Available online at www.sciencedirect.com
ScienceDirect
Progress in Neuro-Psychopharmacology & Biological Psychiatry 32 (2008) 1000–1004
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

Calcineurin A gamma and B gene expressions in the whole blood in Japanese patients with schizophrenia

Masahiko Murata [a,*], Masahiko Tsunoda [b], Tomiki Sumiyoshi [b], Chika Sumiyoshi [c], Tadasu Matsuoka [a], Michio Suzuki [a], Miho Ito [a], Masayoshi Kurachi [d]

[a] *Department of Psychiatry, National Hokuriku Hospital, 5963 Nobusue, Nanto, Toyama, Japan*
[b] *Department of Neuropsychiatry, University of Toyama Graduate School of Medicine and Pharmaceutical Sciences, Toyama, Japan*
[c] *Faculty of Human Development and Culture, Fukushima University, Fukushima, Japan*
[d] *Department of Psychiatric Early Intervention, University of Toyama Graduate School of Medicine and Pharmaceutical Sciences, Toyama, Japan*

Received 26 October 2007; received in revised form 18 January 2008; accepted 19 January 2008
Available online 31 January 2008

Abstract

Calcineurin (CaN) has been regarded as a candidate gene for vulnerability of schizophrenia. Although CaN gene expression has been investigated with postmortem brain specimens or in association studies, little information is available about CaN gene expression levels in peripheral sources.

We obtained whole blood samples from 16 patients with schizophrenia and 16 controls, and total RNA was extracted. CaN A gamma and CaN B genes were analyzed by quantitative RT-PCR. In the patient group, expression levels of both genes were correlated with psychopathology, as measured by the Brief Psychiatric Rating Scale (BPRS), and neuroleptic dose.

No significant differences in CaN A gamma or CaN B gene expression were observed between patients with schizophrenia and normal controls. Linear regression analysis revealed that the CaN A gamma gene expression level was associated with the BPRS score but not with neuroleptic dose. Neither of the clinical variables was associated with the CaN B gene expression level.

The results of this study suggest that the CaN A gamma gene may be an effective predictor of the progression of psychosis. The effect of medications on expression of CaN genes requires further study.

Keywords: Calcineurin A gamma; Calcineurin B; Gene expression; Quantitative RT-PCR; Schizophrenia; Whole blood

1. Introduction

Schizophrenia is a psychiatric illness characterized by positive symptoms (delusions, hallucinations), negative symptoms (blunted affect, social withdrawal), and disturbances of cognitive function (attention, memory). As patients with schizophrenia exhibit various degrees of symptom severity, response to neuroleptic drugs, and prognosis, this disorder is thought to include heterogeneous population. Recent advances in molecular technology indicate that multiple genetic factors associated with neurotransmitters (e.g. dopamine (DA), glutamate) and synaptic functions are involved in the pathophysiology and heterogeneity of schizophrenia (Egan et al., 2001; Halim et al., 2003; Stefansson et al., 2002; Vawter et al., 2002; Vogel et al., 2004).

Calcineurin (CaN), also designated as protein phosphatase 2B (*PPP2B*), has been reported to regulate dopaminergic (Adlersberg et al., 2004; Takeuchi and Fukunaga, 2004) and *N*-methyl D-aspartate (NMDA) receptor-mediated glutamatergic (Lieberman and Mody, 1994; Tong et al., 1995) signal transduction. The CaN gene acts in the downstream of both signaling cascades, and modulates intracellular signal transduction (Greengard et al., 1999). CaN knockout mice show a range of behavioral abnormalities reminiscent of schizophrenic

Abbreviations: CaN, Calcineurin; PPP2B, protein phosphatase 2B; BPRS, Brief Psychiatric Rating Scale; qRT-PCR, quantitative real time polymerase chain reaction.

* Corresponding author.
E-mail address: muratam@hokuriku.hosp.go.jp (M. Murata).

0278-5846/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2008.01.013

symptoms, such as increased locomotor activity, decreased social interaction, impaired prepulse inhibition, and disturbed working memory (Miyakawa et al., 2003; Zeng et al., 2001).

Calcineurin is a Ca^{++}/calmodulin-dependent serine/threonine protein phosphatase (Rusnak and Mertz, 2000), and is densely expressed in the central nervous system (Klee et al., 1998; Shibasaki et al., 2002). Calcineurin protein is a heterodimer, consisting of a 61 kDa catalytic subunit (CaN A) and a 19 kDa regulatory subunit (CaN B) (Aramburu et al., 2000; Klee et al., 1979), and the presence of both subunits is essential for the activity of the phosphatase (Milan et al., 1994; Perrino et al., 1992). There are three isoforms for the CaN A subunit in the mammals, i.e. CaN A alpha, beta and gamma.

Expression of CaN A is decreased in the postmortem hippocampus from subjects with schizophrenia (Eastwood et al., 2005a). Specifically the CaN A gamma gene has been found to be associated with schizophrenia (Gerber et al., 2003; Yamada et al., 2007). The *PPP3CC* gene encoding CaN A gamma subunit and *PPP3R1* gene encoding CaN B subunit are located on human chromosome 8p (Gerber et al., 2003; Kendler et al., 1996; Straub et al., 2002; Wang et al., 1996) and 2p (Camp et al., 2001; Coon et al., 1998; Gerber et al., 2003), respectively. As these loci are implicated in schizophrenia susceptibility, it is hypothesized that altered levels of the CaN A gamma or CaN B gene would contribute to the development of psychotic symptoms.

Recently, blood-derived RNA has been shown to be usable for gene expression analysis (Tsuang et al., 2005; Vawter et al., 2004). Unlike the case with postmortem studies, this method makes it possible to estimate gene expression patterns from easily accessible peripheral sources. This prompted us to investigate if the levels of CaN A gamma and CaN B gene expression were altered in the whole blood RNA from subjects with schizophrenia as compared with normal controls. We also sought to test the hypothesis that expression levels of these genes would be correlated with severity of psychotic symptoms.

2. Materials and methods

2.1. Subjects

The patients and controls' profile are shown in Table 1. Sixteen patients who fulfilled ICD-10 criteria for schizophrenia and sixteen healthy volunteers as control subjects entered the study. The patients were recruited from the Department of Neuropsychiatry, University of Toyama Hospital or National Hokuriku Hospital. Control subjects with no psychiatric or neurological history were recruited from staffs of above hospitals. Diagnosis was made based on structured interview by experienced psychiatrists using SCID. Permission to carry out this study was obtained from the Ethics Committees of National Hokuriku Hospital and University of Toyama. After written informed consent was obtained, blood samples were collected around noon before lunch. All patients were evaluated psychotic symptoms with the Brief Psychotic Rating Scale (BPRS). They were treated with neuroleptics.

Table 1
Profile of subjects

	Patients with schizophrenia	Controls
Gender	7 male, 9 female	9 male, 7 female
Age (years)	34.1±8.6	33.3±9.9
BPRS (points)	23.8±10.1	
Neuroleptic dose[a] (mg/day)	577.4±437.8	

Values are mean±SD.
[a] Chlorpromazine equivalent dose.

2.2. RNA extraction

Total RNA was isolated from 2.5 ml of whole blood with Paxgene Blood RNA Kit (Pre-AnalytiX, Hombrechtikon, Switzerland). Genomic DNA was digested during the RNA isolation procedure with RNase-Free DNase Set (QIAGEN, Tokyo, Japan), according to the supplier's protocol. RNA was collected in approximately 80 μl of BR-5 buffer (PaxGene Blood RNA System) and stored at −80 °C until the preparation for quantitative real time PCR (qRT-PCR). Quality of RNA was confirmed by absorbance A260/A280 ratio, and RNAs with the ratio of more than 1.8 were used for qRT-PCR.

2.3. qRT-PCR

The samples were quantified by the absorbance of A260, and 1000 ng of RNA was used for reverse-transcription template. cDNA was synthesized by using Taqman Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) with an oligo d (T)16 primer. qRT-PCR was performed using Assays-on-Demand Gene Expression Products (Applied Biosystems, Foster City, CA). The primers chosen for RT-PCR were Hs 00194467 for CaN A gamma, and Hs 00168722 for CaN B. Hs 99999903 for beta-actin was used as an internal standard. All probes were synthesized with FAM-dye labeled by the manufacture (Applied Biosystems, Foster City, CA).

Following the manufacturer's amplification protocol (50 °C 2 min for AmpErase UNG activation, 95 °C 10 min for enzyme activation and 40 cycles of PCR, 95 °C 15 s for denature and 60 °C 1 min for annealing/extension), duplicated cDNA samples were applied on ABI PRISM 7700 Sequence Detection System. Beta-actin cDNA corresponding to each subject was also quantified simultaneously to normalize the CaN A gamma or B gene expression levels. The template concentration for the cDNA standard curve was set in a 16-fold dilution series of a reference sample of pooled control sample. All samples were automatically quantified simultaneously during the amplification process by comparison to the standard curve and expressed as a ratio relative to the reference sample. To rule out the genomic DNA contamination, samples were assessed by RNA free negative controls. When the difference between duplicated samples exceeded 20%, these samples were quantified again.

2.4. Statistical analysis

Data were analyzed using SPSS for Windows (version 13.0). *t*-test with Welch's method was carried out to compare CaN