

博士論文（要約）

Analysis of neural development using human iPS cells  
carrying genetic mutations related to neuropsychiatric disorders

（精神神経疾患に関与する遺伝子変異を持つ  
ヒト iPS 細胞を用いた神経発生解析）

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Schizophrenia is a complex disorder with multiple symptoms and is heavy burden not only on individuals but also on the society. Genetic risk factors, prenatal and postnatal development, and life experiences may contribute to its pathophysiology, but their interactions remain mostly unknown. One of the major obstacles to understanding genetic factors in schizophrenia is the limited success of genome-wide association studies (GWAS) in the identification of a clear relationship between specific genetic variation and schizophrenia. Although GWAS studies identified multiple common variants associated with schizophrenia, their effect sizes were still relatively small (median relative risk: 1.08), and in some cases, their reproducibility was questioned. Contrarily, the 22q11.2 deletion syndrome (22qDS) is associated with a 30 to 40% lifetime risk of developing schizophrenia (odds ratio: 67.7) and have been recently focused on.

Previous papers suggested that schizophrenia and 22qDS converge on common pathways such as within synaptogenesis and synaptic functions. But the causal link between these disturbances and behavioral or cognitive symptoms of the patients is hardly confirmed clinically. Also, animal models have been recognized to be not enough to predict drug response in human. Moreover, the unique genomic structure of the human low copy repeats on chromosome 22q11.2 has been recently reported to be an underlying cause of phenotypic variability among patients with 22qDS. In this regard, morphological and functional analysis

using human iPS cells (hiPSCs) has a potential to be a novel method to bridge the gap between animal model and human clinical studies.

However, cell biological studies using a hiPSC-derived single neuron carrying 22qDS have been relatively scarce. The limited number of studies in this direction is partly because the previous production methods of hiPSC-derived neurons were exceedingly time-consuming. To overcome this technical limitation, researchers established the forced expression of a single neural transcriptional factor neurogenin-2 to induce functional neurons from hiPSCs rapidly. In this study, I aimed to establish a basic in vitro culture system for analyzing neural development using hiPSCs carrying genetic mutations.

First, hiPSCs derived from patients with 22qDS and neurotypical (NT) individuals were differentiated into a neuronal lineage by inducible and transient expression of neurogenin-2. RNA-seq of hiPSC-derived neurons indicated the preservation of the overall developmental pattern of gene expression in 22qDS-derived hiPSCs. Within the culture period of four weeks, the transcripts of the neuronal cytoskeleton and presynaptic cell adhesion molecules showed upregulation. In turn, increases in transcripts of postsynaptic cell adhesion molecules and scaffolding molecules were not evident. This delay in upregulation of genes related to postsynaptic proteins exists in rodent neurons in culture, but with a much

faster time-course.

The co-culture of hiPSC-derived neurons with mouse glial cells accelerated the neuronal differentiation. Primary branches of neurites were fewer in neurons differentiated from 22qDS-derived hiPSCs than in neurons from NT individuals. This phenotype appeared only in the presence of glial cells, suggesting that 22qDS-derived and NT-derived neurons responded differently to glia-derived extracellular signals. Glial contribution to the differentiation of hiPSCs is consistent with previous findings in glia-neuron co-culture systems.

This thesis supports the feasibility of morphological phenotyping of psychiatric disease-related genome alterations using hiPSCs-derived neurons in vitro and may help discriminate contributions of cell-autonomous and extrinsic factors. Translational research based on in vitro neural development, combined with other analytical methods, may clarify complex interactions between genetic and environmental factors associated with 22qDS and schizophrenia and to lead to alleviating the burden of individuals and society in the future.