Microendophenotypes of Psychiatric Disorders: Phenotypes of Psychiatric Disorders at the Level of Molecular Dynamics, Synapses, Neurons, and Neural Circuits

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Abstract: Psychiatric disorders are caused not only by genetic factors but also by complicated factors such as environmental ones. Moreover, environmental factors are rarely quantitated as biological and biochemical indicators, making it extremely difficult to understand the pathological conditions of psychiatric disorders as well as their underlying pathogenic mechanisms. Additionally, we have actually no other option but to perform biological studies on postmortem human brains that display features of psychiatric disorders, thereby resulting in a lack of experimental materials to characterize the basic biology of these disorders. From these backgrounds, animal, tissue, or cell models that can be used in basic research are indispensable to understand biologically the pathogenic mechanisms of psychiatric disorders. In this review, we discuss the importance of microendophenotypes of psychiatric disorders, i.e., phenotypes at the level of molecular dynamics, neurons, synapses, and neural circuits, as targets of basic research on these disorders.

Keywords: Animal model, bipolar disorder, depression, endophenotype, microendophenotype, psychiatric disorder, PTSD, schizophrenia.

INTRODUCTION

Psychiatric disorders such as Schizophrenia, Depression, Bipolar disorder and PTSD are major diseases in the world. It is necessary to understand the mechanisms of these disorders and to develop therapeutic strategies. Importantly, the development of psychiatric disorders is not only due to genetic factors; rather they are strongly influenced by interactions between environmental and genetic factors. Furthermore, the mechanisms by which brain functions are controlled at the molecular, cellular, and circuit levels are not understood well. Therefore, unraveling the mechanisms of psychiatric disorders requires a variety of approaches including both basic and clinical studies.

To understand the pathogenic mechanisms of psychiatric disorders, endophenotypes of psychiatric disorders, which are their phenotypes at the psychological, physiological and behavioral levels and reflect some aspect of genetic factors, have been identified to provide objective indices of a psychiatric condition. However, these endophenotypes of psychiatric disorders have barely been characterized at the molecular, cellular and circuit levels. On the basis of this background, we propose to develop "micro-endophenotypes" that are phenotypes of psychiatric disorders at the molecular dynamics, synapses, neurons, and neural circuits levels, as an interface between basic and clinical studies.

LIMITATION OF GENETIC APPROACHES TO IDENTIFY GENETIC FACTORS OF PSYCHIATRIC DISORDERS

Higher concordance rate in monozygotic twins than dizygotic twins suggested the role of genetic factors in mental disorder. Family and adoption studies also supported this view. Based on these findings, linkage analysis has been performed since late '80s. However, they were not successful. The roles of rare chromosomal abnormalities such as 22q11 deletion and balanced translocation causing disruption of DISC1 gene in schizophrenia are well established. However, 22q11 deletion can also confer a risk of other phenotypes such as autism, and mental disorder phenotype in DISC1 family is not limited to schizophrenia but also extends to depression and bipolar disorder [1].

Recent genome wide association studies revealed a number of single nucleotide polymorphisms (SNPs) that are associated with bipolar disorder [2], and schizophrenia [3] at the genome wide significant level. The number of associated SNPs increased with the expansion of the number of subjects. Bioinformatic analysis suggested that thousands of SNPs confer a risk of schizophrenia, and schizophrenia and bipolar disorder significantly share the common associated

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SNPs. On the other hand, the roles of rare copy number variations (CNVs) were revealed in schizophrenia and autism [4]. However, the identified CNVs are shared by schizophrenia, autism, and other neuropsychiatric diseases including epilepsy and attention deficit hyperactivity disorder. More recently, roles of *de novo* mutations, which increase with higher paternal age, are revealed in autism and schizophrenia [5, 6]. Because such *de novo* mutations are rare event, it is hard to statistically prove that these *de novo* mutations are causative for autism and schizophrenia. In addition, the genes identified are not always specific to each disease [1].

Collectively, recent genetic studies clarified the roles of both common SNPs and rare CNVs or mutations, either transmitted or *de novo*. However, these identified factors can explain only a modest part of heritability of mental disorders. Furthermore, it has become apparent that none of these genetic factors are specifically related to one mental disorder, but rather they are common to multiple mental disorders.

Thus, it has become apparent that genetic study alone cannot reveal neurobiological basis of each mental disorder. It is increasingly recognized that neurobiological changes associated with these genetic factors should be investigated to elucidate the etiology of mental disorders.

ENDOPHENOTYPES ARE NOT SUFFICIENT AS TARGETS OF BASIC RESEARCH

Basic studies on the pathogenic mechanisms of psychiatric disorders have been performed based on the current diagnostic criteria. However, diagnoses of psychiatric disorders are currently in a very difficult position because of the following reasons: (1) the biological indices of psychiatric disorders are unknown, which means that their pathologic condition cannot be indexed by biochemical/biological markers; and (2) their pathologic condition cannot be easily visualized at the microscopic level, unlike senile plaques of Alzheimer's disease. Thus, current diagnostic criteria of psychiatric disorders do not define discrete "diseases" based on pathology but only describes "syndromes" based on subjective experience and observable behavioral changes. Identification of common, rather than discrete, genetic factors between mental disorders by genome wide association studies is consistent with this notion [7]. Development of sub-definitions within each primary psychiatric disorder which have biological foundations would be meaningful for both clinical practice and basic research.

To overcome these problems, endophenotypes of psychiatric disorders have been studied. For example, the neurophysiologic impairments in pre-pulse inhibition of acoustic startle, latent inhibition, impaired exploratory eye movements, altered brain morphology by MRI, reduced evoked gamma activity, impaired event related potential have been identified as endophenotypes of schizophrenia [8, 9]. Neurocognitive impairments in working, verbal and visual memories, attention and so on were also proposed as endophenotypes, though they provide objective indices of a psychiatric condition, rather than biological phenotypes.

Since mice with a genetic mutation also show such endophenotypes, these phenotypes have been considered to be promising research tools that connect humans with animal models. However, the pathologic conditions and pathogenic mechanisms of psychiatric disorders have been clarified very rarely with the use of the above endophenotypes. This is because the hitherto identified endophenotypes are still far distant from molecular and cellular levels.

ADVANTAGES AND DISADVANTAGES OF ANIMAL MODELS

Genetically engineered animals enable the analysis of the effects of the loss- or gain-of function of a gene-of-interest in a living animal. Therefore, genetically engineered animals have become a powerful and indispensable tool to clarify pathologic conditions and to understand the pathogenic mechanisms of various disorders, thereby contributing significantly to medical science. In psychiatric disease research, the development of highly applicable disease models would also provide a number of advantages. Importantly, genetically engineered mice simulating chromosomal abnormalities that confer a risk of schizophrenia have been shown to display endophenotypes such as prepulse inhibition similar to those identified in some human psychiatric disorders (Table **1**). However, it is becoming clear that the observation of endophenotypes in mutant mice is not always sufficient as a model of psychiatric disorders, although such endophenotypes have been used as markers to generate and develop mouse models of psychiatric disorders.

Genetically engineered mice can be used to induce a null mutation, to inhibit a series of gene families simultaneously by expressing dominant-negative mutants, and to induce conditional mutations, i.e., generating tissue and/or time-dependent genetic mutations. Accordingly, mutant mice often show the effects of the strong functional inhibition of genes, which occurs rarely in humans. Therefore, it is possible that such mutant animals exhibit phenotypes that include the secondary and tertiary effects of gene mutations to a greater degree than in humans as genetic function is inhibited more strongly. For these reasons, in contrast to human genomic research where the genes responsible for psychiatric disorders has just been emerged, a large number of mutant mice showing endophenotypes of psychiatric disorders have been identified. For example, there are numerous genetically modified mice that have been nominated as model mice of schizophrenia due to the abnormalities of prepulse inhibition and working memory [10], these endophenotypes are exhibited in a considerable number of different mouse models. Furthermore, it is important to note that genetic backgrounds of genetically engineered mice have been known to

Table 1. Examples of endophenotypes in animal models of schizophrenia.

Genetic Mutations Modeled by Mice	Endophenotype	Reference
22q11.2	prepulse inhibition	[18, 59-61]
	Impaired hippocampal-prefrontal synchrony	[62]
	brain structural changes by MRI morphometry	[63]
Chromosomal translocation causing disruption of DISC1	prepulse inhibition brain structural changes by MRI morphometry	[11]
	impairment of latent inhibition brain structural changes by MRI morphometry	[13]
15q13.3 microdeletion	auditory stimulus-evoked gamma activity decreased auditory evoked potentials	[64]
16p11.2 microduplication	brain structural changes by MRI morphometry	[65]

display strong influences on their phenotypes. Therefore, it is possible that these influences of genetic backgrounds on genetic mutation in mice sometimes mask endophenotypes/microendophenotypes that should be observed, or lead to unreasonable and/or unexpected phenotypes.

Additionally, several kinds of mice with mutations in the disrupted in schizophrenia 1 (DISC1) gene, which is one of the candidate genes responsible for schizophrenia, have been generated and analyzed. Interestingly, although most of these mice exhibited schizophrenia-like phenotypes [11-15], some displayed depression-like behavior [14], suggesting that the relationship between the disease and genes is more complicated than a simple one-to-one relationship.

Thus, it is difficult to advance studies of psychiatric disorders relying only on animal models. Nevertheless, it is important that animal models should be developed that exhibit higher validity as models of psychiatric disorders in collaboration with human genomic research to identify the genes responsible for these conditions.

MICROENDOPHENOTYPES OF PSYCHIATRIC DISORDERS

Unlike neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, in which senile plaques and degeneration of substantia nigra, respectively, are observed clearly at the microscopic level, such easy-to-observe pathologic conditions have not been observed in psychiatric disorders. Conversely, as described above, it is difficult to uncover the cause of psychiatric disorders merely by the analysis of genetic factors. Moreover, although endophenotypes at the level of psychology, physiology, and behavior have been identified, their molecular basis has barely been characterized.

The current consensus that psychiatric disorders are not caused only by genetic factors and show no easy-to-observe pathologic conditions strongly suggests that the pathology of psychiatric disorders resides at the level of molecular dynamics, synapses, neurons, and neural circuits. Therefore, it is considered that research on psychiatric disorders should focus on phenotypes at the level of molecular dynamics, cells, and neural circuits. Here, we nominate such phenotypes observable at the microscopic scale the "microendophenotypes of psychiatric disorders".

Though we used the term endophenotype here, it is practically impossible to test the heritability of such phenotypes in humans. Thus, the concept of "microendophenotype" does not always intend to extrapolate the concept of endophenotype, but propose a phenotype of psychiatric disorder observable at the microscopic level.

It is impossible to completely replicate psychiatric conditions exactly and faithfully in animals that cannot have the mental capacity similar to humans. However, it is not necessary for animal models to manifest all of the pathologic conditions of a psychiatric disease. These models are satisfactory if they recapitulate some aspect of a psychiatric condition, namely microendophenotypes. Once microendophenotypes have been identified, clarification of the causes and molecular basis of a psychiatric disease is expected to progress by means of basic research focusing on those microendophenotypes.

For example, one of most promising "microendophenotype" of psychiatric disorders would be the decreased number of parvalbumin positive interneurons or decreased expression of parvalbumin in the prefrontal cortex [16]. This was initially found in postmortem brains, and subsequently verified in several rodent models of schizophrenia [10]. The other candidate microendophenotype of schizophrenia is reduction in the density of dendritic spines in the prefrontal area [17]. Reduction of spines was also detected in animal models of schizophrenia [18]. In this case, understanding the mechanisms by which spine density or shape is altered is expected to reveal the pathogenic mechanisms of schizophrenia.

Recent progresses of molecular neuroimaging studies using such as PET and MR spectroscopy may help to identify microendophenotypes of psychiatric disorders at the circuit and synaptic levels; recent studies suggest that abnormalities of neurotransmissions such as dopamine, NMDA and serotonin are associated with pathophysiology of psychiatric disorders [19, 20]. In line with this perspective, targets of drugs, such as NMDA glutamate receptors antagonist (e.g., Ketamine), used for the

patients of psychiatric disorders may also give hints to identify microendophenotypes at the circuit and synaptic levels [21, 22].

Furthermore, it is assumed that electrophysiologically detectable abnormalities of synapses and/or changes in neuronal plasticity will be identified as aspects of the pathologic states of psychiatric disorders. In such a case, these electrophysiological abnormalities are considered to reflect some changes of molecular dynamics at the synaptic level. Therefore, it is expected that the use of super-resolution molecular imaging, such as stochastic optical reconstruction microscopy (STORM), might uncover microendophenotypes at the level of molecular dynamics. Since this kind of molecular imaging using STORM, if applied together with fluorescence immunohistochemistry, can be applied to postmortem brains, the identification of microendophenotypes at the molecular dynamics level will be of great significance in the collaboration between human and animal studies.

Furthermore, it is important to note that current progresses of psychiatric disorder diagnoses focusing on sub-definitions of primary psychiatric disorders may help to identify microendophenotypes since psychiatric disorders have been started to be defined as syndrome, but not discrete disorders.

POSSIBLE CANDIDATE MICROENDOPHENOTYPES THAT ARE SHARED BY HUMANS AND ANIMALS

In this section, possible microendophenotypes of psychiatric disorders are described as candidates that need to be validated in future studies.

Depression and Bipolar Disorders

Studies of depression and bipolar disorder have been focused on the molecular mechanisms of drugs for these disorders; antidepressants and mood stabilizers.

The prototype antidepressants discovered in 1950s were an inhibitor of monoamine transporters (imipramine) and monoamine oxidase inhibitor (iproniazid), both of which increases neurotransmission of monoamines; serotonin, noradrenaline, and dopamine. Since then, many antidepressants were developed, and all of them increase monoaminergic neurotransmission. However, the evidence to support impaired monoaminergic neutotransmission in depression is scarce. Thus, long term consequence of antidepressants treatment was investigated and the role of increased BDNF signaling, which is the downstream event of altered monoamine signaling, was established. Together with the fact that stress causes dendrite remodeling [23], neuroplastic hypothesis of depression was established [24]. However, recent studies suggest that dendrite remodeling may vary depending on brain regions. In medial prefrontal cortex, spine density is decreased by stress, whereas it is increased in amygdala [25]. Stress decreased BDNF in hippocampus, but BDNF is increased in ventral tegmental area dopaminergic neurons projecting to nucleus accumbens in social defeat model [26]. Thus, when we aim at searching for molecular basis of depression, consideration for biochemistry is not enough, and anatomical consideration is crucial. Anyway, various structural plasticity in specific brain regions related to emotion regulation is the most promising candidate for the microendophenotype of depression. Indeed, altered spine density has been suggested in postmortem brains of patients with depression [27].

In the case of bipolar disorder, effect of lithium on inositol monophosphatase and GSK-3β are well documented. Studies of common molecular effect of several mood stabilizers revealed that mood stabilizers commonly act on molecular cascades related to cell death and neuroplasticity [28]. On the other hand, increased calcium signaling is suggested by studies of peripheral blood cell. Thus, alterations of cellular plasticity and vulnerability are implicated as a pathophysiological basis of bipolar disorder, and mood stabilizers might act on such molecular cascades [29]. However, neural circuit responsible for bipolar disorder is not well studied. Though volume of anterior subgenual cortex and insular cortex is suggested to be decreased in bipolar disorder [30], they are not specific to bipolar disorder. Further studies to identify microendophenotype of bipolar disorder are required.

Post-Traumatic Stress Disorder (PTSD)

PTSD is a psychiatric disease caused by intense fear and terrifying experiences that generate fear memory. A large number of studies suggest that the regulation of fear memory is, at least partially, shared between humans and animals [31-33]. Therefore, as mentioned below, collaborative research on humans as well as animal models has been conducted. For example, findings from studies on fear memory using rodents were immediately verified in humans and applied to exposure therapy for PTSD.

Pavlovian fear conditioning is thought to be a model of fear learning and memory. The mechanisms regulating fear learning and memory have been investigated in humans and rodents. The conditioning procedure in these species is almost identical. In the case of mice, a mouse receives electrical footshocks in the presence or absence of a tone in a small chamber. From this experience, the mouse learns and memorizes an association between a conditioned stimulus (CS), such as context and tone, and an unconditioned stimulus (US), such as an electrical footshock that induces fear [34, 35]. Conversely, in the case of humans, for instance, a person receives a slight electrical shock when a certain pattern is displayed on a computer screen, thereby learning and memorizing an association between fear and the displayed pattern [36]. Fear memory is assessed by analyzing memory retrieval-induced fear reactions such as freezing responses (completely motionless behavior) in mice and changes in skin conductance in humans. In humans, research on fear memory is performed with the combined use of brain imaging

analyses. These imaging studies have shown that humans and rodents use similar brain areas to regulate fear learning and memory.

Fear memory becomes persistent *via* a memory consolidation process. Indeed, short-term memory lasts for several hours and is considered to be unstable and is converted into stable long-term memory through a memory consolidation process that requires gene expression [37, 38]. Studies using rodents have shown that pharmacological or genetic blockage of the signal transduction pathway that activates the gene expression program required for memory consolidation inhibits memory storage, in other words, it disrupts memory formation [39, 40]. Moreover, recent studies have shown the existence of memory processes that control fear memory following the retrieval of consolidated memory. Indeed, when fear memory is retrieved by re-exposure to the CS, the retrieved memory returns to a labile state (i.e., is destabilized), as is the case with short-term memory, and is restored *via* the reconsolidation process that requires the similar gene expression program such as transcription factor cAMP responsive element binding protein (CREB)-mediated transcription with consolidation [41, 42]. Studies using rodents have demonstrated that the blockage of reconsolidation disrupts retrieved fear memory, similar to the case of blocking memory consolidation [41-43].

The retrieval of fear memory by re-exposure to the CS induces fear responses. Importantly, prolonged re-exposure to the CS leads to new inhibitory learning against the fear memory in which animals learn that they do not need to respond to the CS. This phenomenon is called “fear memory extinction” [44]. Interestingly, the most efficacious cognitive behavioral therapy for PTSD is believed to be “exposure therapy”, which cures PTSD through the repeated retrieval of terrifying memories. Therefore, this therapy induces memory extinction in humans and its biological basis was recently appreciated as memory extinction [44-46].

Studies using rodents have identified agents that disrupt fear memory by blocking memory reconsolidation or facilitate fear memory extinction. Therefore, attempts to shorten the duration of exposure therapy are currently being undertaken by combining this approach with blocking memory reconsolidation or facilitating memory extinction using agents that were confirmed to be effective in rodents and were approved for human use. For example, currently used agents include a beta-adrenergic blocker (propranolol) to block reconsolidation [47] and a partial agonist of NMDA-glutamate receptors (D-cycloserine) to facilitate memory extinction [48-50].

Importantly, fear-conditioned rodents display the spontaneous recovery of a fear response even after a fear memory has been extinguished (in some experimental conditions at approximately 1 month after extinction training) [44, 51]. Hence, there is the potential that a fear reaction may relapse even though the fear memory has been extinguished with an effective treatment such as exposure therapy. In an attempt to resolve this problem, it was shown that the spontaneous recovery of a fear reaction did not occur if an animal underwent extinction learning at 10 min-to-several hours after a brief session of memory retrieval that induces memory reconsolidation, during which the retrieved fear becomes labile (reconsolidation-update) [52]. Interestingly, this phenomenon of blocking spontaneous recovery, which was found in rats, was also shown to be the case in human fear conditioning [36]. Therefore, this reconsolidation-update pheno-menon may be applicable for the improvement of exposure therapy.

Thus, studies on the regulation of fear memory have revealed strong correlations between rodents and humans; both species are similar to each other with respect to the regulation of fear memory. Therefore, findings from studies on memory reconsolidation and extinction in rodents can be applied to human clinical studies to improve exposure therapy for the treatment of PTSD.

However, it still remains uncertain whether PTSD is caused by strong consolidation of fear memory or by the failure of memory extinction, although it is considered that PTSD is related, at least in part, to the impairment of the regulation of fear memory. Most importantly, the basis for the regulation of fear memory after memory retrieval at the molecular, cellular, and circuit levels remains to be clarified. For example, as mentioned above, D-cycloserine facilitates fear memory extinction when the fear memory is in the extinction phase after retrieval. However, this agent also enhances memory reconsolidation, leading to the reinforcement of fear memory when the memory is in the reconsolidation phase. Similarly, inhibition of gene expression disrupts fear memory in the reconsolidation phase, whereas this inhibition blocks fear memory extinction in the extinction phase, leaving fear memory unaffected. Thus, it is important to estimate which retrieved fear memory is being reconsolidated or extinguished when treatment with agents is combined with exposure therapy. To do this, the mechanism by which the fate of the retrieved fear memory is determined (reconsolidation or extinction) should be investigated and understood.

Thus, the mechanistic basis of fear memory after retrieval at the molecular, cellular, and circuit levels, i.e., microendophenotypes for the regulation of retrieved fear memory, should be elucidated further to develop therapeutic methods for PTSD that are based on the underlying biological bases. To achieve this aim, those microendophenotypes should be clarified initially using animal models. The progression of such basic study will make a huge contribution to the clarification of the pathogenic mechanisms of PTSD and the development of efficacious therapies.

IDENTIFICATION OF MICROENDOPHENO-TYPES USING INDUCIBLE PLURIPOTENT STEM (IPS) CELLS DERIVED FROM PATIENTS WITH PSYCHIATRIC DISORDERS

As mentioned above, since many of the loss-of-function genes in genetically engineered animals correspond to the effects of a null mutation, the effects

of a gene mutation are thought to have a far-reaching influence. In contrast, what is sought from animal models of psychiatric disorders is the reproduction of the microendophenotypes of psychiatric conditions *in vivo*, not necessarily requiring their reproduction at all levels (from molecular to behavioral). For example, observations at the cellular or circuit level in an animal model should be sufficient to investigate the molecular basis of microendophenotypes. From this perspective, recently developed iPS cells are thought to have the potential to contribute significantly to the identification and characterization of such microendophenotypes [53].

Therefore, iPS technology has a great significance since it is impossible to analyze living neurons inside the brain of patients with psychiatric disorders. iPS cells generated from patients with psychiatric disorders may enable the characterization of the functions of neurons displaying some aspect of psychiatric disorders at the cell culture level. Although it is difficult to generate functional neural circuit relevant to human brain in culture dish, neurodevelopmental process at the cellular level can be mimicked *in vitro*. For example, recent study showed that iPS cells-derived neurons of schizophrenic patients carrying 22q11.2 deletion had increased LINE1 (Long interspersed nuclear elements) copy number similarly to postmortem brains of patients with schizophrenia [54]. LINE-1 retrotransposition occurs during neurodevelopment and thus could be simulated *in vitro* in culture dish. Furthermore, there are at least two strategies to simulate functionl neural circuit using human iPS cells; transplantation (chimera assay) and brain organoids. Transplantation of neuronal stem cells derived from iPS cells of patients generates functional neural circuit in the brain of rodents [55]. This could allow us to observe the nature of the transplanted cells in a living brain. For example, it would be possible to analyze electrophysiologically the functions of the transplanted cells within a neuronal circuit or to analyze their gene expression profiles, even though the number of transplanted cells is small. Self-organized three dimensional structures such as cortical tissues [56], pituitary gland [57] and brain organoid [58] have been generated *in vitro* from embryonic stem cells or iPS cells. Cerebral organoids generated from iPS cells of a patient with microcephaly could recapitulate the features of this disease, such as premature neuronal differentiation, *in vitro*. From those analyses, it may be possible to identify new microendophenotypes of psychiatric disorders.

SUMMARY

Animal models that cannot have the same mental capacity as humans are unable to reproduce the phenotypes of psychiatric disorders faithfully. Conversely, phenotypes at the level of molecular dynamics, cells, or circuits, here we nominated microendophenotypes, can represent some aspects of psychiatric conditions shared in the brains of humans and animals. Once microendophenotypes of psychiatric disorders have been identified, they will facilitate basic studies of psychiatric disorders focusing on microendophenotypes using animal models. We look forward to a time when the concept of microendophenotypes contributes to the future progress of the study of psychiatric disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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