



Association Study of RGS2 Gene Polymorphisms with Panic Disorder in Japanese

Mouri, Kentaro ; Hishimoto, Akitoyo ; Fukutake, Masaaki ; Nishiguchi, Naoki ; Shirakawa, Osamu ; Maeda, Kiyoshi

(Citation)

The Kobe journal of the medical sciences, 55(5):116-121

(Issue Date)

2009

(Resource Type)

departmental bulletin paper

(Version)

Version of Record

(JaLCD0I)

<https://doi.org/10.24546/81002359>

(URL)

<https://hdl.handle.net/20.500.14094/81002359>



Association Study of RGS2 Gene Polymorphisms with Panic Disorder in Japanese

KENTARO MOURI¹, AKITOYO HISHIMOTO^{1*}
MASAAKI FUKUTAKE¹, NAOKI NISHIGUCHI²
OSAMU SHIRAKAWA², and KIYOSHI MAEDA¹

¹ *Department of Psychiatry, Kobe University Graduate School of Medicine, Kobe, Japan*

² *Department of Neuropsychiatry, Kinki University School of Medicine, Osaka, Japan*

Received 18 September 2009/ Accepted 10 November 2009

Key Words: RGS2, panic disorder, polymorphisms, case-control study, linkage disequilibrium

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 play 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 significantly 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.

ASSOCIATION OF RGS2 WITH PD IN JAPANESE

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 $\pm$ SD, 36.4 $\pm$ 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 $\pm$ SD, 42.8 $\pm$ 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)					

ASSOCIATION OF RGS2 WITH PD IN JAPANESE

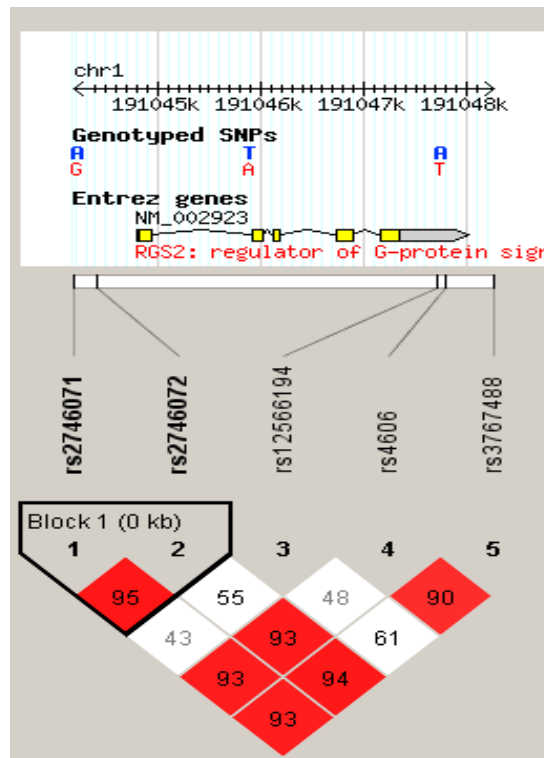


Figure1 Linkage disequilibrium map of the *RGS2* gene. The chromosomal position and the localization of exons, are given in the upper white panel. The lower panel displays the localization of the 5 SNPs genotyped in this study, and the linkage disequilibrium map based on D'. LD numbers represent the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

Table II. Haplotype analysis in the *RGS2* Gene

Haplotype block	Haplotype	Frequency	Chi Square	P Value
Block1: rs2746071 rs2746072	AG	0.514	0.106	0.745
	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 G_q 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-III-R 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 interview. 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.

REFERENCE

1. **Barrett, JC., Fry, B., and Maller, J.** 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*.**21**:263-265.
2. **Cui, H., Nishiguchi, N., and Ivleva, E.** 2008. Association of RGS2 gene polymorphisms with suicide and increased RGS2 immunoreactivity in the postmortem brain of suicide victims. *Neuropsychopharmacology*. **33**:1537-1544.
3. **Dupont, WD., and Plummer, WD Jr.** 1998. Power and sample size calculations for studies involving linear regression. *Control Clin Trials*. **19**:589-601.
4. **Ghavami, A., Hunt, RA., and Olsen, MA.** 2004. Differential effects of regulator of G-protein signaling (RGS) proteins on serotonin 5-HT_{1A}, 5-HT_{2A}, and dopamine D₂ receptor-mediated signaling and adenylyl cyclase activity. *Cell Signal*. **16**:711-721.
5. **Goodwin, RD., Faravelli, C., and Rosi, S.** 2005. The epidemiology of panic disorder and agoraphobia in Europe. *Eur Neuropsychopharmacol*. **15**:435-443.
6. **Gross, V., Tank, J., and Obst, M.** 2005. Autonomic nervous system and blood pressure regulation in RGS2-deficient mice. *Am J Physiol Regul Integr Comp Physiol*. **288**:R1134-R1142.
7. **Hettema, JM., Neale, MC., and Kendler, KS.** 2001. A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am J Psychiatry*. **158**:1568-1578.
8. **Ingi, T., and Aoki, Y.** 2002. Expression of RGS2, RGS4 and RGS7 in the developing postnatal brain. *Eur J Neurosci*. **15**:929-936.
9. **Ingi, T., Krumins, AM., and Chidiac, P.** 1998. Dynamic regulation of RGS2 suggests a novel mechanism in G-protein signaling and neuronal plasticity. *J Neurosci*. **18**:7178-7188.
10. **Leygraf, A., Hohoff, C., and Freitag, C.** 2006. Rgs 2 gene polymorphisms as modulators of anxiety in humans? *J Neural Transm*. **113**:1921-1925.
11. **Neubig, RR., and Siderovski, DP.** 2002. Regulators of G-protein signalling as new central nervous system drug targets. *Nat Rev Drug Discov*. **1**:187-197.
12. **Oliveira-Dos-Santos, AJ., Matsumoto, G., and Snow, BE.** 2000. Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc Natl Acad Sci U S A*. **97**:12272-12277.
13. **Semplicini, A., Lenzini, L., and Sartori, M.** 2006. Reduced expression of regulator of G-protein signaling 2 (RGS2) in hypertensive patients increases calcium mobilization and ERK1/2 phosphorylation induced by angiotensin II. *J Hypertens*. **24**:1115-1124.
14. **Smoller, JW., Acierno, JS., and Rosenbaum, JF.** 2001. Targeted genome screen of panic disorder and anxiety disorder proneness using homology to murine QTL regions. *Am J Med Genet*. **105**:195-206.
15. **Weissmann, MM., Bland, RC., and Canino, GJ.** 1997. The crossnational epidemiology of panic disorder. *Arch Gen Psychiatry*. **54**:305-309.
16. **Yalcin, B., Willis-Owen, SAG., and Fullerton, J.** 2004. Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. *Nat Genet*. **36**:1197-11202.