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Lack of Association between Endoplasmic Reticulum Stress Response Genes and Suicidal Victims

KAORU SAKURAI[1], NAOKI NISHIGUCHI[2], OSAMU SHIRAKAWA[2], HIDEYUKI NUSHIDA[3], YASUHIRO UENO[3], KIYOSHI MAEDA[2], YOSHITAKE HAYASHI[1]

[1]*Division of Molecular Medicine & Medical Genetics, International Center for Medical Research and Treatment (ICMRT), Kobe University Graduate School of Medicine*
[2]*Department of Psychiatry and Neurology, Kobe University Graduate School of Medicine*
[3]*Department of Legal Medicine, Kobe University Graduate School of Medicine*

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As lithium has been shown to have a protective effect against suicidal behavior, genes on which mood stabilizers act may be involved in biological susceptibility to suicide. A recent study showed that endoplasmic reticulum (ER) stress response was impaired in the bipolar disorders and the impairment was ameliorated by a mood stabilizer, valproate. We hypothesized that an alteration of ER stress response is involved in the biological susceptibility to suicide through genetic polymorphisms, and examined the association of polymorphisms of X-box binding protein 1 (*XBP1*) and heat shock 70-kDa protein 5 (*HSPA5*) genes with suicide. We found no significant difference in the distribution of these polymorphisms between the suicide victims and the controls. These results suggest that the polymorphisms examined in this study are not involved in the susceptibility to suicide of the Japanese.

It has been suggested that suicide involves a genetic factor. Many genes involved in transmitter synthesis and degradation, such as the genes for serotonergic transduction, have been examined in case-control studies on suicide. Although some of these studies have reported a positive association with suicide, the results are controversial (1, 4).

Most psychopathological backgrounds for suicidal behavior are estimated to have a relation with mood disorders such as depression. However, the efficacy of using antidepressants to treat suicidal behavior is still controversial. Lithium, one of the major mood stabilizers, has been shown to have a protective effect against suicidal behavior (3). Although there are few reports about other mood stabilizers, it is possible that these medicines have a similar effect to lithium.

Endoplasmic reticulum (ER) is a protein folding system. When unfolded proteins accumulate in the ER as ER stress proteins, ER chaperones, such as heat shock 70-kDa protein 5 (*HSPA5*) also called GRP78, assist in refolding them. The utilization of *HSPA5* proteins induces the expression of target genes, X-box binding protein 1 (*XBP1*) and *HSPA5*. Dissociation of *HSPA5* by the accumulation of unfolded protein also induces the subsequent splicing of *XBP1* mRNA. The spliced mRNA encodes an active form of *XBP1* itself (15, 18). Several studies have focused on the regulation of ER stress proteins by valproate, which is a mood stabilizer (6).

Recently, Kakiuchi et al. reported that, using DNA microarray analysis of lymphoblastoid cells derived from two pairs of twins discordant with bipolar disorder, they

Phone: +81-78-382-5700 FAX: +81-78-382-5719 E-mail: hayashiy@med.kobe-u.ac.jp

found downregulated expression of *XBP1* and *HSPA5* in both the affected twins (11). They also detected a polymorphism (−116C/G) in the promoter region of *XBP1*, which caused an impairment of ER stress response; however, the impairment was rescued using valproate. Moreover, their research showed that −116G was significantly more common in Japanese patients and overtransmitted to affected offspring in trio samples of the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. Chen et al. and Kakiuchi et al. also identified the association of −116C/G polymorphism in *XBP1* gene with schizophrenia (7, 10). Furthermore, *XBP1* is located on 22q12 and *HSPA5* is located on 9q33–34.1; both the genomic regions have been previously linked with bipolar disorder (2, 12, 13, 16).

Wang et al. found that following treatment with the mood stabilizers valproate and carbamazepine, expression of the *HSPA5* increased in rat cerebral cortex (17). Bown et al. observed increased levels of *HSPA5* in the temporal cortex of subjects with major depressive disorder who died by suicide (5). By screening the genomic region of *HSPA5* and genotyping of three SNPs (single nucleotide polymorphisms) in the *HSPA5* gene (rs17840761, rs3216733, and rs12009), Kakiuchi et al. detected a link between a haplotype block and bipolar disorder (14). Their extended research suggested that the haplotypes consisting of four SNPs in the *HSPA5* gene were significantly associated with the bipolar disorder, and the four SNPs were involved in the promoter activity (9).

Thus, we focused on ER stress response involved in the action of mood stabilizers and hypothesized that the polymorphisms of *XBP1* and *HSPA5* genes, which have been reported to be associated with bipolar disorder and schizophrenia, are also associated with suicide. To test this hypothesis, we genotyped −116C/G polymorphism in *XBP1* gene and rs17840761, rs3216733, and rs12009 in *HSPA5* gene, and examined the association between these polymorphisms and suicide.

MATERIALS AND METHODS

Peripheral blood was collected from suicide completers and controls. DNA was extracted from whole blood using the sodium iodide method.

The study population consisted of 188 suicide completers (126 males and 62 females, mean age: 47.7 ± 17.6 years), who were autopsied at the Division of Legal Medicine, Kobe University Graduate School of Medicine, and 184 healthy controls without psychiatric problems (123 males and 61 females, mean age: 44.8 ± 16.6 years). The control subjects were recruited from the general population of the Kobe city area and were of Japanese descent. None of them manifested psychiatric problems when interviewed briefly by psychiatrists.

Genomic regions, including gene polymorphisms, were amplified using polymerase chain reaction (PCR) with a Gene Amp PCR system 9700 (ABI, Foster City, CA). The PCR product was digested with endonuclease for 6 h and then followed by electrophoresis on agarose gel. Primer sets, endonuclease, and concentration of agarose gel, which were used to detect the polymorphisms, are shown in Table 1.

The genotype distribution and Hardy-Weinberg equilibrium were tested with the χ^2 test for quality of fit. Comparisons of the genotype or allele frequencies between the groups were performed with Fisher's extract test. The level of significance was set at $p = 0.05$. This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine.

Table1. Primer sets, endonuclease, and agarose gel concentration.

Genes	SNPs (alleles)	Primer sets	Endonuclease	Gel conc.
XBP1	−116C/G	U; 5'-CGA CAG AAG CAG AAC TTT AGG G-3' L; 5'-GTT GTC AGG CTG AGG TAA TTC TC-3'	*Bst*EII	2%
HSPA5	rs17840761	U; 5'-TAG ATA ACA TCC GCC CCA TC-3' L; 5'-GAT GGA GGA AGG GAG AAC AA-3'	*Mwo*I	4.5%
	rs3216733	U; 5'-GTT GGA GGC CGT TCA TTG-3' L; 5'-GAC AGC TGC TGA ACC AAT GGG ACC AC*-3'	*Bst*YI	4.5%
	rs12009	U; 5'-CAT TGT AGG TCA TCT TTA ATG GA*T-3' L; 5'-CTG CCC AAG GAT AGG TAT TT-3'	*Fok*I	4.5%

U, upper primer; L, lower primer; *, changed base to create a clevage site

RESULTS AND DISCUSSION

There was no significant difference in the genotype and allele distribution of −116C/G polymorphism in *XBP1* between the suicide completers and the control subjects ($p = 0.622$, $p = 0.355$, respectively; Table 2). Similarly, no significant difference was found in the genotype and allele distribution of rs17840761, rs3216733, or rs12009 in the *HSPA5* gene between the suicide completers and the control subjects (Table 2).

All the genotypes and allele distributions are within the Hardy-Weinberg equilibrium. As each genotyping result of the SNPs in the *HSPA5* gene was not informative for haplotype analysis, we omitted the haplotype analysis of these SNPs.

These results suggest that the gene polymorphisms examined in this study are not involved in the susceptibility to suicide of the Japanese. However, as the sample size in this study is small, type II error is considerable on the results.

Although there is a report of no association with bipolar disorder in people of European origin, −116C/G polymorphism in *XBP1* is reported to have an association with bipolar disorder and schizophrenia in the Japanese population (8, 10, 11). Both diseases are believed to contribute to the pathogenesis of suicidal behavior. Based on the results in this study, it is unlikely that the *XBP1* and *HSPA5* genes are involved in the biological susceptibility to suicide in the Japanese population. However, it is possible that the genes involved in schizophrenia and bipolar disorder are different from those involved in suicide.

It has been reported that activating transcription factor 6 (ATF6) plays an important role in ER stress cascade. Therefore, we examined the association of representative SNPs in the ATF6 gene and suicide in a pilot study; however, no possibility of association with suicide was observed (data not shown). We could find no evidence of genetic involvement of ER stress response system in suicide in this study.

Recently, several studies on the mechanisms of mood stabilizer action have been reported. A better understanding of the pharmacological action of mood stabilizers may provide a clue to help elucidate a biological mechanism of human suicidal behavior.

Table 2. Genotype and allele distributions for the SNPs of *XBP1* and *HSPA5* genes in suicide victims and contrls

Genes	SNPs (alleles)		Genotype count (genotype frequency)				Allele count (allele frequency)		
			GG	CG	CC	p	G	C	p
XBP1	−116C/G	Suicide (n = 188)	76 (40.4%)	88 (46.8%)	24 (12.8%)	χ^2 = 1.012, df = 2, p = 0.622	240 (63.8%)	136 (36.2%)	χ^2 = 0.933, df = 1, p = 0.355
		Control (n = 184)	82 (44.6%)	83 (45.1%)	19 (10.3%)		247 (67.1%)	121 (32.9%)	
			CC	CT	TT	p	C	T	p
	rs17840761	Suicide (n = 182)	33 (18.1%)	90 (49.5%)	59 (32.4%)	χ^2 = 0.934, df = 2, p = 0.635	156 (42.9%)	208 (57.1%)	χ^2 = 0.545, df = 1, p = 0.499
		Control (n = 183)	32 (17.5%)	83 (45.4%)	68 (37.2%)		147 (40.2%)	219 (59.8%)	
			CC	C/−	−/−	p	C	−	p
HSPA5	rs3216733	Suicide (n = 128)	20 (15.6%)	47 (36.7%)	61 (47.7%)	χ^2 = 0.739, df = 2, p = 0.705	87 (34%)	169 (66%)	χ^2 = 0.1018, df = 1, p = 0.7745
		Control (n = 116)	14 (11.9%)	49 (47.1%)	55 (46.6%)		77 (32.6%)	159 (67.4%)	
			CC	CT	TT	p	C	T	p
	rs12009	Suicide (n = 186)	50 (26.9%)	86 (63.2%)	50 (26.9%)	χ^2 = 3.12, df = 2, p = 0.211	186 (50.0%)	186 (50.0%)	χ^2 = 1.4, df = 1, p = 0.251
		Control (n = 181)	35 (19.3%)	96 (65.8%)	50 (27.6%)		166 (47.2%)	186 (52.8%)	

p, Fisher's extracted p

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