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Differential gene expression profiles in neurons generated from lymphoblastoid B-cell line-derived iPS cells from monozygotic twin cases with treatment-resistant schizophrenia and discordant responses to clozapine

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ABSTRACT

Schizophrenia is a chronic psychiatric disorder with complex genetic and environmental origins. While many antipsychotics have been demonstrated as effective in the treatment of schizophrenia, a substantial number of schizophrenia patients are partially or fully unresponsive to the treatment. Clozapine is the most effective antipsychotic drug for treatment-resistant schizophrenia; however, clozapine has rare but serious side-effects. Furthermore, there is inter-individual variability in the drug response to clozapine treatment. Therefore, the identification of the molecular mechanisms underlying the action of clozapine and drug response predictors is imperative. In the present study, we focused on a pair of monozygotic twin cases with treatment-resistant schizophrenia, in which one twin responded well to clozapine treatment and the other twin did not. Using induced pluripotent stem (iPS) cell-based technology, we generated neurons from iPS cells derived from these patients and subsequently performed RNA-sequencing to compare the transcriptome profiles of the mock or clozapine-treated neurons. Although, these iPS cells similarly differentiated into neurons, several genes encoding homophilic cell adhesion molecules, such as protocadherin genes, showed differential expression patterns between these two patients. These results, which contribute to the current understanding of the molecular mechanisms of clozapine action, establish a new strategy for the use of monozygotic twin studies in schizophrenia research.

Abbreviations: CLZ, clozapine; iPS, induced pluripotent stem; FDR, false discovery rate; DAVID, Database for Annotation, Visualization and Integrated Discovery; GO, gene ontology.

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1. Introduction

Schizophrenia is a severe neuropsychiatric disease with an approximate worldwide prevalence of 1% (Gaebel and Zielasek, 2015; Millan et al., 2016; Wang et al., 2015). Common symptoms include delusions, hallucinations, impaired cognitive function, emotional blunting and incoherent behavior. Although many typical and atypical antipsychotic drugs have been developed and demonstrated to be effective in the treatment of schizophrenia, 20–30% of patients remain partially or fully unresponsive to two or more adequate trials with antipsychotic drugs and are therefore classified as treatment resistant (Kane et al., 1988; Meltzer, 1997, 2013). Patients with treatment-resistant schizophrenia suffer disability and have a poor quality of life. Despite the social and economic burden of treatment resistance, the molecular pathophysiology of treatment-resistant schizophrenia remains poorly understood.

Clozapine is the only effective drug for treatment-resistant schizophrenia (Hill and Freudenreich, 2013; Meltzer, 2013; Raja and Raja, 2014). At six months, the response rate to clozapine in treatment-resistant schizophrenia patients is 60–70%. Although the efficacy of clozapine for treatment-resistant schizophrenia is remarkable, clozapine has life-threatening side effects, such as agranulocytosis, which requires medical monitoring. These factors limit the use of this compound in clinical practice. To maximize the efficacy and minimize the side effects of clozapine treatment, biologically validated predictors of the clozapine treatment response should be identified. However, considering that inter-individual variability in the drug efficacy of clozapine treatment exists and that the molecular mechanisms of clozapine action remain largely unclear, the current molecular knowledge of drug response predictors of clozapine is extremely limited (Gressier et al., 2016; Kohlrausch, 2013; Muller et al., 2013; Sriretnakumar et al., 2015).

Considering the very limited accessibility to live neurons from patients with schizophrenia, the molecular defects in neurons leading to the initiation and progression of this disease remain obscure. Accordingly, schizophrenia is poorly understood at the molecular and cellular levels. Modeling schizophrenia using induced pluripotent stem (iPS) cells offers an emerging opportunity to examine the mechanisms underlying complex disease pathogenesis (Falk et al., 2016; Imaizumi and Okano, 2014; Okano and Yamanaka, 2014; Wright et al., 2014). Particularly, iPS cell technologies can produce live human neurons with the genetic backgrounds that lead to schizophrenia (Bundo et al., 2014; Hashimoto-Torii et al., 2014; Maekawa et al., 2015; Robicsek et al., 2013; Topol et al., 2016; Wen et al., 2014; Yoon et al., 2014). Considering the high heritability of schizophrenia, schizophrenia patient-derived live neurons with the same genetic information as the patient can be ideal experimental materials for studies of the molecular pathophysiology of schizophrenia.

Monozygotic twins have been useful resources for studying the genetic and environmental factors of schizophrenia (Boomsma et al., 2002; Kato et al., 2005). Molecular biological methods using blood samples and brain imaging methods have been conventionally used in discordant monozygotic twins to investigate the potential mechanisms of schizophrenia. Currently, to the best of our knowledge, there are no reports on the use of iPS cell technology in monozygotic twin studies of schizophrenia. In the present study, to elucidate the molecular mechanism of inter-individual variability in the clozapine response and clozapine action, we focused on monozygotic twin cases with treatment-resistant schizophrenia who were discordant for clozapine treatment, i.e., one twin responded well to clozapine treatment and the other twin did not. We established iPS cells from immortalized B cells of each patient and neurons were differentiated from these iPS cells. We subsequently performed RNA sequencing to compare the transcriptome profiles of the mock or clozapine-treated neurons as well as lymphoblastoid B-cell lines from these patients and observed that several genes, including cell adhesion molecules, showed differential expression patterns between these two patients.

2. Materials and methods

Following a description of the study, written informed consent was obtained from each subject. This study was conducted in accordance with the World Medical Association's Declaration of Helsinki and approved through the Research Ethics Committee of Osaka University. All recombinant DNA experiments were reviewed and approved by the Gene Modification Experiments Safety Committee of Osaka University.

2.1. Subjects

The monozygotic twin patients with treatment-resistant schizophrenia were recruited at Osaka University Hospital. Each subject was diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on a structured clinical interview. Treatment-resistant schizophrenia was defined according to the following criteria mentioned in the clozapine drug information in Japan: 1) No or little response to treatment from at least two adequately dosed antipsychotic trials for at least 4 weeks (including at least 1 second-generation antipsychotic, >600 mg/day of chlorpromazine equivalent) and Global Assessment of Functioning (GAF) scores never higher than 41; or 2) intolerance to at least two second-generation antipsychotics due to extrapyramidal symptoms. The subjects included in the present study met the criterion of little response. Symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Written informed consent was obtained from subjects after the procedures were fully explained.

2.1.1. Clozapine-responder (CLZ-responder)

The patient was a 59-year-old Japanese female diagnosed with treatment-resistant schizophrenia whose symptoms were improved after clozapine treatment. The PANSS and GAF scores prior to clozapine treatment were PANSS positive, 19; negative, 25; general, 44; and GAF, 23. After clozapine treatment, the PANSS and GAF scores were PANSS positive, 14; negative, 19; general, 40; and GAF, 33. Prior to clozapine treatment, the patient could not leave her house and could not perform housework due to delusions. However, after clozapine treatment, the patient could leave the house and perform housework, reflecting the improvement of positive and negative symptoms.

2.1.2. Clozapine-non-responder (CLZ-non-responder)

The patient was a 59-year-old Japanese female diagnosed with treatment-resistant schizophrenia whose symptoms were not improved after clozapine treatment. The PANSS and GAF scores prior to clozapine treatment were PANSS positive, 29; negative, 20; general, 48; and GAF, 23. After clozapine treatment, the PANSS and GAF scores were PANSS positive, 26; negative, 23; general, 51; and GAF, 21. The patient was constantly affected by delusions concerning God before and after clozapine treatment.

2.2. Generation of iPS cells

The generation of iPS cells from lymphoblastoid B-cell lines was performed as previously described (Fujimori et al., 2016). The isolation of lymphocytes from patient blood samples and the immortalization of these cells using Epstein-Barr virus were performed by Special Reference Laboratories, Inc. (Tokyo, Japan) (Yamamori et al., 2011). Immortalized lymphoblastoid B-cell lines obtained from the schizophrenia monozygotic twin were grown in Roswell Park Memorial Institute (RPMI) medium supplemented with 20% fetal bovine serum. The immortalized lymphoblastoid B-cell lines (2×10^6 cells) were electroporated with 0.63 μg of pCE-hOCT3/4, 0.63 μg of pCE-hSK, 0.63 μg of pCE-hUL, 0.63 μg of pCE-mp53DD and 0.50 μg of pCXB-EBNA1 (Addgene, MA, USA) using the Nucleofector 2b Device (Lonza,