Brief Research Communication

Evaluation of RGS4 As a Candidate Gene for Schizophrenia

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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 (884 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 compositions of the three SNP haplotypes has shown significantly associations with the DSM-IV diagnosis of 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.

KEYWORDS: schizophrenia; RGS4; G-protein; haplotype; SNP; Taiwan families


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 Gz-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 [Mirmics 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 [Bzustowicz 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...
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 [Sohell 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 siblings 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 Sheet (SDAS), based upon the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). This study sample included the criteria of the Diagnostic Interview for Genetic Studies (DIGS) [Chen, 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 [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 Sheet (SDAS), based upon the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). This study sample included the criteria of the Diagnostic Interview for Genetic Studies (DIGS) [Chen, 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 I. PCR Primer and Probe Sequences Designed for Each SNP of RGS4

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Allele type</th>
<th>PCR primers and probe sequences (5’-3’ end)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP1 A/G</td>
<td>Forward: ACGTTGGATGTGACCTCTAGTTTTGTAACG</td>
<td>Reverse: ACGTTGGATGCGCTATGTTTGTGAACG</td>
</tr>
<tr>
<td>SNP 4 A/C</td>
<td>Forward: ACGTTGGATGGCCTAGACATGTTTGTGAACG</td>
<td>Reverse: ACGTTGGATGCGCTATGTTTGTGAACG</td>
</tr>
<tr>
<td>SNP7 G/A</td>
<td>Forward: ACGTTGGATGCATCTGGAAAGGTATTGTGAC</td>
<td>Reverse: ACGTTGGATGCGCTATGTTTGTGAACG</td>
</tr>
<tr>
<td>SNP18 A/G</td>
<td>Forward: ACGTTGGATGCGCTATGTTTGTGAACG</td>
<td>Reverse: ACGTTGGATGCGCTATGTTTGTGAACG</td>
</tr>
</tbody>
</table>

### Table II. RGS4 Genotype Description and Single Locus Association Analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>LD</th>
<th>MF</th>
<th>Schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP 4</td>
<td>Ch:1:160220719</td>
<td>0.929</td>
<td>0.692</td>
<td>0.50 (0.5845)</td>
</tr>
<tr>
<td>SNP 7</td>
<td>Ch:1:160221068</td>
<td>0.824</td>
<td>0.534</td>
<td>0.44 (0.5114)</td>
</tr>
<tr>
<td>SNP18</td>
<td>Ch:1:160227154</td>
<td>0.791</td>
<td>0.398</td>
<td>0.47 (0.8659)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Haplotype (SNP4-SNP7-SNP18)</th>
<th>Haplotype frequency</th>
<th>Chi</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-G-A</td>
<td>0.0573</td>
<td>0.10</td>
<td>0.7467</td>
</tr>
<tr>
<td>C-G-A</td>
<td>0.0560</td>
<td>0.05</td>
<td>0.8283</td>
</tr>
<tr>
<td>A-A-A</td>
<td>0.4273</td>
<td>1.18</td>
<td>0.2773</td>
</tr>
<tr>
<td>C-G-G</td>
<td>0.0549</td>
<td>0.02</td>
<td>0.8790</td>
</tr>
</tbody>
</table>

N, number of families with transmissions to affected offspring.

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REFERENCES


