposure to an agonist, suggesting that the mechanisms of desensitization phenomena may be different for antidepressant- and receptor-agonist-induced desensitization.

Accordingly, the role of ß receptor function in the human brain and the mechanisms of ß receptor downregulation should be reassessed from the viewpoint of downstream signal transduction.

The first report by Menkes et al.[3] demonstrated that long-term administration of various antidepressants enhances guanine nucleotide activation of adenylyl cyclase in rat cortex and hypothalamus membranes. They suggested that this augmentation of adenylyl cyclase is associated with a facilitated G protein-catalytic moiety of adenylyl cyclase. Their idea was not apparently in agreement with the opinion that antidepressant-induced subsensitivity of noradrenalin elicited cAMP production in rat brain slices. They explained this obvious inconsistency was due to alpha 2 adrenergic inhibition of adenylyl cyclase enhanced through G protein-catalytic moiety interaction, and that thereby the net cyclic AMP accumulation was decreased by noradrenalin. There are several reports that GTP analogs, NaF or forskolin stimulated-adenylyl cyclase activity is enhanced long-term antidepressant treatment of membranes. Interestingly, Newman and Lerer[4] reported bi-directional results for forskolin-simulated cAMP production of membranes and slice preparations, observing an increase in membranes and a decrease in slices. They suggested that forskolin interacted with different components of the adenylyl cyclase in the two preparations, reflecting the components of the adenylyl cyclase system distal from the receptor caused by chronic antidepressant treatment. Their report is suggestive in that many factors induced uninform results. Slice preparations have tighter coupling of receptors and G proteins than membrane preparations, and therefore seem to reflect receptor changes or the agonist receptor-G protein coupling state. In contrast with slice preparations, membrane preparations appear to reflect the G protein-effector state distal from the receptor. Even in membrane preparations there are complicated effects on the receptor-G protein-effector system.

If it is true that G protein mediated adenylyl cyclase is enhanced subsequently to long-term antidepressant treatment, which postreceptor components are key targets in the action of antidepressants? In general, adenylyl cyclase activity is based on the balance between stimulatory (Gs) and inhibitory (Gi) GTP binding protein function. We separately measured the stimulation and inhibition of adenylyl cyclase by GppNHp.[5,6] GppNHp dependent-stimulation, but not inhibition of adenylyl cyclase, increased without any change in affinity of guanine nucleotide to G proteins, suggesting that chronic antidepressant administration promotes increased coupling between

Gs and the catalytic unit of adenylyl cyclase.[5,6]

One might also expect that the change in the amount of G protein varies with antidepressant effects. Our reports demonstrated that guanine nucleotide binding is unaffected by chronic antidepressant treatment.[5,6] However, one study[7] reported that some antibodies to G protein alpha subunits measured by ELISA showed a change in the amount of G protein caused by chronic antidepressant treatment. Our study and others did not observe any changes in the quantity of Gs and Gi alpha sununits due to chronic treatment with antidepressants as determined by western blotting.[8,9] These studies do not seem to confirm whether the amounts of G proteins change. However, it is suggestive that the amounts of G proteins do not parallel the amount of mRNA; for example, in the brain there are 10 times more Gi and Go proteins than Gs protein, while mRNA of Gs is abundant. These results indicate that turnover of protein synthesis occurs more quickly in Gs than in Gi and Go. Thus, long-term adaptive action such as chronic antidepressant treatment does not seem to be reasonable for protein synthesis of Gs.

Acute effects of several antidepressants except for MAO-inhibitors, antipsychotics or anxiolytics, have demonstrated to increase [^{3}H]-GTP binding in a homogenate of rat cerebral cortex. These effects were partially inhibited by pertussis toxin, suggesting that target sites of antidepressants involve Gi- and Go-like proteins. Furthermore, the same laboratory also reported that the GTPase activity of purified Go and the ratio of [^{35}S]-GTPγS binding to the purified protein were increased by antidepressants, indicating that the drugs enhance the dissociate reaction of G protein heterotrimer subunits.[10] We also examined the *in vitro* effects of antidepressants on functional photoaffinity by labeling GTP binding protein. Saturation binding studies were performed by incubating membranes with increased concentrations of [^{32}P]-AAGTP, followed by UV irradiation and SDS-PAGE. The specifically bound isotherms for each of the G proteins studied showed characteristics of a one-site model. Scatchard analysis revealed increases in the Bmax and Kd of AAGTP binding for each of the G proteins (especially stimulatory G proteins) with the addition of antidepressants such as amitriptyline, clomipramine, desipramine and mianserin except for MAO inhibitors, antipsychotics or anxiolytics. These results suggested that drugs having antidepressive properties may directly affect G protein, especially Gs protein.[11]

Based on current evidence that cationic amphiphilic compounds, including neuropeptides, antidepressants and polyamine, directly modify G protein functions in a receptor-independent fashion, development of new drugs targeting G protein is under discussion for the treatment of neuropsychiatric diseases. Although it will be necessary to extend this preliminary work to a broad spectrum of psychoactive drugs in the near future, we believe the attempt to observe difference in the specificity and selectivity of action sites in each G protein of psychoactive drugs will contribute to the development of novel psycotherapeutics.

Human brain study in mood disorders

Our previous study reported that the quantity of various G protein subunits in postmortem brain samples from the parietal and temporal cortices was same in controls and depressive patients as demonstrated by western blotting.[12] However, photoaffinity GTP labeling of Gi/oα, but not Gsα, was significantly augmented in depressives in both cortex regions. In addition to basal activity, guanine nucleotide-, forskolin- and manganese-stimulated adenylyl cyclase activities were decreased. Besides, an increase in phosphodieseterase binding sites (PDE TYPE 4) was observed, suggesting that degradation of cAMP is enhanced in depression and suicide. Cowburn et al.[13] found that brains of suicide with depressive symptoms revealed a significant decrease to the same extent in basal, guanine nucleotide-, forskolin- and manganese-stimulated adenylyl cyclase activities despite an increase in Gs immunoreactivity. This finding agreed with the results of our study on unipolar depressive disorders, indicating reduced Gi in depressive brains. On the other hand, Young et al.[14] reported that the amounts of Gs- and forskolin-mediated adenylyl cyclase were enhanced in the postmortem human brain of bipolar disorders. These findings indicate that cAMP production through G protein and adenylyl cyclase plays a key role in the process of formation of the manic and depressive conditions, and in the difference between unipolar and bipolar mood disorders.

Imbalance hypothesis of G protein function in mood disorders

Many of the past hypotheses on manic-depressive illness finally have looked for causes of biological dysregulation of brain signaling which controls human emotion. Imbalances in the second-messenger system have been hypothesized in the pathophysiology of mood disorders.[15,16] Instability in the second-messenger signaling of emotions has suggested that mood disorders may be caused by functional disproportion of the two major second signaling systems, with depression resulting from hypofunction of cAMP pathways with absolute or relative dominance of the inositol phosphate (IPs) pathways, and mania resulting from contrasting conditions for depression, hypothesized from the mechanisms of action of therapeutic agents and peripheral tissue studies in mood disorders.[15,16]

On the basis of our and other pharmacological and human brain studies, we proposed the imbalance hypothesis of G protein function in mood disorders (Figure 1). cAMP production by adenylyl cyclase is regulated by the balance between Gs and Gi functions. In addition, Gi and Go-like proteins, which are ADP-ribosylated substrates of pertussis toxin, are presumed to activate phosphoinositide metabolism by Phospholipase C. Hence, our results denote that in proportion to Gs and Gi/o in depressives a state was observed, which occurred during hypofunction of the cAMP system and hyperfunction of the IPs system through distorted equilibrium of G protein functions. Antidepressants may have increased Gs function to normalize this imbalance. Since lithium has the potency to inhibit

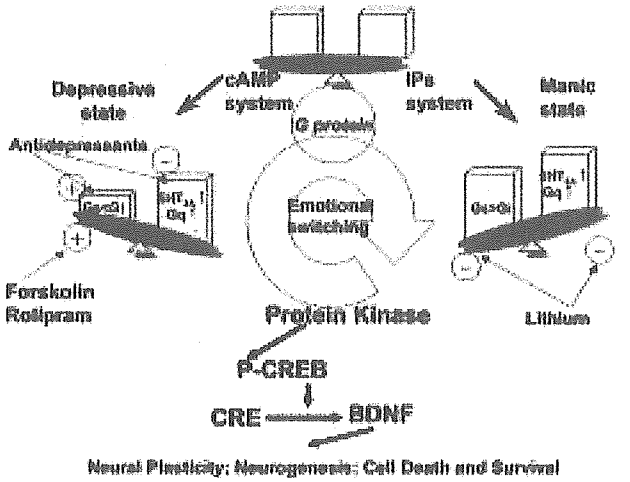

Figure 1. Illustration of the imbalance hypothesis of G protein function in mood disorders.

Gs as well as Gi, this compound suppresses both the manic and depressive states. On the other hand, antidepressants may induce an imbalance in Gs and Gi functions because they promote Gs function rather than other functions of G protein. Therefore, this may cause depression turn to mania and easily induce rapid-cycle formation. A recent postmortem brain study in bipolar mood disorders [17] suggested that there is an imbalance, compared to matched controls, between the activities of the phosphoinositide system via decreasing Gq function and the adenylyl cyclase system via increasing Gs function. Enhanced postsynaptic $5HT_{2A}$ receptor function in depressive patients was reported in the studies of postmortem brain and peripheral tissue. Moreover, it has been reported that chronic treatment with several antidepressants or SSRI can induce downregulation of $5HT_{2A}$ receptor function. Consequently, we hypothesize that the proportion of Gs and Gi/o is altered in the manic state that occurs during hyperfunction of the cAMP system and hypofunction of the IPs system via $5HT_{2A}$ receptor.

This hypothesis is based on the facilitation of Gi function in depressive and manic conditions. In the mammalian brain, Gi (inhibition of adenylyl cyclase) exists 10 times more than does Gs (stimulation of enzyme). As G protein level changes in the developmental period, persons who have a predisposition to absolute or relative dominance of Gi function compared to Gs function may be affected by some environmental or constitutional impacts during a critical period. Such persons may have a tendency to be altered symmetry of G protein function and they may suffer from mood disorders caused by emotional stress.

Transcription factor regulation in mood disorders

In recent times, it was reported that the cAMP response element binding protein (CREB), a transcription factor that mediates many of the actions of the cAMP cascade on gene expression, is upregulated by antidepressant treatment. These findings suggest that CREB is probably a common downstream target of antidepressant treatment.[18]

Dowlatshahi et al.[19,20] suggested that the CREB level in the postmortem temporal cortex was lower in antidepressant-free patients with major depressive disorder than in both antidepressant-treated patients with major depressive disorder and nonpsychiatric control subjects. Furthermore, it is known that chronic antidepressant administration increases the expression of CREB in rat hippocampus.[21] Thome et al.[22] demonstrated that chronic antidepressant administration increases CRE-mediated gene expression and CREB phosphorylation in mice brains. These studies show that the CREB level in the brain is decreased in the depressive state and is increased by chronic antidepressant administration. These previous findings and the present study suggest that alteration in CREB levels may be related to the pathophysiology of major depressive disorder. Meyer et al.[23] suggested that the expression of the CREB gene is positively autoregulated by CREB itself. The present report suggests that the reduced phosphorylation of CREB induces a decline of the transcriptional transactivational activities of CREB, leading to decreased transcription of CREB. Therefore, the decreased level of immunoreactivity of phosphorylated CREB found in postmortem brains of patients with major depressive disorder could lead to a decline in protein levels of CREB, inducing a continuous reduction in CREB-dependent transcription. More recently, we examined immunoreactivities of CREB and phosphorylated CREB in orbitofrontal cortices of human postmortem brains and compared antidepressant-free patients with major depressive disorder and nonpsychiatric control subjects. This was demonstrated by our observations that showed a significant decrease in the level of CREB and phosphorylated CREB in depressive patients. Furthermore, the decreased ratio of phosphorylated CREB immunoreactivity to CREB immunoreactivity in depressive subjects suggests that phosphorylation of CREB is probably impaired in brains of patients with the disease.[24]

A recent report[25] of increased hippocampal BDNF immunoreactivity in postmortem brains of subjects treated with antidepressant medication suggests that BDNF as well as CREB is involved in the pathophysiology of major depressive disorder and in the mechanism of antidepressant action. Yamada et al.[26,27] identified in rat brain a novel gene induced after chronic antidepressant treatment with the RING-H2 finger motif and cysteine string protein. These data may demonstrate a possible role of the altered gene expression in the mechanism of antidepressant action and may imply that antidepressants induce alteration in the neuronal network at the molecular level.

Figure 1 illustrates the imbalance hypothesis of G protein function in mood disorders explained in the last two chapters.

Conclusion

Despite the four decades of research, the molecular basis of mood disorders and the mechanisms of antidepressants are still not clear. However, it has been demonstrated that antidepressants, lithium,

ECT and others modify the coupling receptor-G protein-effector system, causing reorchestration of neurotransmitter dysregulation and neural circuits via upregulation of cAMP-CREB-BDNF signaling in mood disorders. In the future, there will likely be breakthroughs in genetic research in the components of signal transduction and in pharmacological studies on new agents which act directly on such components.

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Structural and Functional Neuroimaging in Posttraumatic Stress Disorder

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1. Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition associated with a constellation of disabling behavioral and emotional symptoms, which occur in a proportion of individuals exposed to severe psychological trauma, such as combat, sexual abuse, natural disasters, and terrorism. For several years, we have pioneered research in neuroimaging of PTSD in Japan. Here we describe some of our findings, which include: (1) a structural magnetic resonance imaging (MRI) study in patients with PTSD recruited from the victims of the Tokyo subway sarin attack; (2) an event-related potential study and its association with structural MRI abnormality in the same sample; (3) a functional MRI study in an independent sample of Japanese PTSD patients.

2. Structural Abnormalities in PTSD

MRI studies using the manual tracing method have shown smaller than normal hippocampal volume in patients with PTSD. However, these studies have yielded inconsistent results, and brain structures other than the hippocampus have not been well investigated. A recently developed, fully automated method called voxel-based morphometry (VBM) enables an exploration of structural changes throughout the brain by applying statistical parametric mapping to high-resolution MRI. In the current study (Yamasue et al. 2003), we employed VBM to explore structural brain differences in the gray matter as well as the white matter between victims of the Tokyo subway sarin attack with and without PTSD. Based upon findings from previous neuroimaging studies, we hypothesized that patients with PTSD would demonstrate volume reduction in medial temporal regions (including the hippocampus) and medial prefrontal cortices (including anterior cingulate cortex, or ACC). The Tokyo subway sarin attack was caused by terrorists belonging to a cult named Aum

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Shinrikyo in Japan on 20 March 1995. About 5500 victims were exposed to sarin, a poisonous gas, and 12 of the victims died. We recruited victims, with or without PTSD, who had never received psychiatric treatment for PTSD due to the attack. In addition, these subjects had little history of psychotropic treatment and no history of alcohol and substance abuse, which can be significant confounding factors in neuroimaging studies of PTSD.

Thirty-six victims were recruited from victims of the Tokyo subway sarin attack who were treated in the emergency room for acute sarin intoxication with follow-up at St. Luke's International Hospital in Tokyo, Japan. Out of the 36 victims, 9 were diagnosed as having PTSD related to the attack [1 man had current PTSD; 8 (4 men and 4 women) had past PTSD]. Ten more victims met more than one of the three symptom clusters of PTSD (including reexperiencing of the event, hyperarousal, and numbing), and 1 more was diagnosed as having current alcohol dependence; these 11 victims were excluded from further analysis. The remaining 16 victims (10 men, 6 women) did not have PTSD or any history of neuropsychiatric disorders. Thus, the final set of subjects in this study included 9 victims with (current or past) full PTSD and 16 victims without PTSD. Three of the 9 victims diagnosed as having PTSD had the following psychiatric comorbidity: current major depression (n =1), current (n = 1) and past (n = 1) panic disorder with agoraphobia. Although 2 of the 9 victims diagnosed with PTSD had received benzodiazepines for insomnia or general anxiety over an approximate 3-month period 2-4 years earlier, the other 23 victims had never received psychiatric treatment before participating in this study. The MRI data were obtained using a 1.5-Tesla scanner (General Electric Signa Horizon Lx version 8.2, GE Medical Systems, Milwaukee, WI, USA). Voxel dimensions were 0.9375 × 0.9375 × 1.5 mm. Image analysis was performed using ANALYZE PC 3.0 (Mayo Foundation, Rochester, MN, USA) and SPM 99 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK).

An area with lower gray matter intensity in victims with PTSD compared with those without PTSD after controlling for the factors of age and sex was found within the left ACC [peak Montreal Neurological Institute (MNI) coordinates (mm) [x, y, z] = [–8, 12, 32], k = 113, z = 4.33, corrected P = 0.015 with small volume correction (SVC) of 56.7 ml]. No other gray matter or white matter regions showed any significant intensity difference between the two groups. These results indicate diminished left ACC gray matter volume in victims with PTSD. It is unlikely that the volume reduction of ACC in patients with PTSD was solely the consequence of the neurotoxic effect of sarin on brain structure, because there was no significant difference in serum cholinesterase concentration between the two groups. (If anything, there was a trend toward slightly higher sarin exposure in the non-PTSD subjects).

Among the patients with PTSD, there was a significant negative correlation between total score on the Clinician-Administered PTSD Scale (CAPS) and left ACC gray matter intensity (peak coordinate [x, y, z] = [–8, 12, 28], z = 4.36, corrected P = 0.025; SVC = 0.904 ml based upon k = 113 obtained in the main analysis) after controlling for the effects of age and sex. This result indicates that the reduced ACC gray matter volume showed a significant inverse relationship with PTSD severity.