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Lack of association between XBP1 genotype and calcium signaling in the platelets of healthy subjects

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Abstract

Dysregulations of calcium (Ca) homeostasis may be involved in the pathophysiology of bipolar disorder. Enhanced Ca response to various agonists in peripheral blood cells is one of a few confirmed biological markers for bipolar disorder. Recently, a polymorphism of XBP1, a pivotal gene in the endoplasmic reticulum (ER) stress response, was shown to contribute to the genetic risk factor for bipolar disorder. Thus, in this study, we examined the relationship between the XBP1 gene polymorphism and the Ca signaling in the platelets of healthy controls. The present results suggest no significant difference in the basal Ca level or 5-HT-induced Ca mobilization among normal subjects with –116C/C, C/G and G/G genotypes. Further investigations are necessary to examine the relationship in the different peripheral blood cells and/or in larger samples from patients with bipolar disorder.

Key words: Calcium; XBP1; Endoplamic reticulum; Bipolar disorder; Polymorphism; Healthy subject

Altered calcium (Ca) signaling has been reported in the peripheral blood cells of patients with bipolar disorder. We have reported that serotonin (5-HT)- or thrombin-induced intracellular Ca mobilization is enhanced in the platelets of unmedicated patients with bipolar disorder [1-3]. Other researchers also indicated similar findings in platelets [4-7], in neutrophils [8], and in transformed lymphoblastoid cells [9].

Our longitudinal follow-up study suggested that the enhanced Ca response is trait dependent [1] and that patients with marked increased Ca response are good responders to mood stabilizers such as lithium and valproate [10]. These findings in non-neuronal cells, which could provide clues to the molecular basis of the disease, suggest that the altered Ca mobilization might be involved in the pathophysiology of bipolar disorder. The enhanced Ca response could be due to altered functioning of endoplasmic reticulum (ER) [9], mitochondria [11] or some other signal transduction pathways [12,13]. ER is the site of complex processes such as Ca storage, Ca signaling, processing and folding of newly synthesized membrane and secretory proteins, and triggering of cell response to severe forms of stress, which interfere with ER functions [14]. Cell injury may develop under conditions where ER Ca homeostasis and/or folding or processing of proteins is disturbed (referred to as ER stress), leading to the activation of the unfolded protein response such as suppression of protein synthesis and expression of ER stress-related genes including *XBP1* and *GRP78/BiP* [14].

Recently, a polymorphism of the XBP1 gene was shown to contribute to the genetic risk factor for bipolar disorder [15]. The polymorphism (-116C→G) in the promoter region of XBP1 was significantly more common in Japanese bipolar patients (odds ratio=4.6) and overtransmitted to affected offspring in trio samples of the NIMH Bipolar Disorder Genetic Initiative [15]. XBP1-dependent transcription activity of the -116G allele was lower than that of the -116C allele, and in the cells with the G allele, induction of XBP1 expression after ER stress was markedly

reduced [15]. Moreover, valproate rescued the impaired response by inducing ATF6, the gene upstream of XBP1 [15]. Darier's disease, an autosomal-dominant skin disorder, which is sometimes accompanied by bipolar disorder, was reported to be caused by mutations in the ER calcium adenosine triphosphatase (Ca-ATPase) gene [16]. In addition, the recent finding that valproate increases expression of the ER stress protein also suggests that the ER plays a role in bipolar disorder [17].

Therefore, it is possible that the altered Ca signaling may be due to the dysfunction of ER stress response by -116G allele of the XBP1 gene. In this study we examined the relationship between the -116C/G polymorphism of the XBP1 gene and the basal Ca level or 5-HT-induced Ca response in the platelets of healthy subjects.

One hundred and seventeen unrelated healthy volunteers were all Japanese recruited from laboratory, office or hospital staff at Hokkaido University. They all underwent a direct interview to exclude psychiatric disorders classified according to DSM-IV. There were 82 males and 35 females, and the average age was 34.1 $\pm$ 8.7 (mean $\pm$ S.D.) years. They had no physical illness and were all drug free for at least 4 weeks before blood sampling. After complete description of the study, informed consent was obtained from all subjects. The research protocol was approved by the ethics committee of Hokkaido University Graduate School of Medicine.

The isolation of platelets and the measurement of intraplatelet Ca concentration were performed as described previously [18]. Briefly, platelet-rich plasma was incubated with 4 uM fura-2-AM (Dojindo, Kumamoto, Japan), a Ca sensitive

fluorescent probe, for 15 min at 37°C. After centrifugation, the resulting platelet pellet was suspended at 1 X 10^8 cells/ml in Krebs-Ringer HEPES buffer. The samples were prewarmed in a cuvette at 37°C for 4 min and then 10 uM 5-HT was added to the incubation medium. Fluorescence was measured on a Hitachi F-2000 fluorometer with excitation at 340 and 380 nm, and with emission at 510 nm. We measured both basal Ca concentration and the maximum Ca increase (initial peak− resting level) induced by 10uM of 5-HT.

DNA was extracted from 20 ml of whole blood by standard methods. For the genotyping of XBP1 gene -116C/G polymorphism, the TaqMan 5'-exonuclease allelic discrimination assay was used. Primers and probes for TaqMan assay were as follows:

primers, XBP1-F, CTGTCACTCCGGATGGAAATAAGTC, and XBP1-R, ATCCCTGGCCAAAGGTACTTG, and probes, XBP1-C, VIC-CTCCCGCACGTAAC-MGB, and XBP1-G, FAM-TCCCGCAGGTAAC-MBG. Amplification was performed under the following conditions: 10 min at 95°C, 40 cycles of 15 sec at 92°C and 1 min at 60°C in an GeneAmp PCR system 9700 thermocycler. Genotypes were determined using an ABI 7000 HT sequence detection system (Applied Biosystems, Foster City, CA, US).

The associations between the XBP1 gene polymorphism and, basal Ca level or 5-HT-induced Ca response were assessed by one-way ANOVA.

The basal Ca concentration and 5-HT-stimulated Ca response sorted by XBP1 gene polymorphism are shown in Table 1. Observed genotype distributions were

consistent with Hardy-Weinberg equilibrium. The distribution of the XBP1 genotypes in our sample was almost same as in another Japanese sample [15]. There was no significant difference in the basal Ca level or 5-HT-induced Ca mobilization among the healthy subjects with –116C/C, C/G and G/G genotypes (Table 1).

The following findings expect the possible relationship between the XBP1 gene polymorphism and the Ca signaling abnormality. First, the enhanced Ca response to various agonists in peripheral blood cells is one of a few confirmed biological markers for bipolar disorder [1-9]. Second, the dysregulations of Ca homeostasis may be involved in the pathophysiology of bipolar disorder, from the fact that the dysinhibition of Ca mobilization in the presence of PKC inhibitor is observed in bipolar disorder [12] and that agonist-induced Ca responses are enhanced in the presence of myosin light chain kinase inhibitor, which are reversed by treatment with lithium [13]. Third, it is possible that the ER dysfunction may result in abnormal Ca homeostasis. The Ca response to thapsigargin, a ER Ca^{2+}-ATPase inhibitor, are reported to be enhanced in bipolar patients compared to unipolar patients and normal controls [19]. Fourth, the DNA microarray analysis of lymphoblastoid cells derived from pairs of twins discordant with respect to bipolar disorder suggests that the XBP1 polymorphism may contribute to the genetic risk factor for the illness [15]. Last, the expression of GRP78/BiP, one of ER stress proteins, plays a direct and important role in Ca mobilization. It is reported that agonist-stimulated Ca response and thapsigargin-induced Ca release are enhanced in GRP78/BiP-transfected cells compared to control cells [20].

Unexpectedly, the present results did not indicate any significant relationship between XBP1 genotype and basal Ca concentration or 5-HT-induced Ca mobilization in the platelets of healthy subjects. Since drug-free samples are indispensable to measure intraplatelet Ca concentration [18], in this preliminary study we first examine the association between XBP1 gene polymorphism and Ca signaling in healthy controls. We have already indicated that the range of the 5-HT-stimulated Ca response in normal subjects is widely distributed and fairly overlapped with that in bipolar disorder [1]. Moreover, depressed patients with a Ca response above 280% of basal which corresponds to the value of the mean + 2SD for normal controls, exhibit a good response to mood stabilizers [10]. Thus, the higher Ca response depressed patients show, the more probably their diagnosis may be bipolar disorder. This parameter seems to be a continuous marker from normal to bipolar disorder. On the other hand, the G allele of XBP1 gene, a risk allele for bipolar disorder, is also observed in normal controls, not only in bipolar disorder [15]. Therefore, it is of significance to examine the relationship between the XBP1 genotype and the Ca response in normal subjects. Further studies are necessary to examine the relationship between them in patients with bipolar disorder. Another limitation of this study is to measure Ca response in the mature platelets that lack a nucleus and do not have a part of ER stress response including the XBP1 loop. Using lymphocytes or lymphoblastoid cells as samples may result in the different finding from the present study.

In conclusion, the present study suggests that the XBP1 gene polymorphism is not associated with the basal Ca level or 5-HT-stimulated Ca response in the platelets of healthy control subjects. Further investigations are needed to examine the relationship in the different peripheral blood cells and/or in larger samples from patients with bipolar disorder.

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Table 1. Basal Ca level and 5-HT-induced Ca response sorted by -116C/G polymorphism of XBP1 gene in Japanese healthy subjects

Ca signalling	Genotype			ANOVA
	C/C (N=12)	C/G (N=58)	G/G (N=47)	
Basal Ca level	63.0±7.8	59.8±2.2	61.4±2.0	F=0.23, p=0.79
5-HT-induced Ca response	76.3±8.0	88.5±4.4	85.8±3.6	F=0.84, p=0.44

Results are expressed as mean±SEM.