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The JSPN Award for Special Contributions to Psychiatric Research Lecture

De novo Mutation Analysis in Bipolar Disorder and Prospects

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Abstract

Psychiatric disorders such as bipolar disorder and schizophrenia are known to have high heritability, but the molecular basis of their genetic factors has long been a mystery. Recent advances in genomics technology have made it possible to comprehensively analyze the information encoded in DNA, enabling us to elucidate genomic features associated with psychiatric disorders. The progress of genomic analysis technology has led to the discovery of rare variants associated with schizophrenia and autism with large effect sizes. However, the understanding of the genetic architecture of bipolar disorder, especially that of rare variants with large effect sizes, relatively lags. Thus, we focused on *de novo* mutations, which include highly rare variants, to detect rare variants associated with bipolar disorder with large effect sizes. We searched for *de novo* mutations from 354 trios with bipolar disorder, the largest sample size in a trio-based bipolar disorder study to date (Nishioka, et al., Nature Communications, 2021). In this study, we found the following results: (i) *de novo* mutations in bipolar disorder are

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characterized by loss-of-function mutations in genes susceptible to natural selection by loss-of-function mutations (high pLI genes, high probability of loss-of-function intolerance), and (ii) *de novo* mutations in bipolar disorder are characterized by deleterious mutations in synaptic and ion channel-related genes, (iii) there are two cases of deleterious mutations in *XKR6* in bipolar disorder, whose deleterious mutations are significantly enriched in a broad spectrum of psychiatric/neurodevelopmental disorders, (iv) deleterious somatic mutations, including an LoF mutation in *KMT2C*, are enriched in the genes causative for developmental disorders if it existed as germline mutations, and two independent mutations in *SRCAP* were found from two unrelated patients with bipolar disorder. Genomic studies to date have clarified the characteristics of variants associated with bipolar disorder. At the same time, the heterogeneity of bipolar disorder and commonalities among bipolar disorder, schizophrenia, and neurodevelopmental disorders have also been clarified. We need to understand the relationship between genomic features and phenotypes with more detailed phenotypic information beyond the conventional diagnostic classifications, possibly with trans-diagnostic analysis. In this article, I describe our analysis of *de novo* mutation research in bipolar disorder with our study's background and prospects.

Keywords: bipolar disorder, *de novo* mutation, exome, somatic mutation, genomics

Introduction

It has long been known that mental disorders such as bipolar disorder and schizophrenia tend to cluster within families. For example, when a parent has a mental disorder, their child has a higher likelihood of exhibiting a similar phenotype compared with the general population. This observation has led to the hypothesis that genetic factors are involved in mental disorders to some extent. This knowledge has also been clinically useful; for example, when a patient presents with depressive symptoms, the presence of a family member with bipolar disorder prompts consideration of that diagnosis. In the research context, the concordance rate among monozygotic twins is particularly important for understanding genetic factors. Since monozygotic twins generally possess identical genetic information, phenotypes or diseases with high

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concordance rates among them suggest that genetic information is closely involved in those phenotypes or diseases. The concordance rate for bipolar disorder is 40-50%, while the lifetime prevalence in the general population is about 1%. This suggests that genetic factors are involved in the onset of bipolar disorder.[7] However, relying solely on such family history information cannot reveal the actual nature of genetic information, limiting understanding to the observed phenomena.

I. Genome Analysis and Mental Disorders

It is well known that genetic information is encoded in the chemical substance DNA. Recent advances in genomics technology have led to marked progress in techniques for reading genetic information from DNA. Devices such as microarrays and next-generation sequencers now enable researchers to comprehensively discern genetic information encoded in DNA, akin to casting a trawl net. Transcribing the genomic information of a single human individual yields approximately 6 billion characters, equivalent to about 60,000 paperback books.[5] Such vast amounts of information cannot be processed by sequentially reading it with the eyes like a conventional book. Parallel to DNA sequencing technology, the development of large-scale information analysis techniques, such as parallel computing systems, has made it practically feasible to process the vast amount of information contained in the human genome. This new analytical approach and academic discipline is called "genomics." The term "genome" combines "gene" and the suffix "-ome" (meaning "totality"), signifying the entirety of genes. This differs from classical genetic analysis in that it aims to capture the entire genetic landscape, like a trawl net, rather than individual genes. Genomic and genetic data exist as the foundation of an individual, and it goes without saying that when examining correlations, we expect them to potentially be causal.

Advances in genome analysis technology have enabled comprehensive exploration of the human genome. However, extracting information potentially related to disease from such vast genomic data requires efficient search strategies. A foundational framework for these strategies is the concept illustrated in Figure 1.[6] This framework represents the concept that the effect sizes of polymorphisms or variants are inversely correlated with disease susceptibility. If a mutation

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significantly impacts a disease, it is disadvantageous to the individual and thus undergoes negative natural selection. Consequently, its frequency in the population remains low, inevitably making it a rare variant. Conversely, common variants, such as common single nucleotide polymorphisms (SNPs) with high frequency across the population, are considered to have little impact on disease. While highly impactful variants may sometimes be explained by a monogenic (single-gene) model where a single variant causes the disease, polymorphisms or variants with small effects do not cause disease alone. Instead, understanding the disease requires a polygenic (multiple-gene) model, where multiple variants collectively contribute to disease susceptibility. Common variants associated with disease have primarily been detected through genome-wide association studies (GWAS) using microarrays (SNP chips). SNP chips can genotype millions of SNPs simultaneously but have the limitation of being restricted to pre-defined SNPs. Rare variants associated with disease have primarily been detected through whole-exome sequencing using parallel sequencers (so-called next-generation sequencers). Exome sequencing facilitates the comprehensive detection of variants in exonic regions (protein-coding regions) and can identify previously unknown variants. However, it is more costly than SNP chips. Whether to target common variants like SNPs or rare variants is a major consideration in analytical strategies.

II. Findings from Genomic Analysis in Major Mental Disorders

Indeed, genomic analysis approaches targeting mental disorders are gradually revealing genetic information associated with these conditions. Regarding schizophrenia, findings are accumulating within the framework shown in Figure 1. This framework is being validated, and research is advancing toward a genetic understanding of schizophrenia and improved diagnostics. At the rare variant end of the spectrum, loss-of-function mutations in approximately 10 genes, including GRIN2A (which encodes an NMDA [N-methyl-D-aspartate] type glutamate receptor subunit), and multiple copy number variations have been identified as having large effect sizes.[16] Results supporting the glutamatergic neurotransmission hypothesis, one of the most compelling hypotheses for the etiology of schizophrenia, have been obtained, including for another gene,

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GRIA3, which encodes the AMPA (α-3-hydroxy-5-methyl-4-isoxazolepropionate) type glutamate receptor, a crucial component of glutamate neurotransmission. At the other extreme, hundreds of genetic loci have been identified as common variants, characterized by a high frequency but small effect size (odds ratio ~1.3).[15)17)] Findings indicate that many of these common variants also involve synapse-related genes, contributing to further advances in understanding the pathology. Research is steadily progressing toward the original goal of elucidating the disease pathology. From the perspective of genomic research, schizophrenia is highly likely to be broadly characterized as a disorder of synaptic dysfunction. *DRD2* is among the associated genes, and it encodes a subunit of the dopamine receptor in postsynaptic structures. One of the most compelling hypotheses regarding the etiology of schizophrenia is dysregulation of the dopaminergic system hypothesis. Findings from genomic research support this hypothesis, which has long been positively viewed in the field of psychiatry.

Numerous findings from genomic analyses have also been accumulated for autism spectrum disorder (ASD). In ASD, the contribution of rare variants has been primarily elucidated, with many genes identified at a level where they can be explained as single causative genes, primarily loss-of-function variants. The contribution of common variants is considered relatively small, and our understanding of ASD is progressing primarily through rare variants. Over 100 genes, including *CHD8*, *SCN2A*, and *ARID1B*, have been identified as associated genes mediated by rare variants in autism spectrum disorder.[14)]

Regarding bipolar disorder, considering schizophrenia as an example, it is anticipated that both common and rare variants are associated. Compared with schizophrenia, bipolar disorder is relatively less severe, suggesting lower natural selection pressure. Consequently, analysis of common variants was prioritized. GWAS studies, including reports by Ikeda, M. et al., have been conducted both domestically and internationally, demonstrating associations in dozens of genomic regions, primarily centered around synapse-related gene regions.[2)8)] However, research on rare variants in bipolar disorder is less advanced compared with schizophrenia or autism spectrum disorder, leaving many

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aspects unexplored. Rare variants represent a relatively unexplored area in understanding bipolar disorder. A meta-analysis of exome data by an international consortium reported an association between the AKAP11 gene and bipolar disorder (specifically, a significant correlation was observed when bipolar disorder and schizophrenia were analyzed as a single group).[13] Furthermore, Kushima, I. et al. reported an association with multiple short copy number variations.[4] While these reports represent important milestones in bipolar disorder research, our understanding of rare variants in bipolar disorder remains incomplete. Further exploration of rare variants is essential to elucidate the genomic architecture of bipolar disorder.

III. Our Genome Analysis in Bipolar Disorder

Therefore, we focused on *de novo* mutations, which are particularly scarce among rare variants, and searched for variants and genes with a significant impact on bipolar disorder.[12]

1. Methods

We primarily enrolled families of sporadic cases where the individual had bipolar disorder and both parents were unaffected, mainly from the Bipolar Disorder Research Network Japan (BDRNJ). A total of 171 families participated. Combining data from other countries, we analyzed *de novo* mutations in 354 families. The study was conducted in compliance with the Declaration of Helsinki and related guidelines, and with ethical approval from the relevant institutions. *De novo* mutations are defined as those present only in the index child and absent in the parents [*de novo* is a Latin expression meaning "newly arising"]. Such variants are expected to be newly generated, particularly scarce among rare variants, and potentially have significant effects. As of 2022, we had conducted *de novo* mutation analysis involving 354 families, the largest number worldwide for a bipolar disorder trio study, with BDRNJ making a significant contribution. Another key feature of this study was the analysis of both germline *de novo* mutations occurring before fertilization (i.e., congenital *de novo* mutations) and somatic *de novo* mutations arising during post-fertilization development (i.e., acquired *de novo* mutations) (Figure 2).

2. *De novo* Mutations in High pLI Genes

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First, regarding congenital *de novo* mutations, we compared families with bipolar disorder to control families to examine the characteristics of *de novo* mutations in bipolar disorder. The results showed a tendency toward a higher number of rare, functionally disruptive *de novo* mutations not observed in the general population. Defining genes where loss-of-function mutations are likely to result in a phenotypic disadvantage as high pLI genes,[3] we found that bipolar disorder is associated with a higher frequency of loss-of-function mutations in high pLI genes. This result is theoretically expected.

3. Biological Characteristics of Rare Disruptive *De novo* Genes

Consistent with previous bipolar disorder studies, genes harboring rare disruptive *de novo* mutations were frequently associated with synapses and calcium ions. Rare *de novo* loss-of-function mutations tend to be found in genes highly expressed in the anterior cingulate cortex among all organs. Analysis using single-nucleus RNA sequencing data (Allen Institute for Brain Science public data) confirmed these genes as highly expressed in a group of subtypes of excitatory neurons. This group of excitatory neurons also showed a tendency toward higher-level expression of genes associated with developmental disorders and tag genes detected by GWAS. Among these overlapping genes, *CACNA1C* was noted, encoding a calcium channel. *CACNA1C* is a gene particularly noted for its association with bipolar disorder. Although this result was unexpected, it is considered to indirectly support the validity of previous analyses and importance of *CACNA1C*.

4. Genes Broadly Associated with Mental and Neurodevelopmental Disorders

We investigated genes broadly associated with mental and neurodevelopmental disorders, not limited to bipolar disorder, to explore genes potentially involved in a wide range of mental symptoms. This revealed that the *XKR6* gene is associated with a broad spectrum of psychiatric and neurological disorders via rare *de novo* functional variants. Although this gene shows elements suggesting an association with bipolar disorder, such as being linked to neuroticism and risk behaviors in GWAS, it was not previously reported and can be considered a newly identified gene associated with mental disorders. *KMT2C* is known as the causative gene

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for Kleefstra syndrome, a severe neurodevelopmental disorder. This study further revealed that loss-of-function variants in *KMT2C* can be widely detected across disorders ranging from bipolar disorder to schizophrenia and autism spectrum disorder, indicating its broad involvement in psychiatric symptoms through multifaceted expression.

5. Somatic Mutations (Acquired *De novo* Mutations) in Bipolar Disorder

However, *KMT2C* loss-of-function mutations in bipolar disorder were actually somatic mosaic mutations, specifically somatic mutations arising during early development (acquired *de novo* mutations). This result led to the hypothesis that "mosaic mutations in neurodevelopmental disorder causative genes might confer milder phenotypes, including bipolar disorder." We therefore comprehensively detected somatic mosaic mutations (acquired *de novo* mutations). As a result, we independently detected function-disrupting mosaic mutations in the same gene, *SRCAP*, in two cases. Since *SRCAP* is the causative gene for Floating-Harbor syndrome, this result supported our working hypothesis. A scenario can be proposed where mutations in *KMT2C* or *SRCAP* occurring in the germline lead to severe neurodevelopmental disorders, while those arising as somatic mutations result in endogenous mental disorders, such as bipolar disorder and schizophrenia.

Conclusion

Although this study provided new insights into *de novo* mutations in bipolar disorder, many challenges remain. While we explored the correspondence between bipolar disorder and genotypes, the genes and mutations actually identified are likely associated not only with bipolar disorder but also with other diseases. Furthermore, the possibility of explaining bipolar disorder with a limited number of genes is considered extremely low. From the perspective of genotype-phenotype correspondence, one key discussion point emerging from this study involves the need to examine more detailed phenotypes and evaluate them along axes different from conventional diagnoses. While the need for refining disease concepts through genomics has been advocated in recent years,[1)] classification based on genomic analysis requires comprehensive examination of both rare and common variants, leaving significant room for future investigations in this regard.

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Moving forward, detailed clinical evaluations starting from genomic features will be required to advance understanding of the genotype-phenotype correspondence.

Furthermore, findings regarding somatic mutations are exploratory; larger sample sizes are needed to sufficiently examine their association with disease.[11] While data on somatic mutations in mental disorders have gradually accumulated in recent years, sample sizes remain insufficient for robust validation, necessitating further data collection.[9][10] If somatic mutations do contribute, determining whether their contribution can be theoretically estimated remains a challenge. Genomics research involving the germline has developed based on heritability, calculated from the diagnostic concordance rate in monozygotic twins, as both a theoretical basis and driving force for exploration. However, the theoretical basis for its association with somatic mutations is insufficient; whether theoretical estimation is possible remains a major future challenge.

Conflict of Interest

The author holds a joint appointment at the Department of Molecular Pathology of Mood Disorders, a collaborative research program between Sumitomo Pharma Co., Ltd. and Juntendo University. However, Sumitomo Pharma Co., Ltd. had no involvement in this research.

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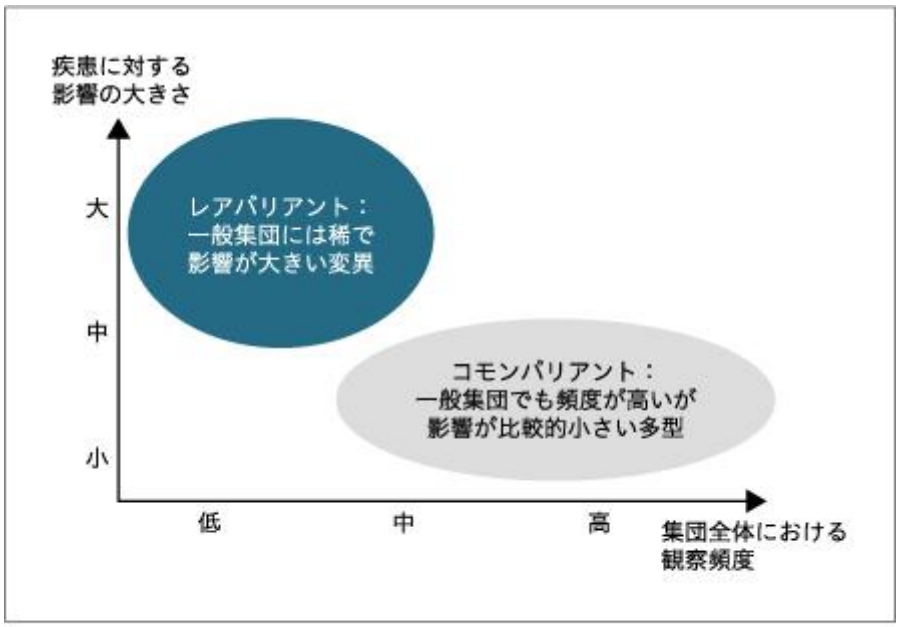

図1　疾患に関連するレアバリアント・コモンバリアントの枠組み
横軸に多型や変異の頻度，縦軸に疾患に対する効果量を示す．

Figure 1 Framework of Rare Disease-associated and Common Variants
The horizontal axis presents the frequency of polymorphisms or variants, and the vertical axis shows the effect size on the disease.

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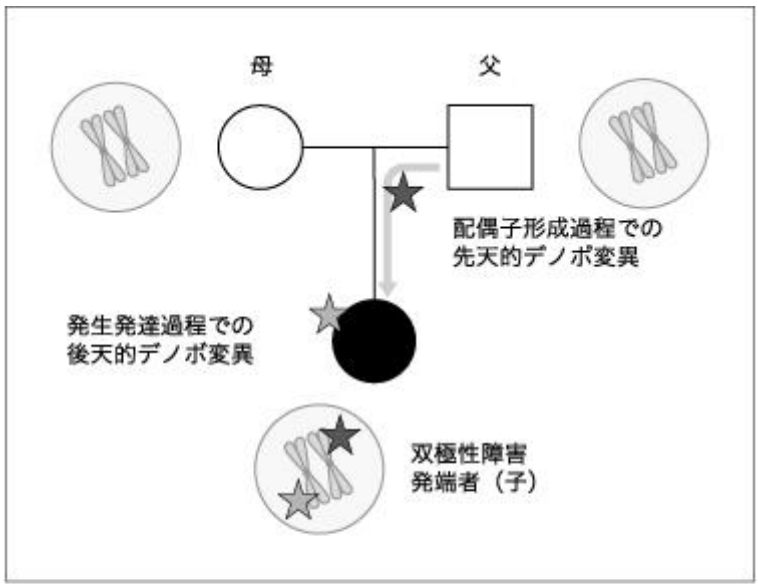

図 2　デノボ変異の概略図

子どもの遺伝情報のほとんどは父親か母親に由来するが，父親・母親から検出されない新生の突然変異が一定数生じ，デノボ変異と呼ばれる．配偶子形成過程で生じる先天的なデノボ変異と，発生発達過程で生じる後天的なデノボ変異の 2 種類がある．

Figure 2: Schematic Diagram of *De novo* Mutations

While most of a child's genetic information is inherited from either the father or mother, a certain number of new mutations, undetectable in either parent, can arise. These are called *de novo* mutations. There are two types: congenital *de novo* mutations arising during gametogenesis, and acquired *de novo* mutations arising during development.

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