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Association Study of RGS2 Gene Polymorphisms with Panic Disorder in Japanese

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Genetic factors for panic disorder have been consistently observed in family and twin studies. Regulators of G-protein signaling (*RGS*) is a family of proteins that negatively regulate the intracellular signaling of G protein-coupled receptors such as dopamine and serotonin receptors. *RGS2*, one of the *RGS* families, has been suggested to plays a role in anxiety and/or aggressive behavior. Polymorphisms in the *RGS2* gene were recently associated with panic disorder, trait anxiety, suicidal behavior, and generalized anxiety disorder. From these findings, we tried to replicate an association between panic disorder and genetic variations of the *RGS2* using a case-control study of 186 patients with panic disorder and 380 controls in Japanese. We genotyped five common single nucleotide polymorphisms (SNPs) by the PCR-RFLP method and the TaqMan Assays. Neither genotype distribution nor allele frequency for five SNPs was significant different between the panic disorder and control groups. We found a relative tight LD block in the 5'- flanking region of *RGS2* gene. One of the common haplotypes, AC of rs2746071 and rs2746072, has shown a nominally significant association with panic disorder (p=0.027). This significance, however, did not remain after correction for multiple testing. These findings suggest that *RGS2* may not be genetically involved in the biological susceptibility to panic disorder in Japanese.

Panic disorder (PD) is a common anxiety disorder with a world-wide life-time prevalence of 1 to 3% (15). It is characterized by sudden, unexpected attacks of intense fear and anticipatory anxiety. In 50% of the cases, panic disorder occurs with agoraphobia, an object- and situation-bound fear (5). Family and twin studies suggest a genetic component in the pathogenesis of PD with an estimated heritability of up to 46% (7).

The regulator of G-protein signaling 2 (*RGS2*) family proteins play a key role in modulating intracellular signaling through the G protein pathway. Their primary functions are to act as GTPase-activating proteins that negatively regulate signaling by G protein-coupled receptors. The *RGS2* protein is expressed in cortical and limbic area in the brain and, therefore, plays an important role in the serotonergic and noradrenergic systems through accelerating deactivation of G proteins to reduce G protein–coupled receptor (*GPCR*) signaling that is suggested to be implicated in the pathophysiology of panic disorder.

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Recent studies suggest that *RGS2* was implicated in anxiety behavior in animal models. For examples, *RGS2* knockout mice show increased GTP mediated neurotransmission, increased excitability in hippocampal CA1 neurons, and are more anxious than their wild-type counterparts (12). Yalcin et al. (16) also identified *RGS2* as a quantitative trait gene that quantitatively measured anxious behavior in mice. In addition, *RGS2* gene polymorphisms have been associated with panic disorder (10) and completed suicides (2). These results suggest that *RGS2* modulates anxiety and/or aggressive behavior not only in mice but also in humans. In addition, a targeted genome screen found modest evidence for linkage between markers in a region including *RGS2* and anxiety disorder proneness (14).

From these findings, we tried to replicate an association between panic disorder and genetic variations of the *RGS2* using a case-control study of 186 patients with PD and 380 controls in Japanese.

MATERIALS AND METHODS

Subject

The subjects consisted of 186 Japanese patients with PD (94 males and 92 females; mean age±SD, 36.4±11.5 years), all of whom met ICD10 criteria for PD. The controls consisted of 380 unrelated volunteers (164 men and 216 women; mean age±SD, 42.8±16.8 years). All subjects were healthy individuals of Japanese descent, and none manifested any psychiatric problems in unstructured interviews with DSM-IV diagnosis criteria by psychiatrists. This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine.

Genotyping

Four of five common SNPs were selected for this study based on the previous genetic association of *RGS2* with completed suicides (2). Genotyping of the four SNPs was performed as described by Cui et al, 2008. Another SNP (rs12566194) is selected from Hapmap Data. We used the following polymerase chain reaction (PCR) and restriction fragment-length polymorphism (RFLP) methods and Taqman Assay for genotyping each polymorphism. rs12566194 was genotyped by TaqMan Assay and rs2746071, rs2746072, rs4606, and rs3767488 were genotyped by PCR-RFLP methods. Detailed information is available on request.

Statistical analysis

The genotype distribution or allele frequency for the PD and the controls was compared by using two-tailed Fisher's exact test. Hardy-Weinberg equilibrium was assessed with the chi-square test. These statistical analyses were performed with the SNPAlyze program v 4.1(http://www.dynacom.co.jp/e/products/package/snpalyze /index.html).

Linkage disequilibrium, allelic/haplotypic frequencies, and the association between SNPs or haplotypes and PD were determined with the Haploview software program v 3.32. (http://www.broad.mit.edu/mpg/haploview/) (1). The PS v2.1.3.1 program (3) was used for the power analysis. The level of significance for all statistical results was set at $P < 0.05$.

RESULTS

The genotypic and allelic distributions of the five SNPs of the *RGS2* gene in the PD and control groups are shown in Table I. The genotypic distribution in all of the groups was in Hardy-Weinberg equilibrium. As shown in Table I, no association with PD was detected with the rs2746071 (p=0.510), rs2746072 (p=0.303), rs12566194 (p=0.510), rs4606 (p=0.109), and rs3767488 (p=0.466). These five SNPs were in linkage disequilibrium with each other (D'=0.15-0.96). The two SNPs (rs2746071 and rs2746072) at the 5'- flanking region of the *RGS2* gene form a tight LD block (D'=0.95) (Figure1). The haplotype AC of rs2746071 and rs2746072 SNPs have shown a nominally significant association with panic disorder (p=0.027) (TableII). However, this significance did not remain after correction for multiple testing. Prevalence of panic disorder is different between male and female. The morbidity of panic disorder in females is twice as likely as that in males. *RGS2*-deficient mouse, however, showed an anxious phenotype only in males (12). We also analyzed an association of *RGS2* with panic disorder in male and female separately. We could not find any significant association between *RGS2* and panic disorder among the male and female subgroups.

Table I. Genotypic and Allelic Frequencies of 5 SNPS in the *RGS2* Gene

SNP ID	**Genotype frequency, N (%)**				**Allele frequency, N (%)**			
		Control	**Panic Disorder**	**P value**		**Control**	**Panic Disorder**	**P value**
rs2746071	G/G	82(0.23)	43(0.24)	0.66	G	337(0.47)	180(0.49)	0.51
	G/A	173(0.48)	94(0.51)		A	379(0.53)	186(0.51)	
	A/A	103(0.29)	46(0.25)					
rs2746072	C/C	58(0.17)	30(0.16)	0.14	C	265(0.39)	158(0.43)	0.30
	C/G	149(0.44)	98(0.53)		G	407(0.61)	212(0.57)	
	G/G	129(0.39)	57(0.31)					
rs12566194	A/A	349(0.93)	170(0.92)	0.77	A	724(0.97)	354(0.96)	0.78
	A/G	26(0.07)	14(0.08)		G	26(0.03)	14(0.04)	
	G/G	0(0)	0(0)					
rs4606	G/G	63(0.18)	36(0.21)	0.19	G	306(0.43)	169(0.49)	0.11
	G/C	180(0.51)	97(0.56)		C	400(0.57)	179(0.51)	
	C/C	110(0.31)	41(0.23)					
rs3767488	C/C	58(0.16)	28(0.15)	0.32	C	289(0.41)	157(0.43)	0.47
	T/C	173(0.49)	101(0.56)		T	419(0.59)	207(0.57)	
	T/T	123(0.35)	53(0.29)					

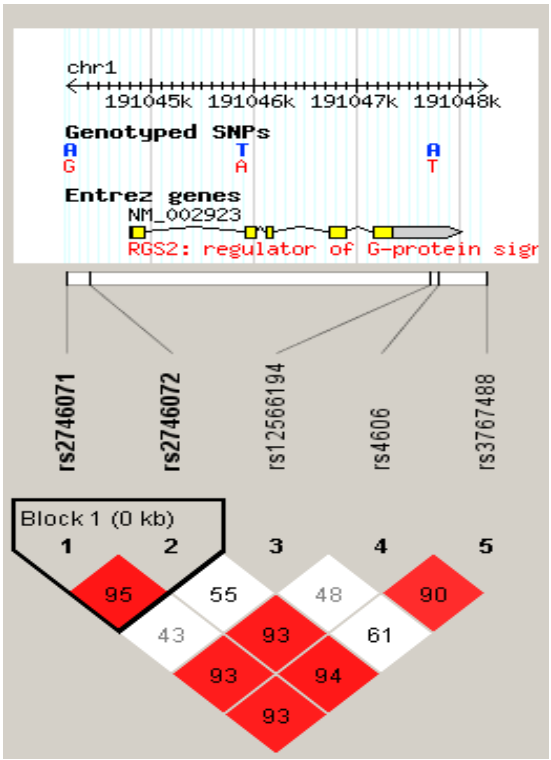

Figure1 Linkage disequilibrium map of the *RGS2* gene. The chromosomal position and the localization of exons, are given in the upper white panel. The lower panel displays the localization of the 5 SNPs genotyped in this study, and the linkage disequilibrium map based on D′. LD numbers represent the D′ values after the decimal point.

Table II. Haplotype analysis in the *RGS2* Gene

Haplotype block	Haplotype	Frequency	Chi Square	P Value
Block1: rs2746071	AG	0.514	0.106	0.745
rs2746072	GC	0.398	1.815	0.178
	GG	0.078	1.014	0.314
	AC	0.011	4.91	**0.027**

DISCUSSION

RGS2 regulates $G_{i/o}$ and Gq and is expressed in brain regions thought to underlie anxiety, including the hippocampus, amygdala, cerebral cortex, hypothalamus, and dorsal raphe nuclei (8, 9, and 11). Neuronal *RGS2* transcription is modulated by plasticity-inducing synaptic stimuli and by agents known to affect anxiety and mood symptoms (9, 11) and *RGS2* expression has been implicated in experience-dependent development of neural circuits (8). *RGS2*-deficient mice exhibit increased anxiety behavior (12), increased sympathetic tone, reduced heart rate variability, and increased urinary norepinephrine excretion (6).

Cui et al. found that rs2746071, rs2746072, rs4606, rs3767488 SNPs in *RGS2* gene were significantly associated with completed suicides (2). Moreover, two SNPs (rs4606, rs3767488) have been associated with panic disorder (10). The anxiety-related G allele of rs4606 has been associated with reduced *RGS2* expression in both peripheral blood mononuclear cells and fibroblasts in hypertensive patients (13). In this study, all five SNPs of the *RGS2* gene investigated, including the rs4606 SNP, were not associated with panic disorder. Although the haplotype AC of rs2746071 and rs2746072 have shown a nominally significant association with panic disorder ($p=0.027$), this significance did not remain after correction for multiple testing. In addition, we could not find a significant association of *RGS2* with panic disorder within the male and female subgroups. However, the sample size of our study may be insufficient to reach any robust conclusions. It needs to increase the number of subjects for analyses when we make a distinction between male and female.

One reason for the discrepancy between our results and those of Leygraf et al. may be due to a difference in population and selection of subjects. They chose subjects which were diagnosed as having panic disorder according to DSM-IIIR or DSM-IV, however we chose the PD patients whom met ICD10 criteria for PD as subjects. Because comorbid psychiatric disorders such as depression, alcohol dependence, etc. occur commonly in PD, we need to evaluate the PD as subjects using a structured interviewed. In this study, the powers of the analysis for each SNPs were 0.057 to 0.264. Considering the *RGS2* gene locus might have a small effect on panic disorder, we cannot completely exclude the possibility that our failure to find an association between these SNPs and panic disorder is due to type II error.

In conclusion, *RGS2* may not be genetically involved in the biological susceptibility to panic disorder in Japanese. As mentioned earlier, the pathophysiology of PD is involved with genetic components even though it should be kept in mind that the pathophysiology of most psychiatric disorders are polygenic and the etiology of most psychiatric conditions is multifactorial. Further studies to explore an association of other promising candidate genes on panic disorder are useful for helping to identify individuals with increased risk for panic disorder and even for helping to match treatment to anti-panic disorder.

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