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Contribution of Copy Number Variants in Severe Eating Disorders

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Abstract

Among eating disorders, anorexia nervosa still lacks well-established treatment, and it has been reported to be associated with a high mortality rate. Many aspects of its brain pathology remain unclear, and research methodologies are currently limited. Epidemiological studies suggest that genetic factors play a role in the onset of eating disorders, and the search for genome variants associated with the onset risk is advancing, backed by the development of genome analysis technology. In this article, we introduce research on single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) among these variants. These genomic studies also show that there are

common genetic factors between eating disorders (anorexia nervosa) and other psychiatric disorders. In the CNV study conducted by the authors, it was suggested that variants were enriched in synapse-related genes, suggesting a possible association with synaptic dysfunction. In the future, large-scale genome analyses will hopefully clarify the genetic factors of eating disorders, and advanced understanding of the pathology will be achieved through analyses of model organisms.

Keywords: eating disorder, copy number variant, synapse

Introduction

Eating disorders are a group of conditions characterized by persistent disturbances in eating behavior that impair physical health and psychosocial functioning. Among these, anorexia nervosa has a reported lifetime prevalence of approximately 1% in women. It is marked by an intense fear of weight gain, a distorted perception of one's own body (body image), and being significantly underweight due to restricted food intake. It is also associated with a high mortality rate (approximately 5% over 10 years). The brain pathologies underlying eating disorders remain largely unclear, and there are currently no effective pharmacological treatments. However, epidemiological studies to date have indicated that genetic factors are involved in the onset of such disorders. First-degree relatives of individuals with anorexia nervosa have been reported to exhibit an 11-fold higher risk of being diagnosed with anorexia nervosa.[16] Twin studies, which compare the concordance rate of the disorder between monozygotic (identical) and dizygotic (fraternal) twins, also indicate that genetic factors strongly influence the development of anorexia nervosa. Epidemiological findings also suggest an overlap in genetic factors with other psychiatric disorders. For example, patients with anorexia nervosa have a significantly higher rate of co-occurrence with autism spectrum disorder (ASD), and relatives of patients diagnosed with anorexia nervosa also show a higher frequency of ASD.[6] This suggests an overlap in genetic factors between anorexia nervosa and ASD.

Building on findings from epidemiological studies of eating disorder, genome analysis studies aimed at identifying risk variants have been reported.[1)3)] We introduce studies focusing on single nucleotide polymorphisms (SNPs) and copy

number variants (CNVs). Research analyses targeting SNPs have involved genome-wide association studies (GWAS) incorporating approximately 17,000 anorexia nervosa patients and 55,000 healthy controls.[18] GWAS have identified SNPs associated with disease risk by analyzing genotype data for hundreds of thousands to millions of SNPs occurring at high frequencies (typically ≥1%) in the population, followed by association analyses of case-control samples. Eight SNPs have been identified that are significantly correlated with anorexia nervosa. The impact of these SNPs on disease onset (odds ratio) is small, below 1.2. Such SNPs with small effects are hypothesized to exist in large numbers across the genome; considering all SNPs together, they are thought to exert a relatively marked influence on disease onset. Furthermore, analyses examining genetic correlations between multiple SNPs across two disorders confirmed that anorexia nervosa shows positive correlations with obsessive-compulsive disorder, depression, anxiety disorders, and schizophrenia, indicating similarities in their genetic basis. Conversely, negative correlations were observed with body fat, BMI, obesity, type 2 diabetes, and insulin resistance, while a positive correlation was found with HDL cholesterol. The fact that anorexia nervosa correlates not only with other psychiatric disorders but also metabolic indicators and metabolic diseases suggests the involvement of metabolic abnormalities in its pathology.

Meanwhile, studies focusing on rare CNVs occurring at frequencies below 1% have also been reported. CNVs refer to changes where a genomic region normally present in two copies is reduced to one copy or less (deletion) or increased to three copies or more (duplication). While healthy individuals also possess numerous CNVs, some are known to be associated with disease risk. Links to psychiatric disorders, including schizophrenia and ASD, have been reported in numerous studies, including those by our group.[9][13] Genes within CNV regions exhibit altered expression levels. Changes in the expression of genes involved in central nervous system development, in particular, are considered to cause abnormalities in brain development, potentially leading to the onset of psychiatric disorders. Previous studies reported anorexia nervosa patients harboring known CNVs associated with schizophrenia and ASD (deletions or duplications located in the chromosomal regions 1q21.1, 15q11.2, 15q13.3, 16p13.1, and 16p11.2, which are also large in size, exceeding 1 million base pairs).[17][19] However, no evidence was found of a significant correlation between CNVs

and the risk of developing anorexia nervosa. To examine this, the authors performed CNV analysis in Japanese patients with severe eating disorders, as described in the next section.[8)]

I. Methods and Results

The authors' study[8)] was conducted with the approval of the Ethics Review Committee of Nagoya University Graduate School of Medicine and after obtaining written informed consent from all participants. High-resolution array CGH was used in this study to identify CNVs, including those with a small size (less than 100,000 base pairs), which have not been sufficiently investigated to date. Note that array CGH became covered by insurance in 2021, and is now available when hereditary diseases caused by CNVs are suspected. We performed whole-genome CNV analysis involving 70 patients with eating disorders and 1,036 healthy controls (all study participants were Japanese women). Patients met DSM-5 criteria for either anorexia nervosa or avoidant/restrictive food intake disorder, and had a minimum post-onset BMI of ≤15 kg/m² (range: 8.0–14.9 kg/m², median: 11.3 kg/m²). Previous studies reported CNVs associated with neurodevelopmental disorders (ASD, ADHD, intellectual disability, etc.) in patients with eating disorders. Therefore, we focused on such CNVs in our analysis.

As a result, we identified neurodevelopmental disorder-associated CNVs in 10% of patients (7/70) and 2.3% of healthy controls (24/1,036). Statistical analysis confirmed a significant correlation with the eating disorder onset risk (odds ratio = 4.69, $P = 0.0023$). CNVs found in patients included 45,X (Turner syndrome) and deletions of seven genes associated with neurodevelopmental disorders (*KATNAL2*, *DIP2A*, *PTPRT*, *RBFOX1*, *CNTN4*, *MACROD2*, and *FAM92B*).

45,X causes Turner syndrome through the loss of one of the two X chromosomes in females. Recent large-scale studies reported that 45,X (Turner syndrome) is associated with risks of intellectual disability, ASD, schizophrenia, and eating disorders.[2)] Furthermore, basic research has shown that four genes: *PTPRT*, *DIP2A*, *RBFOX1*, and *CNTN4,* are involved in the formation and function of synapses in nerve cells. Synapses are junctions connecting nerve cells where information is transmitted via neurotransmitters. Animal experiments have revealed that synapses are closely involved in the mechanisms of learning and memory. It has been reported that about 5% (1,100 genes) of the approximately 20,000 genes in humans are expressed and

function in synapses.[7] Many synapse-related genes are also known to be associated with risks of schizophrenia and ASD.

PTPRT (protein tyrosine phosphatase receptor type T) encodes tyrosine phosphatase and is involved in glutamate receptor expression, membrane translocation, and regulation of GABA neurotransmission at synapses.[11] Mice lacking this gene exhibit reductions in food intake and the body fat percentage, along with resistance to obesity even when fed a high-fat diet.[5] *DIP2A* (disco interacting protein 2 homolog A) is involved in the synthesis of acetylated coenzyme A (one of the essential coenzymes) and primarily expressed in the brain. Dip2a knockout mice exhibit abnormal dendritic spine formation, impaired synaptic transmission, and ASD-like behaviors.[12] *RBFOX1* (RNA binding fox-1 homolog 1) regulates alternative splicing of genes involved in brain development.[10] It also plays a crucial role in the formation of excitatory and inhibitory synapses. *CNTN4* (contactin 4) is involved in synaptic plasticity, and mice lacking this gene show enhanced fear conditioning.[14] Abnormal fear conditioning has been reported to be involved in anorexia nervosa.[15]

To further investigate the involvement of synaptic dysfunction in the pathogenesis of eating disorders, we employed a genetic statistical approach known as gene set analysis. We utilized Synaptic Gene Ontology (SynGO),[7] a database of synaptic molecular functions and biological processes. Based on synaptic-related gene sets registered in SynGO, we examined which gene sets (i.e., synaptic molecular functions and biological processes) patient CNVs accumulated in. The results revealed a significant enrichment of patient CNVs in "synaptic signaling" (GO:0099536) (odds ratio = 2.55, P = 0.0254). Thus, gene set analysis also suggested that impairments in synaptic transmission (information transfer between neurons via synapses) may be involved in the pathogenesis (Figure).

II. Discussion

The authors' findings suggest that: (i) CNVs associated with neurodevelopmental disorders contribute to the risk of severe eating disorders, and (ii) synaptic transmission dysfunction may be involved in the pathogenesis. Patients with eating disorders show a significantly higher comorbidity rate of ASD, and ASD is also more frequent among their blood relatives, suggesting shared genetic factors between anorexia nervosa and neurodevelopmental disorders. This study provides evidence for the involvement of CNVs as a common

genetic factor. Synaptic dysfunction has been supported by numerous studies, including genomic analyses, as contributing to the pathogenesis of psychiatric disorders such as schizophrenia and ASD. Surprisingly, there has been little genetic evidence to date supporting the involvement of synaptic dysfunction in eating disorders. The identification of this association with synaptic dysfunction may advance our understanding of the pathophysiology of eating disorders.

One strength of this study was the comprehensive identification of CNVs, including small-scale CNVs (less than 100,000 base pairs) that were difficult to detect in conventional CNV studies, achieved through the use of high-resolution array CGH. This includes deletions in neurodevelopmental disorder-associated genes (*KATNAL2*, *PTPRT,* and *CNTN4*). However, the sample size analyzed in this study was relatively small (70 eating disorder patients). Therefore, genome analyses using larger cohorts is necessary to confirm the reproducibility of these findings.

Conclusion

The authors' research provides results supporting the involvement of neurodevelopmental disorder-associated CNVs and synaptic dysfunction in the etiology and pathogenesis of eating disorders. Future studies should investigate how risk variants affect synaptic function through analyses of patient-derived iPS cells and model animals. Elucidating the neural circuit-level changes induced by synaptic dysfunction may advance our understanding of the pathophysiology of eating disorders. Previously, the molecular pathophysiology of ASD was unclear, but the identification of risk variants through genomic analysis, followed by model-based studies, has progressed understanding of the disorder.[4] It is hoped that the outcomes of genomic analysis of eating disorders will eventually lead to the development of preventive methods, early diagnostic approaches, and novel therapeutic drugs.

This paper was based on a recent research article[8] published in PCN. At the request of the editorial board, one of the authors rewrote it in Japanese and added commentary on its significance and future prospects.

Conflicts of Interest

Kushima received a research grant from the SENSHIN Medical Research Foundation. Ozaki has received research support, honoraria, or served as a consultant for Sumitomo Pharma Co., Ltd., Eisai Co., Ltd., Otsuka

Pharmaceutical Co., Ltd., Earth Comfort Institute, Inc., Mitsubishi Tanabe Pharma Corporation, Shionogi & Co., Ltd., Eli Lilly Japan K.K., Mochida Pharmaceutical Co., Ltd., Daiichi Sankyo Company, Limited, Japan Mediphysics Co., Ltd., Takeda Pharmaceutical Company Limited, Meiji Seika Pharma Co., Ltd., EA Pharma Co., Ltd., Pfizer Japan Inc., MSD K.K., Lundbeck Japan K.K., and Taisho Pharmaceutical Co., Ltd. The other authors declare no conflicts of interest related to this article.

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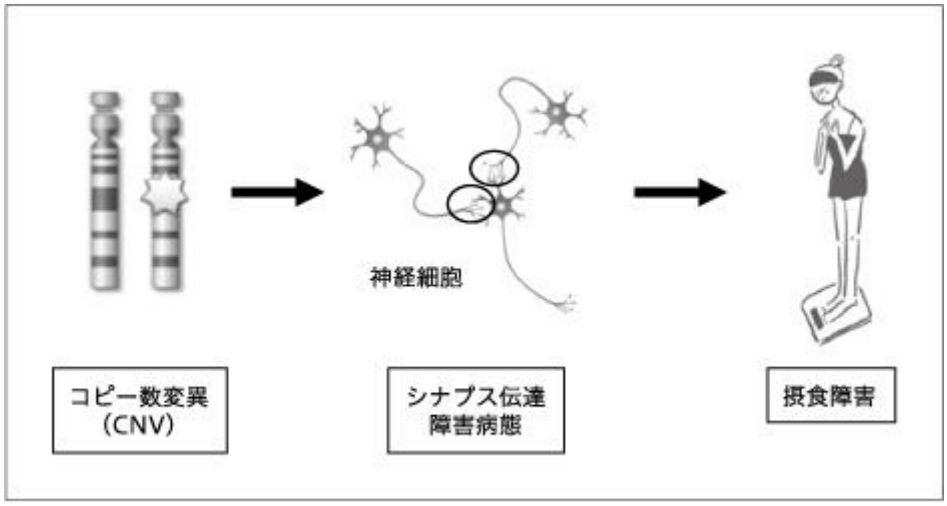

図　摂食障害の病態におけるシナプス伝達障害の関与

Figure: Involvement of synaptic transmission disorders in the pathophysiology of eating disorders