Genotype Distributions and Allele Frequencies of Possible Major Depressive Disorder-Associated Single Nucleotide Polymorphisms, Cyclic Adenosine Monophosphate Response Element Binding Protein 1 rs4675690 and Piccolo rs2522833, in a Japanese Population

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It is known that the onset of major depressive disorder (MDD) would be associated with genetic factors. To investigate the susceptibility to psychiatric disorders, e.g. MDD, schizophrenia etc., it is necessary to compare the genetic differences of objective polymorphisms between in patients and in relative control subjects. Recently, an increasing number of studies focused on the role of cyclic adenosine monophosphate response element binding protein 1 (CREB1) and Piccolo (PCLO) on MDD. However, there was no report about genetic characterization of polymorphisms in between MDD patients and healthy subjects in Japanese population. We analyzed genotype distributions and allele frequencies of CREB1 rs4675690 and PCLO rs2522833 polymorphisms in 267 Japanese subjects, respectively. In CREB1 rs4675690, C allele frequency (0.59) was lower than T allele (0.59). While in PCLO rs2522833, A allele frequency (0.45) was lower than C allele (0.55). Our findings may be useful for investigating the genetic factors concerning the susceptibility to MDD in Japanese population.

Key words cAMP response element binding protein 1; Piccolo; polymorphism; Japanese; major depressive disorder

Major depressive disorder (MDD) is a familial disorder and its familiarity mostly or entirely results from genetic influence. To investigate disease susceptibility affected by genetic polymorphism, it is often performed with comparison of genotype distributions and allele frequencies of objective polymorphism in patients with in its relative control subjects.

Recently, an increasing number of studies focused on the role of transcription factor, cyclic adenosine monophosphate response element binding protein (CREB1) and presynaptic cytomatrix protein, Piccolo (PCLO) on MDD. CREB1 was reported that the association with neuronal signal transduction involved in synaptic plasticity and antidepressant response. In Caucasian population, CREB1 rs4675690 polymorphism was associated with anger expression in male MDD patients and suicidal behavior in MDD patients. While PCLO was localized in the presynaptic active zone and involved in monoamine neurotransmission. PCLO polymorphism rs2522833 was related with hypothalamic-pituitary-adrenocortical (HPA) regulation during antidepressant treatment and the susceptibility to MDD in Caucasian population.

It was suggested that these polymorphisms may be associated with the susceptibility to MDD and therapeutic efficacy of antidepressants in Japanese population. However there was no report about CREB1 rs4675690 and PCLO rs2522833 polymorphisms in Japanese population. In this study, we examined genotype distributions and allele frequencies of CREB1 rs4675690 and PCLO rs2522833 polymorphisms in 267 Japanese healthy subjects.

MATERIALS AND METHODS

Japanese Subjects This study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and approved by the Ethics Committee of the University of Shizuoka, Shizuoka, Japan (approved number 18-2). A total of 267 Japanese subjects (137 males and 130 females, mean age 35.9±12.7 years olds) were recruited. Written informed consent was obtained from an individual after a detailed briefing of the study purposes and protocols.

Genotyping Procedure Venous blood (5mL) was obtained from a subject using ethylenediaminetetraacetic acid (EDTA)-2Na Venoject II tubes (Terumo, Tokyo, Japan). Leukocyte genomic DNA was extracted directly from the blood specimen using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) followed by its instruction. The genotype of CREB1 rs4675690 was analyzed by newly established allele-specific polymerase chain reaction (PCR)-based method. In brief, genomic DNA (100ng) was amplified in PCR buffer containing 200μM deoxynucleotidetriphosphate (dNTP) mixture (Applied Biosystems), 1μM each of forward primer (5′-CTC AAC CTC CTG AGT AGC TAG-3′) and allele-specific reverse primer (for wild A allele: 5′-TGG TGC GAT CTC ATC TGT CTG ATC TGT CTG TGT TCT GTT TGT TCA-3′, for mutant G allele: TGT CTG ATC TGT TCT GTT TGT TG-3′), 1.25 units HotStar Taq plus DNA polymerase (Qiagen), and 1.5mM MgCl2. Amplification was performed by i-Cycler thermal cycler (Bio-Rad, Hercules, CA, U.S.A.). The PCR condition consisted of an initial denaturation/ enzyme activation step at 95°C for 5min, amplification for 33 cycles at 94°C for 30s, 59°C for 30s, and 72°C for 30s and final extension step at 72°C for 5min. The PCR products were electrophoresed on 3% agarose gel and genotype was identified by the appearance of amplified band (291bp) either with wild A allele reverse primer or with mutant G allele reverse primer (Fig. 1a).

Accuracy of allele-specific PCR method was confirmed by direct sequencing of PCR products amplified with primer sets (forward: 5′-TGG TGC GAT CTC AGC TCA CT-3′, reverse: 5′-ATG TGA CTT ACT GCC TCT CAG-3′) (Fig. 1b).
The genotype of \textit{PCLO} rs2522833 was determined by newly established PCR-restriction fragment length polymorphism (RFLP)-based method. In brief, genomic DNA (100 ng) was amplified in PCR buffer containing 200 $\mu$M dNTP mixture, 1 $\mu$M each of forward primer (5'-TGA CTG GAA TGA GAC TTG CCA-3') and reverse primer (5'-GTT TCC AGT ATC ATT GGT GAA G-3'), 1.25 units HotStar Taq plus DNA polymerase, and 1.5 mM MgCl$_2$. Amplification was performed by i-Cycler thermal cycler. The PCR condition consisted of an initial denaturation/enzyme activation step at 95°C for 5 min, amplification for 35 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s and final extension step at 72°C for 10 min. PCR products were digested by \textit{Alu} (New England BioLab., Beverly, Mass., U.S.A.). Digested products were electrophoresed on 3% agarose gel and the genotype was assigned by digestion patterns (wild \textit{A} allele: 278 bp, mutant \textit{C} allele: 196+82 bp) (Fig. 2a). Accuracy of PCR-RFLP method was confirmed by direct sequencing of amplified PCR product (Fig. 2b).

\textbf{Statistical Analyses} Genotype and allele frequencies were compared using the chi-square test with SPSS version 14.0 (SPSS Inc., Chicago, IL, U.S.A.). Differences were considered significant when $p<0.05$. Chi-square test was also used to compare results obtained with expected allele frequencies (Hardy–Weinberg equilibrium).

\textbf{RESULTS AND DISCUSSION} In this paper, we firstly reported the genotype distributions and allele frequencies of \textit{CREB1} rs4675690 and \textit{PCLO} rs2522833 polymorphisms in Japanese population. The results of \textit{CREB1} rs4675690 genotype distributions and allele frequencies were shown in Table 1. The wild \textit{CC} genotype frequency was lower than that of mutant \textit{TT} genotype in Japanese population. These results were in good accordance with the expected genotype distributions, calculated from...
Table 1. The Genotypes and Allele Frequencies of CREB1 rs4675690 and PCLO rs2522833 Polymorphisms in Japanese Subjects

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>n (%)</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREB1 rs4675690</td>
<td>CC</td>
<td>39 (14.6)</td>
<td>C: 0.410</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>141 (52.8)</td>
<td>T: 0.590</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>87 (32.6)</td>
<td></td>
</tr>
<tr>
<td>PCLO rs2522833</td>
<td>AA</td>
<td>47 (17.6)</td>
<td>A: 0.448</td>
</tr>
<tr>
<td></td>
<td>AC</td>
<td>145 (54.3)</td>
<td>C: 0.552</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>75 (28.1)</td>
<td></td>
</tr>
</tbody>
</table>

Hardy–Weinberg equilibrium ($p=0.559$). Serretti et al. reported that $C$ and $T$ allele frequencies in 76 healthy Caucasians were 0.61 and 0.39, respectively. In our study, $C$ and $T$ allele frequencies in Japanese population were 0.41 and 0.59, respectively. It was known that CREB1 plays an important role in MDD and antidepressant treatment. Although it is not clear the function at a molecular level in CREB1 rs4675690, Perlis et al. reported that individuals who are homozygous for the $T$ allele in rs4675690 were display less negative blood oxygenation level-dependent (BOLD) signals by functional magnetic resonance imaging (fMRI) than those carriers for angry and fearful faces. In other papers, Perlis et al. reported that $T$ allele in rs4675690 was associated with greater internal effort at anger control, greater risk of treatment-emergent suicidal ideation in MDD. There were reports that depressed patients are significantly greater levels of anger than normal controls and anger attacks are improved by antidepressant treatment. Anger might overlap only partially with their effects on depression. $T$ allele frequency of CREB1 rs4675690 in Japanese is higher than in Caucasian. We suggest it is more necessary to analyze the genotype of rs4675690 in between Caucasian and Japanese for examination of the susceptibility to MDD and the response of antidepressants. Moreover, it was known that the mutation of $S^r$ promoter of CREB1 gene was associated with the activation of cAMP pathway in culture cells. Rs4675690 was located at the $S^r$ region of CREB1 gene and may associate with the regulation of CREB1 expression. It was necessary to investigate the molecular function and the influence to MDD susceptibility in rs4675690. On the other hand, the results of PCLO rs2522833 genotype distributions and allele frequencies were shown in Table 1. The wild AA genotype frequency was lower than that of mutant CC genotype in Japanese population. These results were in good accordance with the expected genotype distributions, calculated from Hardy–Weinberg equilibrium ($p=0.735$). PCLO rs2522833 was known as a non-synonymous coding polymorphism in the C2A calcium-binding-domain of PCLO. There was a report that overexpression of PCLO C2A domain induced depression like behavior in transgenic mouse. $C$ allele in rs2522833 is suggested as a causal risk factor for MDD in Caucasian. Although it is not clear the function at a molecular level in rs2522833, Schuhmacher et al. reported that this polymorphism is associated with HPA system to antidepressant treatment in MDD. They reported that the individuals of homozygous for the $A$ allele in rs2522833 only displayed a decrease of cortisol and adrenocorticotropic hormone (ACTH) values during antidepressant treatment, whereas $C$ allele carriers did not. In Caucasian population, $C$ allele frequency of MDD and healthy population were 0.49 and 0.43 respectively. In our study, $C$ allele frequency in Japanese population was 0.55. $C$ allele frequency in rs2522833 in Japanese is higher than in Caucasian. We suggest it may be more important to analyze the genotype of rs2522833 in Japanese than in Caucasian for examination of the susceptibility to MDD and the response of antidepressants. We consider that it is necessary to investigate genetic frequencies of CREB1 rs4675690 and PCLO rs2522833, respectively, in MDD. Genotype distributions and allele frequencies of CREB1 rs4675690 and PCLO rs2522833 reported in this paper would be useful for examining the susceptibility to MDD in Japanese population.

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REFERENCES


