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ORIGINAL ARTICLE

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Expression of *HSPF1* and *LIM* in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia

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Abstract We have previously reported the altered expressions of *HSPF1* and *LIM* in the lymphoblastoid cell lines (LCLs) derived from Japanese patients with bipolar disorder (bipolar I disorder). The altered expression at the LCL level would be useful for developing diagnostic markers as well as a cellular model for bipolar disorder. In this study, we extended our previous study by measuring their expressions using the following samples: (1) larger number of LCLs from Japanese subjects, (2) LCLs from Caucasian subjects, and (3) LCLs from patients with bipolar II disorder or schizophrenia. We confirmed the increased expression of *HSPF1* ($P=0.009$) and decreased expression of *LIM* ($P=0.001$) in the LCLs from patients with Japanese bipolar I disorder. These altered expressions were also observed in those from patients with Japanese bipolar II disorder ($P=0.002$ for *HSPF1* and $P=0.072$ for *LIM*). We also found the altered expressions of *HSPF1* in LCLs from Caucasian patients with bipolar II disorder ($P=0.011$) and *LIM* in those from patients with schizophrenia ($P=0.001$).

Keywords Lymphoblastoid cells · Heat shock protein (HSP) · *LIM* · Mental disorder

Introduction

Bipolar I disorder (manic-depressive illness) is one of the major mental disorders and affects 1% of populations. It is characterized by recurrent depressive and manic episodes with the increased risk for suicide (Goodwin and Jamison 1990). Twin, adoption, and family studies clearly show that genetic factors are involved in the pathogenesis of bipolar disorder (Gershon and Cloninger 1994). Pharmacological evidence suggests the involvement of monoaminergic systems and intracellular second messenger systems in bipolar disorder (Manji and Potter 1997). However, the etiology or biological pathogenesis of bipolar disorder has not been established. Although many linkage and molecular genetic studies have been performed, the uncertainty of phenotype definition and complex mode of inheritance impede the understanding of bipolar disorder at the molecular level by conventional strategies (Kato 2001a).

Gene expression analysis such as DNA microarray has a great advantage to identify genes or cascades involved in the complex diseases in a nonbiased manner (Mirnics et al. 2001). We have previously performed DNA microarray analysis of postmortem brains of patients with mental disorders including bipolar disorder, major depression, and schizophrenia, and identified several altered gene expressions in patients with bipolar disorder (Iwamoto et al. 2004). By utilizing the microarray data, we searched the potential molecular markers for bipolar disorder in the lymphoblastoid cells lines (LCLs). Although LCLs transformed by Epstein-Barr virus are not neuronal cells, they have been used as a cellular model for human diseases such as hypertension (Gruska et al. 1997), diabetes mellitus (Pietruck et al. 1998), Alzheimer's disease (Panov et al. 1999), Huntington's disease (Panov et al. 1999), and bipolar disorder (Emamghoreishi et al. 2000; Kato et al. 2003). During the course of the previous study, we found that *HSPF1* and *LIM* showed altered gene expressions in both postmortem brains and LCLs from Japanese patients with bipolar I disorder. Thus, these genes may be biologically and genetically important candidate genes for bipolar disorder (Iwamoto et al. 2004).

HSPF1 (HSP40) modulates the activity of HSP70 and directs unfolded proteins to HSP70, which leads to the

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translocation of proteins into mitochondria and endoplasmic reticulum (ER) (Fewell et al. 2001). The expression of *HSPF1* was up-regulated in postmortem brains and the LCLs from patients with bipolar I disorder. LIM protein plays an important role in regulating the intracellular calcium level by linking calcium channel beta and protein kinase C (Maeno-Hikichi et al. 2003). In postmortem brains, the expression of *LIM* was up-regulated in all mental disorders tested (bipolar disorder, major depression, and schizophrenia). Conversely, the expression of *LIM* was significantly down-regulated in the LCLs from patients with bipolar I disorder (Iwamoto et al. 2004).

In this study, we tested the altered expression of these genes in a larger number of Japanese LCLs and LCLs from different ethnic groups. In addition, we examined the expressions of these genes in bipolar II disorder and schizophrenia. While bipolar I disorder involves major depressive episodes and manic state, bipolar II disorder is associated with major depressive episodes and hypomania.

Materials and methods

Subjects

Japanese patients with bipolar I disorder and bipolar II disorder as well as healthy volunteers that had no history of psychiatric illness were recruited. Of the 33 control subjects and 26 patients with bipolar I disorder, 11 and 14 samples, respectively, were reported in the previous report (Iwamoto et al. 2004). Diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (American Psychiatric Association). Blood was collected when patients were in the euthymic state. Written informed consent was obtained from all the subjects. LCLs from Caucasian subjects with bipolar I disorder ($n=10$), bipolar II disorder ($n=14$), schizophrenia ($n=12$), and unaffected control ($n=13$) were obtained from National Institute of Mental Health (NIMH) genetics initiative pedigrees. Controls were subjects who were married into the bipolar or schizophrenia pedigrees. They had no mental disorders or offspring with bipolar disorder or schizophrenia. The characteristics of patients and control subjects are listed in Table 1. This study was approved by the Ethics Committee of the Brain Science Institute, RIKEN.

Lymphoblastoid cells

LCLs were established using Epstein-Barr virus (Kato et al. 2002). Briefly, lymphocytes were separated from peripheral blood and cultured with RPMI 1640 medium containing 20% fetal bovine serum (FBS), appropriate antibiotics, and supernatant of the B95-8 cell culture infected by Epstein-Barr virus. The cells were passaged every week until the cell line was established. Thereafter, the cells were passaged three times a week using similar medium, except for addition of 10% FBS. The cells were kept frozen until the experiment.

Table 1 Subject characteristics

	Number of subjects	Age (mean ± SD)	Gender (F:M)
Japanese			
Control	33	48.6 ± 12.7	11:22
Bipolar I disorder	26	50.5 ± 15.3	11:15
Bipolar II disorder	10	58.8 ± 11.0	8:2
Caucasian			
Control	10	55.0 ± 10.6	3:7
Bipolar I disorder	14	56.6 ± 9.9	4:10
Bipolar II disorder	12	54.0 ± 12.1	6:6
Schizophrenia	13	51.8 ± 13.8	4:9

RNA extraction, cDNA synthesis, and real-time quantitative PCR

Total RNA of LCL was extracted using Trizol reagent (Invitrogen, CA, USA) and then was treated with DNase I. Five micrograms of total RNA was used for cDNA synthesis by SuperScript II reverse transcriptase (Invitrogen). Real-time quantitative PCR using SYBR/GREEN I (Applied Biosystems, CA, USA) was performed with ABI PRISM 7900HT (Applied Biosystems). Comparative Ct method was employed for quantification according to the manufacture's protocol (Applied Biosystems). GAPDH was used for normalization. Measurement of Ct was performed at least in triplicate. Measured expression values were divided by the mean expression value of control subjects. Amplification of a single product in RT-PCR was confirmed by monitoring the dissociation curve and agarose gel electrophoresis. Primer sequences were described before (Iwamoto et al. 2004).

Statistical analysis

Statistical analysis was performed using SPSS 11.0J software (SPSS Japan, Tokyo, Japan). Mann-Whitney *U* test was employed in the statistical analysis of expression levels. The Bonferroni correction was employed for multiple comparison. Spearman's correlation coefficient was employed to examine the correlation between age and expression levels. $P<0.05$ (two tailed) was considered significant.

Results

In concordance with our previous study, the expression of *HSPF1* in LCLs was significantly up-regulated ($P=0.009$) and the expression of *LIM* was down-regulated ($P=0.001$) in Japanese patients with bipolar I

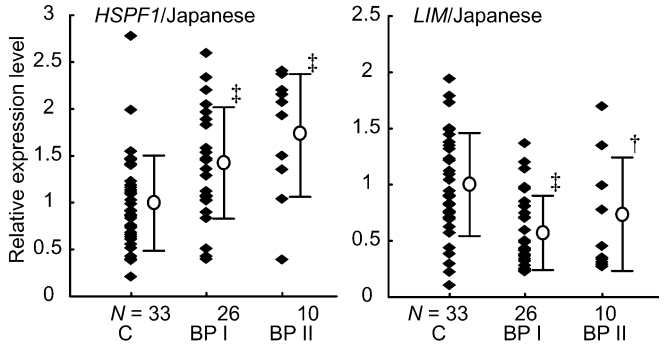

Fig. 1 Expressions of *HSPF1* and *LIM* in limphoblastoid cell lines (LCLs) from Japanese subjects. Real-time quantitative PCR using SYBR/GREEN I was performed. Each expression value was divided by the mean expression value of control subjects. *Open circle* represents the mean of each diagnostic category. *Bar* indicates standard deviation. *C* control; *BP I* bipolar I disorder; *BP II* bipolar II disorder; †, $0.050 \leq P < 0.100$; ‡, $P < 0.050$

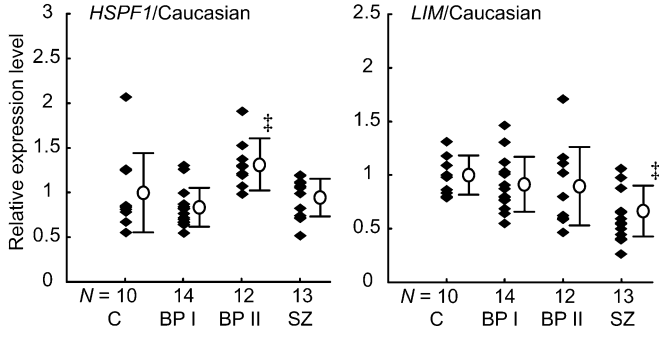

Fig. 2 Expressions of *HSPF1* and *LIM* in lymphyblastoid cell lines (LCLs) from Caucasian subjects. Each expression value was divided by the mean expression value of control subjects. *Open circle* represents the mean of each diagnostic category. *Bar* indicates standard deviation. *C* control; *BP I* bipolar I disorder; *BP II* bipolar II disorder; *SZ* schizophrenia; ‡, $P < 0.050$

disorder compared with control subjects (Fig. 1). We found that the expression of *HSPF1* was also up-regulated ($P = 0.002$) and the expression of *LIM* tended to be down-regulated in Japanese patients with bipolar II disorder ($P = 0.072$) compared with control subjects (Fig. 1). In the Caucasian samples, however, the expressions of *HSPF1* and *LIM* were not statistically different ($P = 0.371$ and $P = 0.235$, respectively) between control subjects and patients with bipolar I disorder (Fig. 2). In Caucasian patients with bipolar II disorder, the expression of *HSPF1* was significantly up-regulated ($P = 0.011$) and the expression of *LIM* was not different from control subjects ($P = 0.418$) (Fig. 2). In Caucasian patients with schizophrenia, the expression of *HSPF1* was not altered ($P = 0.927$), but we found significant down-regulation of *LIM* compared with control subjects ($P = 0.001$) (Fig. 2).

When the Bonferroni correction was employed for multiple comparison, expression differences were still significant in *HSPF1* ($P = 0.036$ for Japanese patients with bipolar I, $P = 0.008$ for Japanese patients with bipolar II, and $P = 0.044$ for Caucasian patients with bipolar II disorders) and *LIM* ($P = 0.004$ for Japanese patients with bipolar I disorder, $P = 0.004$ for Caucasian patients with schizophrenia). We concluded that all of our major findings were still significant after this correction.

We next assessed the effects of age and gender on expressions of *HSPF1* and *LIM*. We found no significant correlations between age of control subjects and expression of *HSPF1* ($R = -0.067$, $P = 0.709$, $n = 33$ in Japanese control subjects; $R = -0.158$, $P = 0.663$, $n = 10$ in Caucasian control subjects), or *LIM* ($R = -0.011$, $P = 0.953$, $n = 33$ in Japanese control subjects; $R = 0.243$, $P = 0.491$, $n = 10$ in Caucasian control subjects). We also found no correlations between age and expression levels when all samples were included in statistical analyses ($R = 0.004$, $P = 0.968$, $n = 118$ for *HSPF1*; $R = 0.118$, $P = 0.222$, $n = 118$ for *LIM*). There was no significant difference in the expression of *HSPF1* or *LIM* with regard to gender in Japanese ($P = 0.534$ for *HSPF1* and $P = 0.114$ for *LIM*) or Caucasian control subjects ($P = 0.517$ for *HSPF1* and $P = 0.517$ for *LIM*). We also found no significant gender difference of the expression of *HSPF1* or *LIM* when all samples were included in statistical analyses ($P = 0.190$ for *HSPF1*, $P = 0.103$ for *LIM*). We thus concluded that the expressions of *HSPF1* and *LIM* in LCLs were not influenced by age or gender.

Discussion

Several groups have reported endophenotypes at the peripheral blood cell level in bipolar disorder (Yamawaki et al. 1998). Most of them imply the anomaly of intracellular calcium metabolism in bipolar disorder. These include the enhanced responses to serotonin (Okamoto et al. 1995; Berk et al. 1996; Hough et al. 1999), thrombin (Dubovsky et al. 1989; Kusumi et al. 1992; Hough et al. 1999), and platelet activating factor (Dubovsky et al. 1989) in platelets, enhanced calcium signaling (Hough et al. 1999), and decreased response to phytohemagglutinin in T lymphocytes (Eckert et al. 1994; Emamghoreishi et al. 1997). These results suggest that certain endophenotypes of bipolar disorder can be studied by using nonneuronal samples such as peripheral blood cells. At the LCL level, increased cytosolic calcium levels and altered calcium signaling responses regulated by store-operated calcium channel, mitochondria, and ER have been reported (Emamghoreishi et al. 1997; Emamghoreishi et al. 2000; Kato et al. 2003). In addition, several genes such as *TRPC7*, *IMPA2* (Yoon et al. 2001a; Yoon et al. 2001b), and *NDUFV2* (Washizuka et al. 2003) have been proposed to be differentially expressed in LCLs from patients with bipolar disorder.

In the previous study, we reported the altered expressions of *HSPF1* and *LIM* in postmortem brains and LCLs from patients with bipolar disorder, both of

which are considered to play important roles in the regulation of calcium signaling (Iwamoto et al. 2004). Since *HSPF1* is involved in the translocation of proteins into mitochondria and ER (Fewell et al. 2001), the increased expression of *HSPF1* might be involved in the aberration of protein translocation systems into mitochondria and ER, which in turn affects the functions of these organelles found in patients with bipolar disorder (Kato 2001b; Kakiuchi et al. 2003). On the other hand, LIM protein regulates the intracellular calcium level by linking calcium channel beta and protein kinase C (Maeno-Hikichi et al. 2003).

In the present study, we extended our previous study by increasing the number of Japanese LCL samples, and we confirmed the altered expressions of *HSPF1* and *LIM* in patients with bipolar I disorder. Moreover, their altered expressions were also found in LCLs from Japanese bipolar II disorder. However, we could not confirm their altered expressions in the Caucasian bipolar I disorder samples. This is likely due to the ethnic difference, because the average expression level of *LIM* (but not *HSPF1*) in Caucasian controls was higher than in Japanese controls ($P = 0.006$). Considering the limited number of Caucasian samples, however, we could not rule out the possibility that sample number was not enough to detect the expression differences. Otherwise, since Caucasian controls in our study were mainly selected from NIMH genetics initiative pedigrees, this might reflect a genetic predisposition to bipolar disorder in spouses of patients with bipolar disorder. There is a possibility that patients with bipolar disorder tend to be married to a subject with the same illness, called "assortative mating" (Mathews and Reus 2001). This finding, however, is controversial (Mathews and Reus 2001).

As predicted, the expression of *HSPF1* was not increased in LCLs from patients with schizophrenia, since increased expression was found only in postmortem brains of patients with bipolar disorder and not in those with schizophrenia (Iwamoto et al. 2004). In addition, we found that the expression of *LIM* was down-regulated in patients with schizophrenia. Decreased expression of *LIM* in LCLs from patients with schizophrenia was in agreement with the previous microarray results that showed altered expression of *LIM* in postmortem brains of three mental disorders (bipolar disorder, major depression, and schizophrenia) (Iwamoto et al. 2004). This suggests that there may be differences of certain phenotypes at the LCL level such as intracellular calcium metabolism in schizophrenia (Lidow 2003). Decreased expression of *LIM* in schizophrenia, however, should be examined in a larger LCL sample and those derived from different ethnic groups.

Considering the fact that gene expressions in LCLs may reflect their genetic backgrounds (Cheung et al. 2003), the results should be carefully interpreted with regard to the effects of interindividual and ethnic differences. Indeed, we found such considerable differences in the expression values of LCLs. Our discriminant analysis using expression data of *HSPF1* and *LIM* could not correctly classify the control subjects and patients with bipolar disorder (data not shown). Use of more differentially expressed genes, such as *IMPA2*, *TRPC7* and *NDUFV2*, might improve the diagnostic values of gene-expression analysis.

In conclusion, we could confirm the altered expressions of *HSPF1* and *LIM* in LCLs from Japanese patients with bipolar I disorder. These altered expressions were also found in Japanese patients with bipolar II disorder. In the Caucasian samples, although we could not confirm all of our previous findings, we found the altered expressions of *HSPF1* in LCLs from patients with bipolar II disorder and *LIM* in those with schizophrenia.

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