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The cell cycle-related genes as biomarkers for schizophrenia

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ABSTRACT

Background: Recent studies suggest that genomic abnormalities such as single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) may elevate the risk of schizophrenia. Such genomic abnormalities often occur during chromosomal DNA replication in the S phase of cell cycle. In addition, several studies showed that abnormal expressions of several cell cycle-related genes are associated with schizophrenia. Therefore, here we compared mRNA expression levels of cell cycle-related genes in peripheral blood cells between patients with schizophrenia and healthy controls.
Method: mRNA expression levels of cell cycle-related genes in peripheral blood cells from patients with schizophrenia and healthy controls were measured with quantitative reverse transcription polymerase chain reaction (Q-RT-PCR). The discovery, replication and intervention studies with Q-RT-PCR were performed as follows: discovery (40 cases and 20 controls), replication (82 cases and 74 controls) and intervention (22 cases and 18 controls).
Result: Nine genes were identified in the discovery and replication stages as schizophrenia-associated genes. Moreover, the combination of mRNA expression levels of *CDK4*, *MCM7* and *POLD4* was identified as a potential biomarker for schizophrenia with multivariate logistic regression analysis. The intervention stage revealed that the mRNA expression levels of these three genes were significantly decreased in the acute state of schizophrenia, and *CDK4* was significantly recovered in the remission state of schizophrenia.
Conclusion: The combination of mRNA expression levels of three cell cycle-related genes such as *CDK4*, *MCM7* and *POLD4* is expected to be a candidate for useful biomarkers for schizophrenia. Especially, the mRNA expression changes of *CDK4* may be potential as both trait and state markers for schizophrenia.

1. Introduction

Schizophrenia is a chronic and disabling psychiatric illness affecting around 1% of the general population (Mueser and McGurk, 2004). Numerous genetic studies including twin researches suggest that a large number of genetic and environmental factors contribute to the development of schizophrenia (Harrison and Owen, 2003; Sullivan et al., 2003). In addition, recent genome-wide association studies (GWASs) and structural variation studies suggest that a lot of single nucleotide polymorphisms (SNPs) and genomic copy number variations (CNVs) contribute to the risk of schizophrenia (Bassett et al., 2010; Schizophrenia Working Group of the Psychiatric Genomics, 2014). However, it remains unclear which genomic abnormalities are essential in the pathophysiology of schizophrenia.

It is well established that genomic abnormalities including SNPs and CNVs likely occur during chromosomal DNA replication in cell cycle (Gu et al., 2008; Koren et al., 2012; Symington and Gautier, 2011). In addition, CNVs are produced by *de novo* mutation events in cell cycle and filtered out by natural negative selection and carriers of some CNVs have fewer offspring (Kirov, 2015). Nevertheless, approximately 1% of the population is affected by schizophrenia worldwide. These suggest that perturbation of cell cycle may be associated with schizophrenia. In fact, a growing body of evidence has accumulated in support of the hypothesis that perturbation of cell cycle is associated with schizophrenia. For example, the upregulation of cyclin D1 gene expression,

Abbreviations: AUC, area under the curve; BPRS, Brief Psychiatric Rating Scale; CDK, cyclin-dependent kinase; CNV, copy number variation; GABA, gamma-aminobutyric acid; GAF, Global Assessment of Functioning; MCM, mini-chromosome maintenance; POLD, polymerase (DNA-directed), delta; Q-RT-PCR, quantitative reverse transcription polymerase chain reaction; ROC curve, receiver operating characteristic curve; SNP, single nucleotide polymorphism; WBC, white blood cell.

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accompanied by increased cyclin D/cyclin-dependent kinase (CDK) 4-dependent phosphorylation of retinoblastoma protein, acting as a checkpoint for G1/S phase transition, has been observed in the postmortem cingulate gyri of patients with schizophrenia, suggesting abnormal cell cycle re-entry in postmitotic oligodendrocytes (Katsel et al., 2008). The expression of cyclin D2 is upregulated in hippocampal gamma-aminobutyric acid (GABA) cells in the brains of patients with schizophrenia (Benes et al., 2009). Olfactory cell cultures derived from biopsied nasal mucosa of patients with schizophrenia expressed higher levels of cyclin D1/E/A2, and had increased cell proliferation rates (Fan et al., 2012; Feron et al., 1999; McCurdy et al., 2006). Chronic hyperdopaminergic activity of schizophrenia is associated with increased ΔFosB levels and CDK5 signaling in the nucleus accumbens (Cantrup et al., 2012). Altered cell cycle and abnormalities in cell cycle-related genes have also been reported in fibroblasts derived from patients with schizophrenia (Wang et al., 2010). In addition, several past reports had shown that the numbers of atypical lymphocytes were increased in peripheral blood samples of patients with schizophrenia (Fessel and Hirata-Hibi, 1963; Kokai et al., 1998; Tachibana et al., 1981). These studies implicated the aberrant regulation of cell cycle-related genes in different types of tissues such as brain and peripheral blood in patients with schizophrenia. Therefore, the alteration of cell cycle-related genes in peripheral blood may be potential biomarkers for schizophrenia.

To test the possibility of cell cycle-related genes as biomarkers for schizophrenia, mRNA levels of cell cycle-related genes in peripheral blood were measured by quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) and compared between patients with schizophrenia and healthy controls.

2. Materials and methods

2.1. Subjects

This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine. All of the participants were Japanese descent and were recruited in the Kobe city area of Japan. After complete description of the study to the subjects, written informed consent was obtained.

The psychiatric assessment of each participant was performed as previously described (Okazaki et al., 2014; Yoshida et al., 2012). The patients with schizophrenia were diagnosed by at least two psychiatrists according to the DSM-IV criteria for schizophrenia based on unstructured interviews and reviews of their medical records at each hospital. The control groups were consisted of healthy volunteers. None of the control subjects had any present, past, or family (first-degree relatives) histories of psychiatric disorders or substance abuse excluding nicotine dependence. All of the control subjects were interviewed and screened for psychiatric disorders based on an unstructured interview performed by a psychiatrist.

A mRNA expression study with Q-RT-PCR was performed to identify cell cycle-related genes associated with schizophrenia (Fig. 1). We used independent cohorts in the discovery, replication and intervention stages, respectively. Demographic and clinical characteristics are shown in Table 1. The discovery cohort consisted of 40 unrelated patients with schizophrenia (20 males: 46.2 ± 12.2 years and 20 females: 50.7 ± 10.9 years) and 20 unrelated healthy controls (10 males: mean age ± SD, 51.3 ± 7.0 years and 10 females: 53.5 ± 4.6 years). The sex and age distributions were matched between the schizophrenia and control groups ($\chi^2 < 0.01$, $p = 1.00$ and $t = 1.42$, df = 58, $p = 0.16$; respectively). We tried to replicate the discovery stage results using the replication cohort consisting of 82 unrelated patients with schizophrenia (38 males, 61.1 ± 11.9 years and 44 females, 60.9 ± 16.1 years) and 74 unrelated healthy controls (44 males, 62.3 ± 7.1 years and 30 females, 65.2 ± 3.9 years). The sex and age distributions were matched between the schizophrenia and control groups ($\chi^2 = 2.68$, $p = 0.10$ and $t = 1.39$, df = 153, $p = 0.17$; respectively). In order to further investigate our results of the replication stage, we performed the experiments using the intervention cohort consisting of 22 unrelated patients with schizophrenia (12 males, 39.0 ± 13.2 years and 10 females, 47.9 ± 15.2 years) and 18 unrelated healthy controls (9 males, 39.8 ± 5.5 years and 9 females, 38.8 ± 3.9 years). The sex and age distributions were matched between the schizophrenia and control groups ($\chi^2 = 0.082$, $p = 0.78$ and $t = 1.05$, df = 38, $p = 0.30$; respectively). In the intervention stage, the patients with schizophrenia were admitted into the hospital with acute psychosis and followed until they were discharged after remission. The peripheral blood samples were collected in both the acute state (Global Assessment of Functioning [GAF], 28.1 ± 11.2 and Brief Psychiatric Rating Scale [BPRS], 60.7 ± 13.2) and remission state (GAF, 59.4 ± 9.3 and BPRS, 32.6 ± 12.4). A paired *t*-test revealed a significant difference in the GAF and BPRS scores between the two stages ($t = 10.9$, df = 21, $p < 0.001$ and $t = 12.4$, df = 21, $p < 0.001$; respectively).

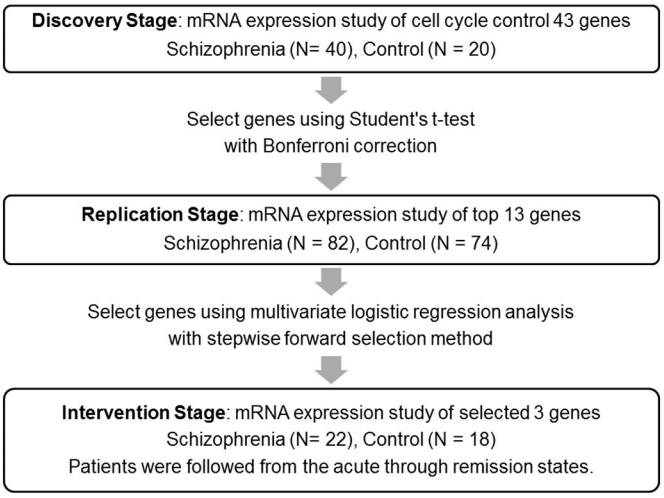

Fig. 1. Design of mRNA expression study with Q-RT-PCR.

Additionally, we performed DNA methylation and potential copy number study using a cohort consisting of 40 unrelated patients with acute schizophrenia (20 males, 40.2 ± 13.1 years and 20 females, 46.2 ± 11.0 years), 40 unrelated patients with chronic schizophrenia (20 males, 41.0 ± 10.5 years and 20 females, 41.8 ± 10.1 years) and 40 unrelated healthy controls (20 males, 41.3 ± 11.9 years and 20 females, 41.1 ± 11.4 years). Demographic and clinical characteristics are shown in Table 2. The sex and age distributions were matched among the three groups ($\chi^2 = 5.99$, $p = 1.00$ and F [2117] = 0.26, $p = 0.77$; respectively). Some participants were overlapped with the mRNA expression study.

2.2. mRNA expression study with Q-RT-PCR

In all the stages, total cellular RNA from whole-blood samples stocked in PAXgene RNA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) was manually prepared with the PAXgene Blood RNA kit (Qiagen, Hilden, Germany). Single-strand cDNA was synthesized from total RNA using SuperScript III First-Strand Synthesis System kit (Invitrogen, Carlsbad, CA). Q-RT-PCR was performed with TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA) using an ABI 7500 Real Time PCR System (Applied Biosystems). The PCR reaction was performed as follows: the initial step was 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. In the discovery stage, the primers and probes were from TaqMan Array Plate of Human Cell Cycle Control of Chromosomal Replication kits (Applied Biosystems). In the replication and intervention stages, TaqMan Gene Expression Assays (Applied Biosystems) were used as primers