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Functional polymorphisms of HSPA5: Possible association with bipolar disorder

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Abstract

Altered endoplasmic reticulum stress (ER) response signaling is suggested in bipolar disorder. Previously, we preliminarily reported the genetic association of *HSPA5* (*GRP78*/*BiP*) with bipolar disorder. Here, we extended our analysis by increasing the number of Japanese case-control samples and NIMH Genetics Initiative bipolar trio samples (NIMH trios), and also analyzed schizophrenia samples. In Japanese, nominally significant association of one haplotype was observed in extended samples of bipolar disorder but not in schizophrenia. In NIMH trios, no association was found in total samples. However, an exploratory analysis suggested that the other haplotype was significantly over-transmitted to probands only from the paternal side. The associated haplotype in Japanese or NIMH pedigrees shared three common polymorphisms in the promotor, which was found to alter promotor activity. These findings suggested promotor polymorphisms of HSPA5 may affect the interindividual variability of ER stress response and may confer a genetic risk factor for bipolar disorder.

Keywords: Bipolar disorder; Schizophrenia; Endoplasmic reticulum stress; HSPA5/GRP78; Association study; Promotor assay

Bipolar disorder is a severe mental disorder characterized by recurrent episodes of mania and depression, affecting about 0.5–1% of the population [1]. Although the contribution of genetic factors has been evidenced by family, twin and adoption studies, the molecular pathophysiology of the illness has been controversial [2,3]. Recently, we suggested that the endoplasmic reticulum (ER) stress response signaling is one of candidate cascades related to pathology of the illness [4].

In our previous study, *XBP1* and *HSPA5* were down-regulated in the lymphoblastoid cells of monozygotic twins with bipolar disorder compared with healthy co-twins by DNA microarray analysis. Induction of *XBP1* and *HSPA5* mRNA by thapsigargin was reduced in the patients' cell lines and valproate induced *ATF6* mRNA expression and enhanced the ER stress response in SHSY5Y cells [4]. Although we also reported that a functional polymorphism of *XBP1* (−116C/G) altering the ER stress response was

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associated with bipolar disorder, the genetic association was not replicated in Caucasian bipolar samples and Taiwanese samples [5,6]. On the other hand, the association of *XBP1* −116C/G polymorphisms with schizophrenia was observed in Chinese samples and Japanese samples [7,8]. Schizophrenia is another major mental disorder sharing common clinical features and genetic background with bipolar disorder [9]. The chromosomal region of *XBP1*, 22q, is one of common linkage loci for these two disorders. Thus, altered ER stress response signaling may contribute to the pathophysiology of both of these major mental disorders.

When unfolded proteins accumulate in endoplasmic reticulum (ER) by some reasons, ER stress response begins. ER stress response consists of four signaling cascades: (1) induction of ER chaperon such as *HSPA5* (*GRP78*/*BiP*), which promotes the folding of unfolded proteins (unfolded protein response, UPR), (2) inhibition of protein synthesis, (3) induction of ER-associated degradation pathway, which promotes the processing of unfolded proteins, and (4) induction of apoptosis when this system could not process the unfolded proteins [10,11].

In the previous paper, we focused on *XBP1* since it is a transcription factor regulating the mRNA expression of ER chaperon genes such as *HSPA5*. However, initial reaction eliciting ER stress response is the consumption of HSPA5. When HSPA5 proteins are consumed to fold unfolded proteins, dissociation of HSPA5 from ATF6 protein on the ER membrane causes cleavage of ATF6. Cleaved ATF6 protein induces the expression of ER chaperons and *XBP1*. In parallel, dissociation of HSPA5 from IRE1 protein on the ER membrane causes dimerization of IRE1, which splices *XBP1* mRNA. The spliced *XBP1* mRNA encodes active form XBP1 that strongly induces the expression of chaperon genes including *HSPA5* as well as *XBP1* itself [10]. In this regard, HSPA5 is a key protein regulating ER stress response.

HSPA5, *TRA1* (*GRP94*), and *CALR* (*Calreticulin*) are known to be increased in the temporal cortex of depressed subjects who died by suicide [12]. Anti-malarial drug mefloquine, which is known to cause psychiatric symptoms including bipolar disorder in susceptible individuals [13], is reported to induce ER chaperons including HSPA5 in rat neurons [14]. Methamphetamine (MAP), a psychostimulant causing manic state, is also known to induce *HSPA5* and other ER chaperon genes in the mouse brain [15]. Induction of *HSPA5* by mefloquine or MAP is interpreted that these drugs cause ER stress, since they also induce ER stress pathway other than UPR, such as apoptosis. On the other hand, valproate, one of the mood stabilizers, is known to increase *HSPA5* expression and have neuroprotective effects by enhancing the UPR [16–20,4]. Recently, the other mood stabilizer, lithium, was also shown to protect the rat PC12 cells against ER stress by inducing the *HSPA5* mRNA [21].

HSPA5 gene is located on 9q33–34.1, on which significant evidence for linkage with bipolar disorder was observed by several studies [22–24].

In this study, we examined whether or not genetic variations of *HSPA5* contribute to the pathophysiology of bipolar disorder and schizophrenia.

Part of the data presented in this paper (data on 3 of 6 SNPs in 195 of 439 patients with bipolar disorder and 254 of 492 controls in case-control studies, and 88 of 240 trios in transmission disequilibrium test) was reported in the reply to correspondence [5].

Materials and methods

Subjects. For the case-control study, 439 patients with bipolar disorder (50.5 ± 13.4 years old, 208 males and 231 females), 229 patients with schizophrenia (46.0 ± 14.9 years old, 131 males and 98 females), and 492 controls (41.7 ± 14.4 years old, 246 males and 246 females) were analyzed. In addition to the samples previously reported in the reply to correspondence [5], we increased the number of case-control samples including the independently collected sample set described previously as "MPS samples" for replication study [25]. MPS samples include 239 patients with bipolar disorder (51.0 ± 13.1 years old, 131 males and 108 females) and 234 controls (51.6 ± 10.7 years old, 117 males and 117 females). They were diagnosed according to the DSM-IV criteria (American Psychiatric Association). Controls were selected from students, nurses, office workers, and doctors in participating institutes, and their friends. A senior psychiatrist interviewed them and they did not have major mental disorders. Only a part of them were interviewed using a structured interview, Mini-International Neuropsychiatric Interview (M.I.N.I.) [26]. In Japanese, no significant population stratification has repeatedly reported in several studies including a part of our samples [4,27–29]. For transmission disequilibrium test, we analyzed total 240 trio samples (227 trios with BPI proband and 12 trios with BPII proband) from NIMH Genetics Initiative Pedigrees (http://zork.wustl.edu/nimh/), including 88 trios previously reported in the reply to correspondence [5]. Only one trio was obtained from one family. The criteria, by which the trio was selected from a pedigree, were, (1) DNA is available for parents and the proband, (2) if multiple complete trios were found in one pedigree, the trio with younger generation was selected, (3) if multiple trios were available in one generation, elder sibling was selected as the proband. Data and biomaterials of the NIMH pedigrees were collected in four projects that participated in the NIMH Bipolar Disorder Genetics Initiative. From 1991 to 1998, the Principal Investigators and Co-Investigators were: Indiana University, Indianapolis, IN, U01 MH46282, J. Nurnberger, M. Miller, and E. Bowman; Washington University, St. Louis, MO, U01 MH46280, T. Reich, A. Goate, and J. Rice; Johns Hopkins University, Baltimore, MD, U01 MH46274, J. R. DePaulo, Jr., S. Simpson, and C. Stine; NIMH Intramural Research Program, Clinical Neurogenetics Branch, Bethesda, MD, E. Gershon, D. Kazuba, and E. Maxwell. Written informed consent was obtained from all the subjects. Postmortem brains were donated by The Stanley Medical Research Institute's Brain Collection courtesy of Drs. Michael B. Knable, E. Fuller Torrey, Maree J. Webster, Serge Weis, and Robert H. Yolken. The Ethics Committees of the Brain Science Institute (RIKEN) and participating institutes approved the study.

Mutation screening of the HSPA5 genes and genotyping of flag SNPs. Polymorphisms of all exons and the upstream region (1 kb) of *HSPA5* (GenBank Accession No. NT_008470) were screened by sequencing in 24 patients with bipolar disorder and eight patients with schizophrenia. Primer sequences are available on request. Genotyping was performed using commercially available TaqMan probes and ABI7900HT according to the protocol recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA).

cDNA synthesis. To genotype the 3′-UTR of *HSPA5* mRNA, we generated cDNA. RNA samples were extracted from the postmortem brain tissues and single-strand cDNA was synthesized by the same method described previously [30].

Cell culture. We cultured SHSY5Y cells in the medium of DMEM (Sigma, Saint Louis, Missouri) containing 10% fetal bovine serum (FBS) and used for degradation assay and promotor assay.

Degradation assay. We generated constructs for mRNA degradation assay. We amplified a 3778 bp fragments excluding SV40 polyA signal region (1242 to 680) of pCMV-tag3 vector (Stratagene, La Jolla, CA, USA), a 1653 bp fragment (coding sequence of luciferase) of pGL3-basic vector (Promega, Madison, WI, USA), and a 1741 bp fragment of 3′-UTR of HSPA5 with SNP5 T. Using BD In-Fusion Technique (BD Biosciences Clontech, San Jose, CA, USA). We fused the three PCR fragments and generated a 7152 bp construct having CMV promotor, coding sequence of luciferase, and 3′-UTR of *HSPA5*. In this construct, start codon of pCMV-tag3 vector fell on that of luciferase coding sequence and the stop codon of luciferase coding sequence fell on the stop codon of the HSPA5 gene. Next we made mutation in SNP5 from T to C using QuickChange Site-Directed Mutagenesis Kit (Stratagene) and generated point mutated construct only in SNP5. The sequence of coding region and 3′-UTR was confirmed by sequencing. We transfected SHSY5Y cells cultured in a 96-well plate using Superfect (Qiagen, Valencia, CA, USA) with 0.2 μg of the reporter plasmid (SNP5 T or C) and 0.05 μg of a reference plasmid (pRL-CMV). After a 48-h incubation, with pre-incubation by the medium containing 5 μg/ml actinomycin D (Wako, Osaka, Japan) for 0, 2, 4, and 6 h before luciferase assay, we measured luciferase activities with the aid of Dual-Glo Luciferase assay system (Promega). In this assay, mRNA degradation was compared by the degradation of mRNA of generated vector and mRNA of pRL-CMV vector. By incubation with actinomycin D for 6 h, the activity of reporter or reference vector decreased to the approximately one-third level compared with the activity not-treated with actinomycin D. The assay was performed independently four times.

Promotor assay. We amplified a 548-bp fragment (−554 to −7, the numbers indicate the nucleotide positions from the transcription start site) of the *HSPA5* gene by PCR and cloned into the *Mlu*I/*Bgl*II site of pGL3-Basic vector (Promega). As a template, genomic DNA derived from control having heterozygotes of haplotype 1 and haplotype 3, or haplotype 2 and haplotype 4, was used and we prepared four kinds of reporter plasmids, having the sequence corresponding to haplotype 1, haplotype 2, haplotype 3 or haplotype 4. We also cloned the spliced *XBP1* cDNA into the *Bam*HI/*Hin*dIII site of pcDNA3.1 vector (Invitrogen, San Diego, CA, USA) to construct a spliced *XBP1*-expressing vector. For the experiments of thapsigargin treatment, we transfected SHSY5Y cells cultured in a 96-well plate with 0.2 μg of the reporter plasmid or pGL3-Basic vector carrying no insert and 0.1 μg of a reference plasmid (pRL-CMV) using Superfect (Qiagen). For the experiments to examine the effects of XBP1, we transfected SHSY5Y cells with 0.4 μg DNA containing 0.2 μg of the reporter plasmid, 0.2 μg pcDNA3.1 vector with or without the insert of spliced *XBP1*, and 0.1 μg of a reference plasmid (pRL-CMV). After a 48-h incubation, thapsigargin (300 nM) or vehicle was added. Three hours after the thapsigargin stimulation, we measured the luciferase activities with the aid of Dual-Glo Luciferase assay system (Promega). The assay was performed independently four times.

Data analysis. Linkage disequilibrium (LD) patterns were assessed by the standardized disequilibrium coefficient (D') and squared correlation coefficient (r^2) calculated by COCAPHASE and TDTPHASE programs (http://www.rfcgr.mrc.ac.uk/fdudbrid/software/unphased/). Analysis of haplotypic distribution, haplotype frequencies, and haplotype TDT analysis was performed using COCAPHASE and TDTPHASE programs. Global significance was calculated by random permutation test for 10,000 times using COCAPHASE and TDTPHASE programs. Two-sample t test was used for comparison among the haplotypes in the promotor assay and mRNA degradation assay.

Results

Mutation screening and identification of the haplotype block of HSPA5

We first performed mutation search of *HSPA5* gene by screening all exons and the upstream region (1 kb) in 24 patients with bipolar disorder and eight patients with schizophrenia. Four single nucleotide polymorphisms (SNPs) [rs391957 (A/G SNP1), rs17840762 (C/T SNP2), rs17840761 (C/T SNP3), and rs3216733 (G/deletion SNP4)] in the upstream region and 3 SNPs [rs16927997 (T/C SNP5), rs1140763 (C/T SNP6), and rs12009 (C/T SNP7)] in the 3′-untranslated region were identified (Fig. 1A). In these 32 samples, SNP1 and SNP4, SNP3 and SNP6 were completely linked. None of two non-synonymous (rs11542738 and rs11542739) and one synonymous

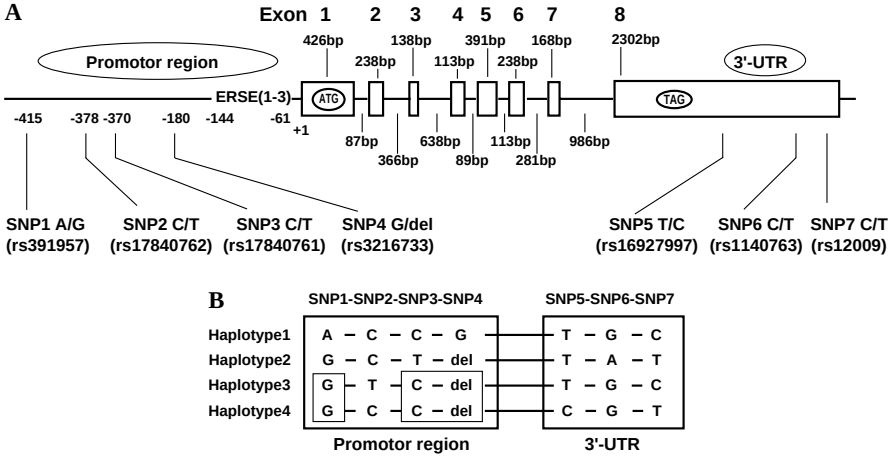

Fig. 1. *HSPA5* gene structure. (A) Genomic structure and the location of single nucleotide polymorphisms. (B) Haplotypes of 7 SNPs of *HSPA5*. Three SNPs in the upstream are common to haplotype 3, the risk for NIMH trios when transmitted paternally, and haplotype 4, the risk for Japanese population.

polymorphisms (rs11542736) registered in dbSNP database were detected in these patients. We genotyped six SNPs, except for SNP6 in 457 samples (257 control samples and 200 bipolar samples), and analyzed by COCAPHASE program. We confirmed that these six SNPs were in linkage disequilibrium (Table 1). We performed a genetic association study for four haplotypes consisted of these SNPs (haplotype 1; A-C-C-G-T-G-C, haplotype 2; G-C-T-del-T-A-T, haplotype 3; G-T-C-del-T-G-C, and haplotype 4; G-C-C-del-C-G-T) (Fig. 1B).

Case-control study of Japanese bipolar disorder and schizophrenia samples

By genotyping three flag SNPs (SNP1, SNP3, and SNP5), we performed association studies in Japanese case-control samples (control $n = 492$, bipolar disorder $n = 439$, and schizophrenia $n = 229$). Haplotype 4 was significantly associated with bipolar disorder ($p = 0.0489$) (Table 2), but not with schizophrenia. Information of family history was available in only a part of bipolar samples. As per our preliminary report [5], the association was much stronger in patients with family history of affective disorders including bipolar disorder and depression in their first-degree relatives ($p = 0.00099$). Extended samples included independently collected sample set, "MPS sample" as described in Materials and methods. In this sample set, no significant association of any haplotype was observed (data not shown).

TDT analysis in NIMH trios

We performed transmission disequilibrium test (TDT) in 240 parents and proband trios of bipolar disorder obtained from NIMH Genetics Initiative pedigrees (NIMH trios). In NIMH trios, mainly originated from Caucasians, only three haplotypes were identified. Haplotype 4 was not found and SNP5 was not polymorphic in NIMH samples. By haplotype TDT using TDTPHASE program, no significant over-transmission was observed (Table 3).

Exploratory analysis of sex-specific transmission

Next, TDT was also performed separately in paternal and maternal transmission, because the parent of origin

Table 1
Linkage disequilibrium (LD) patterns in this region

Control	D'					
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP7
r^2						
SNP1		1	1	1	1	1
SNP2	0.07541		1	1	1	1
SNP3	0.4547	0.1026		1	1	1
SNP4	1	0.07541	0.4547		1	1
SNP5	0.04775	0.01065	0.06494	0.04775		1
SNP7	0.6222	0.1402	0.7317	0.6222	0.07596	
Case	D'					
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP7
r^2						
SNP1		1	1	1	1	1
SNP2	0.07608		1	1	1	1
SNP3	0.3462	0.1061		1	1	1
SNP4	1	0.07608	0.3462		1	1
SNP5	0.07116	0.02182	0.09927	0.07116		
SNP7	0.5706	0.1775	0.598	0.5706	0.1229	

D' and r^2 were calculated by COCAPHASE.

Table 2
Association of HSPA5 haplotype with bipolar disorder in Japanese case-control samples

	BP				Sch		Control
	Total	p value	With FH[a]	p value		p value	
Haplotype 1	282(0.32)	0.0902	40(0.29)	0.0868	145(0.32)	0.1625	352(0.36)
Haplotype 2	373(0.42)	0.831	54(0.38)	0.322	204(0.45)	0.444	422(0.43)
Haplotype 3	116(0.13)	0.362	19(0.14)	0.555	49(0.11)	0.5903	116(0.12)
Haplotype 4	107(0.12)	0.0498*	27(0.19)	0.00099*	54(0.12)	0.1385	92(0.093)
	(Global 0.16)		(Global 0.0028)*		(Global 0.32)		

p values are calculated by COCAPHASE for only haplotypes which are certain at least once. BP, bipolar disorder; Sch, schizophrenia; FH, family history of mood disorders.

[a] The patients' population is the same as that reported in [5], in which SNP3, 4, and 7 were genotyped.

* $p < 0.05$.

Table 3
Transmission disequilibrium test of HSPA5 haplotype in NIMH trios

	Total			Maternal transmission			Paternal transmission		
	T	NT	*p* value	T	NT	*p* value	T	NT	*p* value
Haplotype 1	199(0.41)	195(0.40)	0.792	81(0.41)	77(0.39)	0.681	82(0.41)	84(0.42)	0.838
Haplotype 2	243(0.51)	254(0.53)	0.476	108(0.55)	107(0.54)	0.919	97(0.49)	108(0.55)	0.268
Haplotype 3	36(0.075)	29(0.060)	0.368	9(0.045)	14(0.070)	0.281	19(0.096)	6(0.030)	0.00597*
Haplotype 4	—	—	—	—	—	—	—	—	—
	(Global 0.59)			(Global 0.43)			(Global 0.0054)*		

p values are calculated by TDTPHASE. T, transmitted; NT, not-transmitted.
* $p < 0.05$.

effect has been shown in bipolar disorder [2]. This should be considered as an exploratory and hypothesis-generating analysis. In this study, significant over-transmission of haplotype 3 was found only in paternally transmitted alleles (transmitted, 19; not transmitted, 6; $p = 0.0059$) (Table 3).

Nominally significantly associated haplotype was observed as haplotype 4 in Japanese and haplotype 3 in NIMH trios. Three SNPs in the upstream region are common to these two haplotypes (Fig. 1B). When the association was re-analyzed using the haplotype of three SNPs (SNP1, 3, and 4) in the upstream region, significant association was observed in Japanese samples ($p = 0.031$ in total samples and $p = 0.0029$ in the samples with family history of mood disorder).

We hypothesized two possibilities. One is the contribution of SNP5 in Japanese samples. SNP5 is located in the 3′-UTR. Around the SNP, there are four ATTTA motifs with AT-rich contents (Fig. 2A), which suggested that the region is so-called "AU-rich element." AU-rich element is associated with mRNA stability, in most cases it promotes degradation of mRNA [31]. SNP5 is located next to the ATTTA motif and substitution from T to C reduces the contents of AT, which made us hypothesize that the substitution was associated with the stability of *HSPA5* mRNA. To test this hypothesis, we performed mRNA degradation assay. The other hypothesis is the contribution of haplotype in the upstream region, which was common to Japanese samples and NIMH trios. To test this hypothesis, we performed a promotor assay.

Degradation assay

We performed mRNA degradation assay using luciferase assay system. We generated two kinds of constructs which commonly have a CMV promotor and firefly luciferase coding sequence, with the 3′-UTR of *HSPA5*, either with T or C at SNP5. SHSY5Y cells were transfected by the construct and the degradation was examined by the relative activity of firefly luciferase of the reporter plasmid to that of *Renilla* luciferase of a reference plasmid co-transfected. We treated the transfected cells for 0, 2, 4, and 6 h with actinomycin D (5 μg/ml), which inhibits the transcription of mRNA, and the relative rate of degradation was calculated. No difference was observed between the construct with SNP5-T and the construct with SNP5-C (Fig. 2B).

Promotor assay

We cloned the promotor region of each haplotype into the pGL3-basic vector and generated four kinds of con-

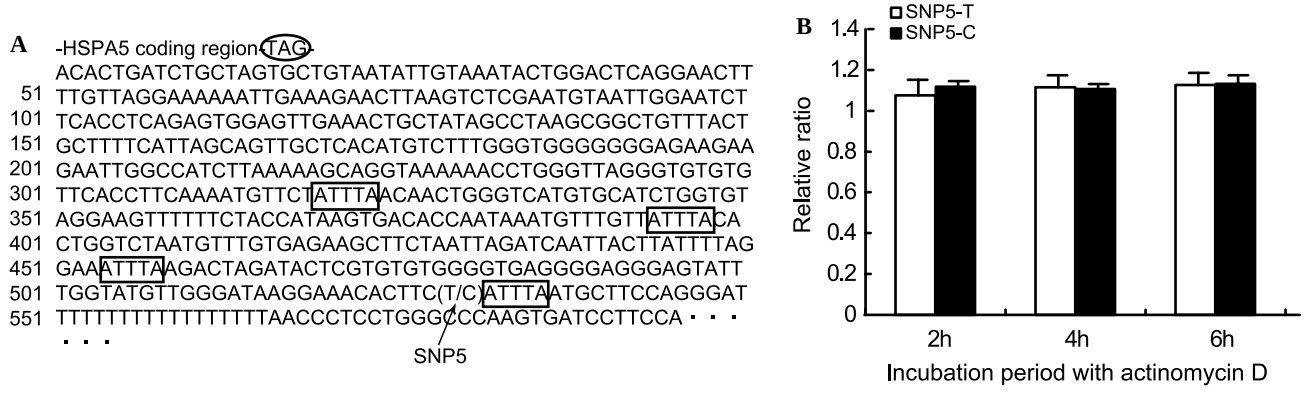

Fig. 2. Degradation assay. (A) 3′-UTR of *HSPA5* gene. There are four ATTTA motifs and SNP5 is located next to the fourth motif. (B) Degradation assay for the examination whether SNP5 is associated with mRNA degradation. No difference between SNP5-T construct and SNP5-C construct. *X*-axis indicates the incubation time with actinomycin D (5 μg/ml) and *Y*-axis indicates the relative ratio of relative activities compared with the activities at 0 h. Values are means ± SD. Assay was performed independently four times.

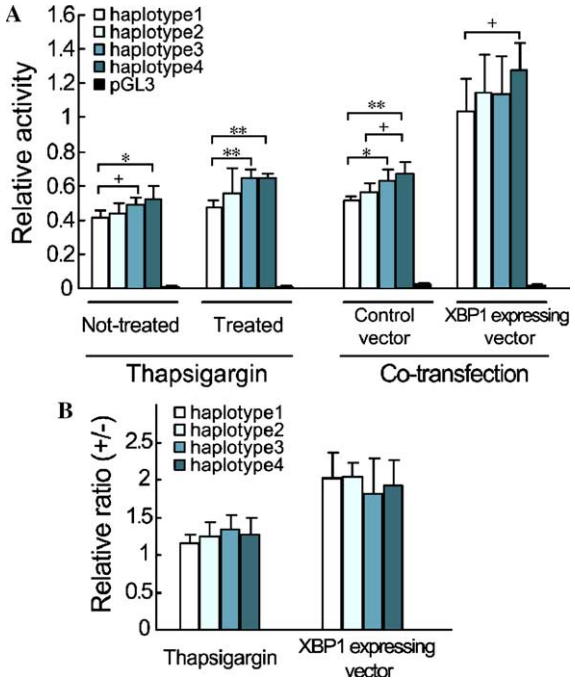

Fig. 3. Effects of haplotype for promotor activity. (A) Basal activity and the activity treated with 300 nM thapsigargin of the construct having haplotype 3 or haplotype 4 were significantly higher than that of haplotype 1, or show a tendency higher than that of haplotype 2. *Y*-axis is relative activity of firefly luciferase of reporter plasmid to *Renilla* luciferase of reference plasmid. Values are means ± SD. Asterisk indicates $p < 0.05$, double asterisk, $p < 0.01$ and plus sign $0.05 < p < 0.1$ (Two-sample *t* test). Assay was performed independently four times. (B) The response to ER stress or XBP1 co-transfection. There was no difference among four haplotypes in the response rate. Vertical axis indicates the relative ratio of the activity treated with thapsigargin to the activity not treated, or the ratio of the activity in the cells co-transfected with spliced form XBP1-expressing vector to that in the cells with control vector. Values are means ± SD.

structs. SHSY5Y cells were transfected by the constructs with pRL-CMV vectors and the relative activity of firefly luciferase (pGL3) to *Renilla* luciferase (pRL) was examined. Among four constructs, the activity of constructs having haplotype 3 and haplotype 4, which are the risk for Japanese bipolar samples and NIMH trios, were significantly higher than that of haplotype 1 (Fig. 3A). Similar difference was observed during the stimulation by 300 nM thapsigargin, the ER Ca^{2+}-ATPase inhibitor. The response to ER stress did not differ between haplotypes. Co-transfection with spliced form XBP1-expressing vector enhanced the promotor activity. This enhancement was observed similarly in these four constructs (Fig. 3B).

Genotyping of the genomic DNA and mRNA in the brain samples

The result of genetic analysis in NIMH trios is compatible with a hypothesis that the haplotype 3 confers a risk only when it is paternally transmitted. A possible explanation for such a phenomenon, that is parent-of-origin effect, is genomic imprinting [2]. To test whether or not HSPA5 shows monoallelic expression in the brain, we genotyped the SNP7 at 3′-UTR in genomic DNA and cDNA obtained from postmortem brain tissues [prefrontal or frontal cortex of bipolar disorder ($n = 41$), schizophrenia ($n = 46$), depression ($n = 12$), and control ($n = 41$)]. The 81 samples having heterozygous genotype in genomic DNA also showed heterozygous genotype in cDNA, which did not suggest monoallelic expression.

Discussion

In this study, we found that there are four haplotypes in the promotor region of HSPA5. A promotor assay revealed that these polymorphisms affect the promotor activity. We also demonstrated that the haplotype of *HSPA5* (*GRP78*/*BiP*) gene was nominally associated with bipolar disorder in Japanese population. However, because (1) the global *p* value was not significant, (2) the association was not replicated in an independent sample set, and (3) no association was found in NIMH trio samples, this could be a type I error. Since the global *p* value was significant in the samples with family history of mood disorder, it would be interesting to test the association in an independent sample set of bipolar disorder with family history. Because parent-of-origin effect is suggested in bipolar disorder [2,32–34] although controversial [35–38], we hypothesized that parent-of-origin effect might confound the findings in NIMH trios. Thus, we performed the hypothesis-generating analysis, which showed significant over-transmission of haplotype 3 from paternal side. This finding made us to hypothesize that HSPA5 is subjected to genomic imprinting. However, monoallelic expression was not observed in the frontal cortex. This did not support our hypothesis. Because imprinting is dependent on the region of the brain [39,40], a possibility that HSPA5 is imprinted in the specific brain region cannot be totally excluded. However, before considering such possibility, this finding of nominal association in paternal transmission should be tested in an independent sample set.

The haplotype in the upstream region showing the nominal association in Japanese and NIMH altered the promotor activity. In this experimental condition, these haplotypes were associated with higher promotor activity. HSPA5 is known to have three ER stress response elements (ERSE, consensus sequence; CCAAT-N9-CCACG) (ERSE1–3) in the promotor region [41]. All the SNPs (SNP1–4) located not within but upstream of ERSE, and thus are unlikely to alter ER stress response. Indeed, there was no difference of response rate among haplotypes against ER stress induced by thapsigargin, while the risk haplotype showed higher activity both in the basal level and after induction of ER stress. In addition to ERSE, *HSPA5* expression is controlled by binding sites of many transcription factors such as ATF4 and AP-1 [42,43]. However, the three SNPs in the risk haplotype do not alter the binding sites of known transcription factors. Unknown transcription factor may determine the basal promotor

activity. Higher promotor activity of the risk is seemingly inconsistent with the reported evidence that valproate improves the ER stress response, and *HSPA5* response to ER stress was impaired in bipolar disorder [4]. This may be due to the cell-type difference or because we used artificial vectors having only promotor regions. It is also possible that higher basal promotor activity might paradoxically result in impaired ER stress response. Although precise mechanism is still unclear, altered regulation of *HSPA5* may contribute to the pathophysiology of bipolar disorder.

The role of ER stress response in the brain is little clarified. Recently, ER stress response was reported to be critical for trafficking of AMPA-type glutamate receptors [44,45]. GluR1 accumulated in the ER of mutant *Caenorhabditis elegans* lacking XBP1 or IRE1. On the other hand, the role of lithium and valproate in AMPA GluR1 receptor trafficking was reported [46]. Altered ER stress response system may contribute to the pathophysiology of bipolar disorder via altered trafficking of AMPA receptors.

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