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Genetic association of the *EGR2* gene with bipolar disorder in Korea

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Abbreviations: ANK3, ankyrin G; BD, bipolar disorder; CNS, central nervous system; DIGS, Diagnostic Interview for Genetic Studies; EGR, early growth response gene; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; LTD, long-term depression; LTP, long-term potentiation; MAF, minor allele frequency; MeCP2, methyl-CpG-binding protein 2; NMDA, N-methyl D-aspartate; SPR, schizophrenia

Abstract

The *early growth response gene 2* (*EGR2*) is located at chromosome 10q21, one of the susceptibility loci in bipolar disorder (BD). *EGR2* is involved in cognitive function, myelination, and signal transduction related to neuregulin-ErbB receptor, Bcl-2 family proteins, and brain-derived neurotrophic factor. This study investigated the genetic association of the *EGR2* gene with BD and schizophrenia (SPR) in Korea. In 946 subjects (350 healthy controls, 352 patients with BD, and 244 with SPR), nine single nucleotide polymorphisms (SNPs) in the *EGR2* gene region were genotyped. Five SNPs showed nominally significant allelic associations with BD (rs2295814, rs61865882, rs10995315, rs2297488, and rs2297489), and the positive associations of all except rs2297488 remained significant after multiple testing correction. Linkage disequilibrium structure analysis revealed two haplotype blocks. Among the common identified haplotypes (frequency > 5%), 'T-G-A-C-T (block 1)' and 'A-A-G-C (block 2)' haplotypes were over-represented, while 'C-G-G-T-T (block 1)' haplotype was under-represented in BD. In contrast, no significant associations were found with SPR. Although an extended analysis with a larger sample size or independent replication is required, these findings suggest a genetic association of *EGR2* with BD. Combined with a plausible biological function of *EGR2*, the *EGR2* gene is a possible susceptibility gene in BD.

Keywords: bipolar disorder; EGR2 protein, human; genes, immediate early; genetic association studies; schizophrenia

Introduction

Early growth response (*EGR*) genes, which include *EGR1*, *EGR2*, *EGR3*, and *EGR4*, encode DNA-binding transcription factors that contain cys_2-his_2 zinc fingers (Beckmann and Wilce, 1997; O'Donovan *et al.,* 1999). The *EGR* genes encode a family of immediate early gene transcription factors that mediate the transcription of various genes related to neuronal development and plasticity, cognition, circadian rhythm, and social behaviors (Beckmann and Wilce, 1997; Morris *et al.,* 1998; O'Donovan *et al.,* 1999; Davis *et al.,* 2003; Knapska and Kaczmarek, 2004; James *et al.,* 2006; Baumgartel *et al.,* 2008; Poirier *et al.,* 2008), which have been implicated in the pathophysiology of schizophrenia (SPR) and bipolar disorder (BD). *EGRs* are involved in rodent models of psychotic disorders

(Gallitano-Mendel *et al.,* 2007, 2008), as well as in the action mechanisms of antipsychotics and electroconvulsive seizures (Yamagata *et al.,* 1994; Jung *et al.,* 1996; Verma *et al.,* 2007).

Moreover, downregulation of *EGR1*, *EGR2*, and *EGR3* transcripts in the postmortem brains of patients with SPR has been reported (Yamada *et al.,* 2007). In particular, *EGR3* has a strong, consistent association with SPR in both family-based and case-control association studies in Japanese cohorts (Yamada *et al.,* 2007). We replicated this in an independent association study in Korea (Kim *et al.,* 2010b), while a negative association has been reported in a Chinese population (Liu *et al.,* 2010). Additionally, a genetic association between *EGR3* and bipolar disorder was recently reported (Mansour *et al.,* 2009).

Although *EGRs* encode closely related transcription factors that contain cys_2-his_2 zinc fingers that can bind to the same cognate GC-rich consensus DNA binding motif, individual *EGR* genes have little sequence homology outside the common DNA-binding domain. Furthermore, they are regulated by different signal pathways and serve different functions (Beckmann and Wilce, 1997; Herdegen and Leah, 1998; Poirier *et al.,* 2008). In certain circumstances, they may even have antagonistic functions; for example, Egr1 and Egr2 exert opposing influences on adipocyte differentiation (Boyle *et al.,* 2009) and T cell receptor-induced changes in T cell function (Collins *et al.,* 2008). Additionally, *Egr2* has been reported to have different effects on cognitive function than other *Egr* genes (Poirier *et al.,* 2007).

The human *EGR2* gene resides at chromosomal location 10q21.3, which the linkage analysis has identified as a susceptibility locus for BD (Venken *et al.,* 2008). The linked regions could contain multiple susceptibility genes showing association (Straub and Weinberger, 2006). For example, *ankyrin G* (*ANK3*), one of the most promising susceptibility genes for BD, is also located at chromosome 10q21 (Ferreira *et al.,* 2008; Schulze *et al.,* 2009; Smith *et al.,* 2009). On the other hand, Egr2 is involved in regulating Bcl-2 family protein and mitochondrial function (Unoki and Nakamura, 2003; Lauritsen *et al.,* 2008), which are thought to be involved in the pathophysiology of BD (Kato, 2006; Marmol, 2008; Andreazza *et al.,* 2010; Kim *et al.,* 2010a). These findings suggest the possible involvement of *EGR2* in BD.

EGR2 plays important roles in peripheral nerve myelination, T cell maturation, hindbrain segmentation, and lipid biosynthesis (Schneider-Maunoury *et al.,* 1993; Swiatek and Gridley, 1993; Topilko *et al.,* 1994; Leblanc *et al.,* 2005). As noted above, the involvement of Egr2 in cognitive behavioral phenotypes have been reported. An attention set shifting task induced the expression of *Egr2*, but not *Egr1* or *Egr3*, in the frontal cortex of mice (DeSteno and Schmauss, 2008). Forebrain-specific *Egr2*-deficient mice demonstrated enhanced long-term object recognition memory and superior implicit motor skill learning abilities (Poirier *et al.,* 2007), in sharp contrast to the behavioral phenotypes of *Egr3*-deficient mice, which had poor long- and short-term memory (Gallitano-Mendel *et al.,* 2007). These findings suggest that *EGR2* has different effects of on brain functions than other *EGR* genes.

Given the possible relationship between *EGR2* and BD, we investigated the association of *EGR2* with BD in a Korean population. We also extended the investigation to the association of *EGR2* with SPR, which showed negative associations in previous Korean and Chinese case control association studies (Kim *et al.,* 2010b; Liu *et al.,* 2010) and positive findings in a family-based association study done in Japanes population (Yamada *et al.,* 2007).

Results

Nine SNPs within the 15-kb region surrounding the *EGR2* gene were selected as described in Methods. SNP information, including the relative location of the SNPs on the *EGR2* gene, is presented in Figure 1. Of the nine investigated SNPs of *EGR2*, rs2295814 (SNP3), rs61865882 (SNP4), rs10995315 (SNP6), rs2297488 (SNP7), and rs2297489 (SNP8) showed significant allelic associations with BD (P = 0.0027, 0.0008, 0.0020, 0.0103, and 0.0011 for each SNP, respectively). These SNPs also showed genotypic associations with BD (P = 0.0026, 0.0011, 0.0068, 0.0240, and 0.0048 for each SNP, respectively). The frequencies of the A, C, A, A, and G alleles in SNP 3, 4, 6, 7, and 8, respectively, were significantly increased in the BD group compared to the controls, and all withstood multiple testing correction with 10,000 permutations ($P_{permutation}$ = 0.0164, 0.0056, 0.0127, 0.0460, and 0.0074 for each SNP, respectively). Additionally, the positive associations with BD in SNP 3, 4, 6, and 8 remained significant after the Bonferroni correction ($P < 0.0028$). The other four SNPs of *EGR2* [rs9990 (SNP1), rs1509963 (SNP2), rs45602133 (SNP5), and rs224278 (SNP9)] did not show significant associations with BD in allele-wise or genotype-wise analyses. Additionally, no significant association was found between the examined *EGR2* SNPs and the SPR group. Association results for rs2295814

Table 1. Allele and genotype distribution of SNPs in the *EGR2* gene

SNP	Group	N	Allele 1/2	Allele frequency (%)		OR	χ^2	*P*	$P_{permutation}$	Genotype 1/2/3	Genotype frequency (%)			χ^2	*P*	HWE *P*
				1	2						1	2	3			
SNP1	CTR	350	C/T	58.3	41.7					CC/CT/TT	34.4	47.9	17.8			0.5513
rs9990	BD	352		54.0	46.0	1.193	2.6714	0.1022	0.4195		27.8	52.3	19.9	3.5156	0.1724	0.9437
	SPR	244		52.9	47.1	1.244	3.3587	0.0668	0.3050		25.4	55.0	19.6	5.4069	0.0670	0.5692
SNP2	CTR	350	G/A	65.8	34.2					GG/GA/AA	43.7	44.3	12.1			0.3995
rs1509963	BD	352		64.9	35.1	1.040	0.1224	0.7265	0.9994		42.6	44.6	12.8	0.1228	0.9405	0.8819
	SPR	244		63.2	36.8	1.119	0.8177	0.3659	0.8934		39.1	48.3	12.6	1.2583	0.5330	0.8567
SNP3	CTR	350	G/A	92.9	7.1					GG/GA/AA	86.3	13.1	0.6			0.4710
rs2295814	BD	350		88.2	11.8	1.748	8.9817	**0.0027**	**0.0164**		76.6	23.1	0.3	11.8998	**0.0026**	0.9037
	SPR	244		90.0	10.0	1.451	3.1172	0.0775	0.3337		80.7	18.4	0.8	3.2943	0.1926	0.9482
SNP4	CTR	350	T/C	92.3	7.7					TT/TC/CC	85.4	13.8	0.9			0.3995
rs61865882	BD	352		86.8	13.2	1.815	11.1892	**0.0008**	**0.0056**		74.4	24.7	0.9	13.5684	**0.0011**	1.0000
	SPR	244		90.4	9.6	1.270	1.2985	0.2545	0.7578		81.6	17.6	0.8	1.5969	0.4500	0.4445
SNP5	CTR	350	T/G	88.1	11.9					TT/TG/GG	78.2	19.8	2.0			0.3969
rs45602133	BD	352		87.5	12.5	1.059	0.1213	0.7276	0.9994		76.7	21.6	1.7	0.4186	0.8112	0.9411
	SPR	242		89.7	10.3	0.846	0.7658	0.3815	0.9064		81.6	16.3	2.1	1.1279	0.5690	0.1641
SNP6	CTR	349	G/A	90.4	9.6					GG/GA/AA	82.2	16.4	1.4			0.6755
rs10995315	BD	352		84.9	15.1	1.664	9.5297	**0.0020**	**0.0127**		72.2	25.6	2.3	9.9742	**0.0068**	0.2173
	SPR	242		89.4	10.6	1.116	0.3138	0.5754	0.9900		79.2	20.4	0.4	2.8926	0.2354	1.0000
SNP7	CTR	350	G/A	90.1	9.9					GG/GA/AA	81.7	16.9	1.4			1.0000
rs2297488	BD	350		85.7	14.3	1.527	6.4911	**0.0103**	**0.0460**		73.1	25.1	1.7	7.4584	**0.0240**	0.0732
	SPR	244		89.1	10.9	1.114	0.3127	0.5760	0.5592		78.7	20.9	0.4	2.9107	0.2333	0.4625
SNP8	CTR	350	C/G	90.4	9.6					CC/CG/GG	82.2	16.3	1.4			0.8234
rs2297489	BD	352		84.7	15.3	1.707	10.5786	**0.0011**	**0.0074**		71.9	25.6	2.6	10.6791	**0.0048**	0.7613
	SPR	244		88.8	11.2	1.183	0.7552	0.3848	0.9090		78.5	20.7	0.8	2.1678	0.3383	0.6668
SNP9	CTR	350		70.1	29.9					TT/TC/CC	49.9	40.4	9.7			0.8314
rs224278	BD	352	T/C	67.6	32.4	1.121	0.9756	0.3233	0.8547		45.5	44.3	10.2	1.3887	0.4994	0.3985
	SPR	242		70.5	29.5	0.979	0.0268	0.8698	1.0000		50.6	39.7	9.6	0.0339	0.9832	0.1477

Significant *P*-values are shown in bold. CTR: control, BD: bipolar disorder, SPR: schizophrenia.

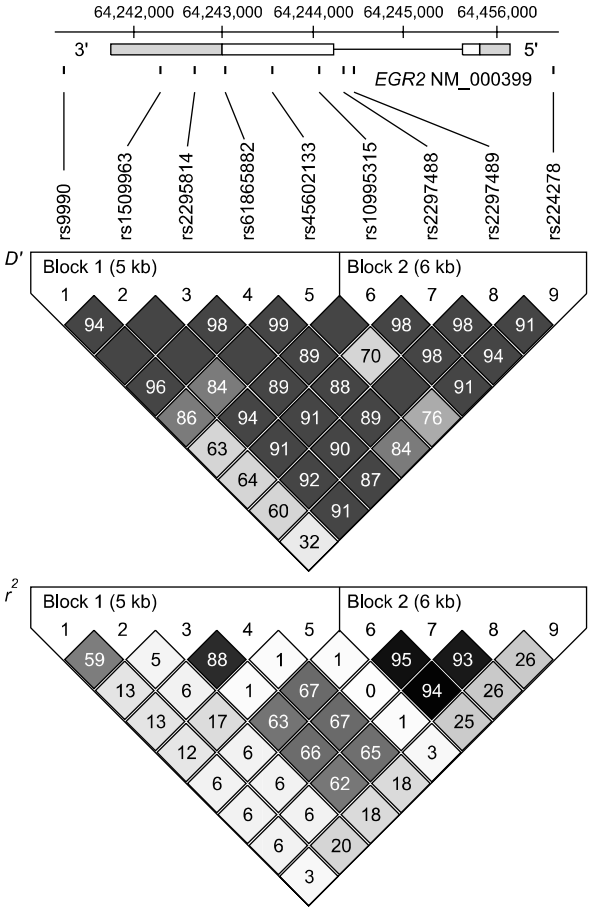

Figure 1. Location of the SNPs on the *EGR2* gene and the linkage disequilibrium structure of the locus. Graphic representation of the genomic structure and location of the *EGR2* SNP sites examined and the linkage disequilibrium (LD) structure of the *EGR2* haplotype block. Exons are denoted by boxes, with untranslated regions in white and translated regions in gray. The LD between SNPs was analyzed using the pair-wise LD measures *D'* and r^2 and the haplotype block was constructed using the solid spine haplotype algorithm (*D'* > 0.8). Each diamond either represents the *D'* or r^2 measure of LD (*D'*: darker shades of red represent greater *D'* values, r^2: darker shades of black represent greater r^2 values).

and rs2297488 with the SPR group from our previous study (Kim *et al.,* 2010b) are included here. The genotypic distributions of all SNPs were found to be in Hardy-Weinberg equilibrium (HWE) in the control, BD, and SPR groups. The allelic and genotypic frequencies of the nine SNPs of *EGR2* in the three groups are summarized in Table 1.

The linkage disequilibrium (LD) among the nine SNPs was investigated. Pair-wise marker LD statistics were determined using Haploview. High levels of LD between SNPs 2 and 3, and among SNPs 6, 7, and 8 were found, as reflected by the squared correlation coefficients (r^2), for the SNPs that showed significant positive associations with BD. The LD block structure was calculated using the solid spine method (D' > 0.8) implemented within Haploview, which demonstrated that five SNPs (SNPs 1 to 5) and the remaining four SNPs (SNPs 6 to 9) formed distinct haplotype blocks (blocks 1 and 2). The LD pattern of *EGR2* is presented in Figure 1.

We performed a haplotype analysis of the haplotype blocks formed by the LD structure of the *EGR2* gene. Regarding haplotype block 1, four common haplotypes (frequency > 5%) were analyzed and the 'C-G-G-T-T (SNP1-5)' haplotype was significantly under-represented (*P* = 0.0422, χ^2 = 4.126, OR = 0.805), while the 'T-G-A-C-T' haplotype was significantly over-represented (*P* = 0.0035, χ^2 = 8.548, OR = 1.736) in the BD group compared with the controls. Additionally, among the three common haplotypes (frequency > 5%) of haplotype block 2, the 'A-A-G-C (SNP5-8)' haplotype was significantly over-represented in the BD group compared to the controls (*P* = 0.0091, χ^2 = 6.807, OR = 1.543). The haplotype association with BD was consistent with the allelic association findings. However, there was no significant haplotypic association with the SPR group (data not

Table 2. Analysis of common haplotypes of the *EGR2* gene in the patients with BD

	Marker					Frequency		OR	χ^2	*P*
	rs9990	rs1509963	rs2295814	rs61865882	rs45602133	Case	Control			
Block 1	C	G	G	T	T	0.509	0.563	0.805	4.126	**0.0422**
	T	A	G	T	T	0.221	0.227	0.966	0.075	0.7843
	T	A	G	T	G	0.107	0.105	1.021	0.017	0.8973
	T	G	A	C	T	0.114	0.069	1.736	8.548	**0.0035**
	Marker					Frequency		OR	χ^2	*P*
	rs10995315	rs2297488	rs2297489	rs224278	-	Case	Control			
Block 2	G	G	C	T	-	0.658	0.696	0.840	2.290	0.1302
	G	G	C	C	-	0.182	0.204	0.868	1.171	0.2792
	A	A	G	C	-	0.138	0.094	1.543	6.807	**0.0091**

The common haplotypes (frequency > 5%) are shown with significant *P*-values in bold.

shown). The frequencies of the *EGR2* haplotypes in the control and BD groups are summarized in Table 2.

Discussion

In this study, we found a genetic association between *EGR2* and BD in Korea. Of the nine *EGR2* SNPs examined, five [SNP3 (rs2295814), SNP4 (rs61865882), SNP6 (rs10995315), SNP7 (rs2297488), and SNP8 (rs2297489)] showed significant allelic and genotypic associations with BD. Additionally, the 'T-G-A-C-T (SNP1-5)' and 'A-A-G-C (SNP6-9)' haplotypes were over-represented, while the 'C-G-G-T-T (SNP1-5)' haplotype was under-represented in the BD subjects. In contrast, there was no association between the examined SNPs of *EGR2* and SPR. These findings provide provisional evidence that *EGR2* may be a susceptibility gene for BD.

To our knowledge, this is the first report of a positive association of *EGR2* with BD. Five SNPs in the *EGR2* gene, which showed a LD trend, were found to be associated with BD. Significant positive associations of four *EGR2* SNPs (SNPs 3, 4, 6, and 8) remained after the multiple testing correction with permutation analysis and the Bonferroni correction. Minor alleles of the associated SNPs, A, C, A, A, G in SNP 3, 4, 6, 7, and 8, respectively, were over-represented in the BD group. Consistently, the haplotypes containing these alleles, 'T-G-A-C-T (SNP1-5)' in haplotype block 1 and 'A-A-G-C (SNP6-9)' in haplotype block 2, were significantly over-represented in the BD group. Although an extended analysis with a larger sample size or independent replication is required, these findings suggest that *EGR2* may be related to the development of BD.

Of the associated *EGR2* SNPs, SNPs 7 and 8 (rs2297488 and rs2297489) are located on intron1 of *EGR2*. *EGR2* expression is upregulated through intronic CpG islands methylation (Unoki and Nakamura, 2003), and the binding of methyl-CpG-binding protein 2 (MeCP2), which plays important roles in neurodevelopment (Gonzales and LaSalle, 2010), to this region upregulates *EGR2* expression (Swanberg *et al.,* 2009). The C/G polymorphism in SNP8 is associated with a sequence change from CC to CG [5'-CCACC(C/G)CCATC-3'], which can add a CpG site for methylation. Changes in nucleotide sequences related to polymorphisms creating additional CpG sites can affect the DNA methylation and transcriptional activity (Moser *et al.,* 2009). These findings suggest a possible function of BD-associated intronic SNPs of *EGR2* regulating gene expression *via* alterations in DNA methylation, which requires further examination.

In the central nervous system (CNS), Egr2 expression is abundant in neurons (Herdegen *et al.,* 1993), and it is expressed strongly in layers II and III of the cortical regions of the brain (Beckmann and Wilce, 1997), where the reduction in neuronal density is most prominent in the postmortem brains of patients with mood disorders (Rajkowska, 2000). Forebrain-specific knockout of *Egr2* in mice induced enhanced motor skill learning and long-term memory, indicating an inhibitory role of *Egr2* in certain cognitive functions (Poirier *et al.,* 2007, 2008). *Egr2* is induced in the prefrontal cortex of mice performing attention set- shifting (DeSteno and Schmauss, 2008), dysfunction of which has been implicated as an endophenotype of BD (Clark *et al.,* 2005). *Egr2* is also involved in Nmethyl D-aspartate (NMDA) receptor-mediated long-term potentiation (LTP) and long-term depression (LTD) in the mouse hippocampus (Coba *et al.,* 2008). These findings suggest the involvement of *EGR2* dysregulation in the cognitive dysfunction of BD through altered levels in the brain.

The function of Egr2 as an intracellular signaling molecule supports its potential role in BD. Egr2 is involved in the signal pathways possibly responsible for the pathogenesis of psychotic disorders, such as SPR and BD, including intracellular signal transduction related to the neuregulin-ErbB receptor (Jacobson *et al.,* 2004; He *et al.,* 2010), calcineurin (Hildeman *et al.,* 2003; Kao *et al.,* 2009), and brain-derived neurotrophic factor (Glorioso *et al.,* 2006). *Neuregulin 1* (*NRG1*) gene has been reported to be associated with SPR and BD (Stefansson *et al.,* 2002, 2003; Cassidy *et al.,* 2006; Thomson *et al.,* 2007; Georgieva *et al.,* 2008). Egr2 acts as a downstream effector of the neuregulin-ErbB receptor signal, which plays a critical role in peripheral nerve myelination in Schwann cells (Kao *et al.,* 2009; He *et al.,* 2010). In the CNS, a different role of the neuregulin signal in brain development has been suggested, such as guiding axon pathfinding (Birchmeier, 2009). These findings suggest a different role of Egr2 in the CNS as a downstream effector of neuregulin signals, which requires further investigation. Egr2 is also involved in apoptosis and mitochondrial functions *via* the regulation of Bcl-2 family proteins (Unoki and Nakamura, 2003; Lauritsen *et al.,* 2008), and the involvement of dysregulation of the Bcl-2 family proteins or mitochondrial function in the pathogenesis of BD has been suggested (Kato, 2006; Marmol, 2008; Andreazza *et al.,* 2010; Kim *et al.,* 2010a).

In this study, no association was found with SPR. Recently, we reported the association of *EGR3* with SPR in a case-control association study in Korea (Kim *et al.,* 2010b), replicating a previous Japanese report (Yamada *et al.,* 2007). However, two *EGR2* SNPs (rs2295814 and rs2297488) did not show positive associations with SPR (Kim *et al.,* 2010b), although they were associated with SPR in a Japanese family-based association analysis (Yamada *et al.,* 2007). Additionally, in the current study, seven more examined *EGR2* SNPs did not show associations with SPR. A recent case-control association study in a Han Chinese population also demonstrated that *EGR2* did not show a significant association with SPR (Liu *et al.,* 2010). The possible association of *EGR2* with SPR and the common and differential involvement of *EGR* genes, especially *EGR2* and *EGR3*, require further clarification.

As our study had a limited sample size, further extended analysis with a larger sample size or an independent replication study is required to confirm the positive association of *EGR2* with BD. The strong association of *EGR2* SNPs with BD, withstanding multiple testing corrections and a *P* value < 0.001 (in the case of rs61865882), are noteworthy, and reduce the chance of this being a false-positive finding and predict future replication (Lohmueller *et al.,* 2003). Although the possible involvement of population stratification was not tested in this study, it seems unlikely in our sample because the Korean population is relatively homogeneous.

In conclusion, we found a genetic association between *EGR2* and BD in Korea. Of the nine SNPs of *EGR2* examined, five showed nominal significant allelic and genotypic associations with BD, and the positive association of four SNPs remained significant after multiple testing correction. Additionally, the corresponding haplotypes were found to be associated with BD. The human *EGR2* gene is located at chromosome 10q21, one of the loci related to the pathophysiology of BD. *EGR2* plays important roles in cognitive function, the myelination process, and intracellular signaling, making involvement in the pathogenesis of BD plausible. These findings suggest that the *EGR2* gene is related to the pathogenesis of BD.

Methods

Subjects

This study enrolled 946 individuals: 350 controls (174 men, 176 women; mean age 25.9 ± 6.5 years), 352 patients with BD (149 men, 203 women; average age 33.5 ± 12.0 years), and 244 patients with SPR (147 men, 97 women; average age 32.7 ± 7.8 years). The participants were all ethnic Koreans. All patients met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), criteria for the diagnosis of BD or SPR. Patients were interviewed individually by trained researchers using the Korean translation of the Diagnostic Interview for Genetic Studies (DIGS) (Joo *et al.,* 2004). Meetings with at least two psychiatrists were held regularly to arrive at consensual decisions about the participants' final diagnoses and to eliminate subjects with organic brain disease, alcohol or drug problems, or other general medical conditions possibly manifesting as psychiatric symptoms. Healthy subjects included in the control group were recruited from hospital staff members and college students who volunteered to participate. Interviews conducted by a psychiatrist enabled the exclusion of subjects with histories or current evidence of psychiatric illnesses, organic mental disorders, substance abuse, or any medical conditions that might lead to mental symptoms. Control subjects who had any first-degree relatives with suspected psychiatric illnesses were also excluded. All subjects in this study were unrelated.

The study protocol was approved by the ethics committee of Seoul National University Hospital, and this study was conducted in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from each patient before enrollment, and only subjects able to provide informed consent were included in the study.

SNPs selection and genotyping

Tag SNPs within the 15-kb region surrounding the *EGR2* gene (from 64237 to 64252 kb) were selected in the international HapMap database (Data release #27, http://hapmap. ncbi.nlm.nih.gov/). Based on the close genetic backgrounds of the Korean and Japanese populations (Kim *et al.,* 2005; Tian *et al.,* 2008), the genotypes downloaded were restricted to those of the Japanese population of Tokyo, Japan (JPT). Five tag SNPs (rs9990, rs1509963, rs2295814, rs10995315, and rs224278) with minor allele frequencies (MAF) above 10% and a minimum value of 0.8 for the r^2 parameter were selected using the tagger algorithm (de Bakker *et al.,* 2005) implemented in Haploview (ver. 4.2) (Barrett *et al.,* 2005). SNP rs2297488, which was included in previous studies of SPR in Japan (Yamada *et al.,* 2007), Korea (Kim *et al.,* 2010b), and China (Liu *et al.,* 2010) was included. Three additional *EGR2* SNPs (rs6186-5882, rs45602133, and rs2297489) with MAF above 10% in East Asian populations in the NCBI database (http://www.ncbi.nlm.nih.gov/projects/SNP/) were included in the analysis. SNP information, including the relative location of the SNPs on the *EGR2* gene, is presented in Figure 1. DNA was extracted from whole blood samples using a DNA isolation kit (Roche Applied Science, Indianapolis, IN). Genotyping assays were performed using the TaqMan method (Applied Biosystems, Foster City, CA), and high genotyping rates for all markers were achieved. The genotyping failure rates for all markers were less than 1% for the control, BD, and SPR groups. SNPs rs2295814 and rs2297488 in the control and SPR groups were genotyped in our previous study (Kim *et al.,* 2010b),

and are presented together with the current findings.

Statistical analyses

Contingency chi-square tests or Fisher's exact test were performed to compare allele and genotype frequencies between patients and controls. Correction for multiple testing was performed using a correction with 10,000 permutations with the Haploview software (ver. 4.2) and the Bonferroni correction. To avoid false-positive results due to multiple testing, we used the Bonferroni correction for the nine independent loci genotyped and for the comparisons of the control group with the SPR and BD groups. Significant *P*-values were changed to $P = 0.0028$ (0.05/18). Haplotype frequencies and the significance of haplotype associations were calculated with Haploview. Rare haplotypes were excluded from the analysis, while frequencies exceeding 5% were included for the analysis. Pair-wise LD statistics and HWE were also assessed with Haploview (ver. 4.2).

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