

Brief Research Communication

Evaluation of *RGS4* As a Candidate Gene for Schizophrenia

Yu-Li Liu,¹ Cathy Shen-Jang Fann,² Chih-Min Liu,¹ Jer-Yuarn Wu,³ Shuen-Iu Hung,³ Hung-Yu Chan,⁴ Jiahn-Jyh Chen,⁴ Chin-Yu Lin,² Shih-Kai Liu,¹ Ming H. Hsieh,¹ Tzung-Jeng Hwang,¹ Wen-Chen OuYang,^{4,5} Chun-Ying Chen,⁶ Jin-Jia Lin,⁷ Frank Huang-Chih Chou,⁸ Ching-Mo Chueh,⁹ Wei-Ming Liu,¹⁰ Ming-Min Tsuang,¹¹ Stephen V. Faraone,¹² Ming T. Tsuang,¹³ Wei J. Chen,¹⁴ and Hai-Gwo Hwu^{1,14,15*}

¹Department of Psychiatry, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

²Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

³National Genotyping Center, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

⁴Taoyuan Psychiatric Center, Taoyuan, Taiwan

⁵Chia-Nan Psychiatric Center, Tainan, Taiwan

⁶Tsaotun Psychiatric Center, Tsaotun, Taiwan

⁷Department of Psychiatry, Chimei Medical Center, Tainan, Taiwan

⁸Kai-Suan Psychiatric Hospital, Kaohsiung City, Taiwan

⁹Yuli Veterans Hospital, Yuli, Taiwan

¹⁰Yuli Hospital, Yuli, Taiwan

¹¹Ju-Shan Mental Hospital, Taoyuan, Taiwan

¹²Medical Genetic Research, Child and Adolescent Psychiatric Research, SUNY Upstate Medical University

¹³Harvard Institute of Psychiatric Epidemiology and Genetics, and Departments of Epidemiology and Psychiatry, Harvard University, Boston, Massachusetts and Institute of Behavioral Genomics, University of California, San Diego, California

¹⁴Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan

¹⁵Department of Psychology, College of Science, National Taiwan University, Taipei, Taiwan

Several studies have suggested that the regulator of G-protein signaling 4 (*RGS4*) may be a positional and functional candidate gene for schizophrenia. Three single nucleotide polymorphisms (SNP) located at the promoter region (SNP4 and SNP7) and the intron 1 (SNP18) of *RGS4* have been verified in different ethnic groups. Positive results have been reported in these SNPs with different numbers of SNP combinatory haplotypes. In this study, these three SNP markers were genotyped in 218 schizophrenia pedigrees of Taiwan (864 individuals) for association analysis. Among these three SNPs, neither SNP4, SNP7, SNP18 has shown significant association with schizophrenia in single locus association analysis, nor any combinations of the three SNP haplotypes has shown significant associations with the DSM-IV diagnosed schizophrenia. Our results fail to support the *RGS4* as a candidate gene for schizophrenia when evaluated from these three SNP markers. © 2006 Wiley-Liss, Inc.

KEY WORDS: schizophrenia; *RGS4*; G-protein; haplotype; SNP; Taiwan families

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Regulator of G-protein signaling (RGS) is a family of proteins modulating the G-protein signaling pathways [Hollinger and Hepler, 2002; Ishii and Kurachi, 2003]. RGS proteins have one major role as GTPase-activating proteins (GAP), which accelerate G α -catalyzed GTP hydrolysis and shorten the G protein mediated intracellular signaling [Dohlman and Thorner, 1997]. There are at least 20 RGS family members identified; the subtypes expressed in human brain include *RGS4*, *RGS7*, *RGS8*, *RGS11*, and *RGS17* [Erdely et al., 2004; Larminie et al., 2004]. The roles of RGS proteins in the central nervous system have not been extensively characterized, the *RGS4* gene has been suggested as a candidate gene for schizophrenia [Chowdari et al., 2002].

RGS4 has been supported as a candidate gene in schizophrenia by several studies. *RGS4* expression was decreased across the cerebral cortex of patients in microarray analysis [Mirnic et al., 2001a,b]. *RGS4* regulated the G-protein signaling pathways relevant to several schizophrenia-associated receptors such as dopamine and glutamate receptors [Saugstad et al., 1998; Taymans et al., 2003, 2004]. *RGS4* had a genomic position close to 1q21–q22, which had previously been implicated in schizophrenia by linkage studies [Brzustowicz et al., 2002; Hwu et al., 2003; Owen et al., 2004]. Furthermore, two proband-parent trio samples from the US, and from India, and a third small sample recruited by the NIMH Collaborative

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*Correspondence to: Hai-Gwo Hwu, Department of Psychiatry, National Taiwan University Hospital, No. 7, Chung San South Road, Taipei, Taiwan, 100. E-mail: haigohwu@ha.mc.ntu.edu.tw

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TABLE I. PCR Primer and Probe Sequences Designed for Each SNP of *RGS4*

SNP ID	Allele type	PCR primers and probe sequences (5'–3' end)
SNP1	G/A	Forward: ACGTTGGATGTGACCTCTAGTTTTGTAACG Reverse: ACGTTGGATGACCAGAATGTTATGCTAAAG Probe: GTTTTGTAACGTGCCCTG
SNP 4	A/C	Forward: ACGTTGGATGGGCCTAGACATCAATGAGAG Reverse: ACGTTGGATGTGGTATACAGCATCCTCCAG Probe: CAATGAGAGTTCAGTTATTGA
rs951436	G/A	Forward: ACGTTGGATGCATCTGGAAGGTATTGTTGAC
SNP 7		Reverse: ACGTTGGATGATAGTTCACATCCTGCTGTG Probe: GGCTGAATGTCTGCCCC
rs951439	A/G	Forward: ACGTTGGATGGTGAAGTTACTGCACCACC
SNP18		Reverse: ACGTTGGATGGAAGGTACCCAGAATGAG Probe: ACCACCACACGGAAAGC
rs2661319		

Genetics Initiative reported significant associations between schizophrenia and *RGS4* [Chowdari et al., 2002]. The involved haplotype encompassing four SNPs in the 5' flanking region of SNP1, SNP4, and SNP7 and the first intron of SNP18 of *RGS4* in each of the US samples [Chowdari et al., 2002]. However different risk haplotypes have appeared across different ethnic groups and neither significant association was obtained for the Indian sample [Chowdari et al., 2002], nor for the Caucasian study [Sobell et al., 2005]. In this study, the four SNP markers of the *RGS4* gene were tested for association in the population of Taiwan.

This research project was approved by the Institutional Review Board of National Taiwan University Hospital. All genomic DNA samples were collected from the family subjects with at least two affected sibling after obtaining written informed consent. The subjects were recruited from two research programs; the multidimensional psychopathology study of schizophrenia (MPSS) [Hwu et al., 2002] from 1993 to 2001 and the Taiwan schizophrenia linkage study (TSLs) [Hwu et al., 2005] from 1998 to 2002. The 86 families of MPSS subjects were interviewed by the research psychiatrists using the Psychiatrist Diagnostic Assessment (PDA) [Hwu, 1999]. The 132 TSLs families were interviewed by well-trained assistants using the Mandarin Chinese version of the Diagnostic Interview for Genetic Studies (DIGS) [Chen, 1999]. For both studies, the final diagnostic assessment was formulated by integrating either the PDA or the DIGS data with clinical information from medical records using the specialist diagnostic assessment sheet (SDAS), based upon the criteria of the diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV). This study sample included 218 schizophrenic nuclear families with at least two affected siblings, which include of 434 probands, 569 sib subjects, and 295 parent subjects. A total of 864 subjects participated in this genotyping study.

All SNP markers were genotyped by the method of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Table I). A DNA fragment (100–300 bp) encompassing the SNP site was amplified using the polymerase chain reaction (PCR) GeneAmp 9700 thermocycler

(Applied Biosystems, Foster City, CA) according to the manufacturer's instruction. After PCR amplification and neutralization of the deoxynucleotide triphosphate (dNTP), the primer extension was performed by adding the probe, Thermo Sequenase (Amersham Pharmacia, Piscataway, NJ) and appropriate dideoxynucleotide triphosphate (ddNTP)/dNTP mixture. Different extension products were differentiated by mass through MALDI-TOF.

We used the procedure ALLELE in SAS/GENETICS release 8.2 [SAS Institute, 2002] to assess Hardy–Weinberg equilibrium. Family relationships were verified by PEDCHECK version 1.1 [O'Connell and Weeks, 1998] and UNKNOWN version 5.23 [Terwilliger and Ott, 1994] to detect deviations from Mendelian inheritance. Linkage disequilibrium of intermarkers was measured using coefficient D' [Hedrick, 1987] which was also used to define haplotype blocks. The D' and Delta coefficients were calculated by the GOLD software [Abecasis et al., 2000]. Both single point and haplotype association analyses were carried out using TRANSMIT version 2.5.4 [Clayton, 1999].

Four *RGS4* SNP markers (SNP1, SNP4, SNP7, and SNP18) were first validated in a small independent 92 individuals to insure the existence of these SNPs in the Taiwan ethnic group before typing the rest of the genomic samples. A SNP was considered valid if the frequency of minor allele was larger than 10% and genotyping missing rate was smaller than 30%. The SNP 1 could not be assessed accurately by MALDI-TOF MS method. The three remaining SNP markers were compatible with the Hardy–Weinberg equilibrium distribution (Table II). As SNP 1 is 498 bp close to SNP4, the significant association between SNP 1 and schizophrenia were controversial in Caucasian [Morris et al., 2004; Williams et al., 2004], and the SNP 1 did not show significant association with schizophrenia in the Han Chinese population [Zhang et al., 2005]. We suspect that this SNP is not critical in schizophrenia.

The SNP4, SNP7, and SNP18 of *RGS4* were analyzed by the TRANSMIT program version 2.5.4 [Clayton, 1999] which can utilize data from all families even when parental genotypes are unknown. No significant associations were found in either the single locus association analysis (Table II) or the haplotype

TABLE II. *RGS4* Genotype Description and Single Locus Association Analysis

SNP	Location	LD		MF		Schizophrenia	
		D'	Delta	(H-W)	N	Chi	P -value
SNP 4	Ch1:160220719	0.929	0.692	0.50 (0.5845)	215	1.40	0.2369
SNP 7	Ch1:160221068	0.824	0.534	0.44 (0.5114)	216	0.85	0.3560
SNP18	Ch1:160227154	0.791	0.398	0.47 (0.8659)	215	0.72	0.3966

LD, linkage disequilibrium of adjacent SNPs represented by D' and Delta; N, the number of informative family; MF, minor allele frequency; H–W, Hardy–Weinberg P -value; Chi, the test statistics.

TABLE III. Haplotype Analyses of all Families Using Transmit v2.5.4 Program

Haplotype (SNP4-SNP7-SNP18)	Haplotype frequency	Schizophrenia N = 216	
		Chi	P-value
A-G-A	0.0573	0.10	0.7467
C-G-A	0.0560	0.05	0.8283
A-A-A	0.4273	1.18	0.2773
C-G-G	0.4409	0.74	0.3911
A-A-G	0.0185	0.02	0.8790

N, number of families with transmissions to affected offspring.

association analysis (Table III) in the DSM-IV diagnosed schizophrenia.

Our result did not support the *RGS4* promoter regions (SNP4, SNP7) and intron 1 (SNP18) to be the susceptible region for schizophrenia. As this region is located at the declining region of the genome wide mapping 1q21–22, we suspect that the *RGS4* gene may be not the major positional candidate gene for schizophrenia in this region.

Most of the replication studies with significant association of schizophrenia and *RGS4* were presented in Caucasian samples [Morris et al., 2004; Williams et al., 2004], but not in Indian [Chowdary et al., 2002] and Taiwan. It is possible that the *RGS4* is not involved in the pathogenesis of schizophrenia in the Asian population. These results suggest that if *RGS4* is a candidate gene for schizophrenia, it seems to have much less influence on the ethnic group of Taiwan Chinese.

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