Polymorphisms and Haplotypes of the Regulator of G Protein Signaling-2 Gene in Normotensives and Hypertensives

Evan L. Riddle, Brinda K. Rana, Kenton K. Murthy, Fangwen Rao, Eleazar Eskin,
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Abstract—Regulator of G protein signaling (RGS) proteins stimulate the GTPase activity of G proteins, thereby negatively regulating G protein-coupled receptor signaling. RGS2, which preferentially alters Goα-mediated signaling, may be important for cardiovascular health, because knockout of RGS2 in mice is associated with altered smooth muscle relaxation and hypertension. In this study, we determined genetic variation in the human RGS2 gene by sequencing DNA in normotensive and hypertensive populations of whites (n = 128) and blacks (n = 122). We identified 14 single nucleotide polymorphisms and 2 two-base insertion/deletions (in/del; 1891 to 1892 TC and 2138 to 2139 AA). Although most of the genetic variants were found at low allelic frequency, in particular in coding regions, the 1891 to 1892 TC and 2138 to 2139 AA intronic in/del were in linkage disequilibrium and were associated with hypertension in blacks (P < 0.05). We defined several haplotypes for the RGS2 gene, certain of which showed striking differences between whites and blacks. Additionally, 2 haplotypes had significantly different frequencies between hypertensive and normotensive black groups (P < 0.05). We conclude that RGS2 is genetically conserved within coding regions but that the intronic in/del define ethnicity-specific haplotypes. Moreover, certain RGS2 variants that occur at greater frequency in hypertensive blacks may serve as ethnicity-specific genetic variants for this disease. (Hypertension. 2006;47:1-6.)

Key Words: hypertension, genetic □ G proteins □ signal transduction □ blood pressure

G protein–coupled receptor (GPCR) signaling via heterotrimeric G proteins and G protein–regulated effector molecules is critical for normal cardiovascular function, and abnormalities in GPCR signaling have been noted in numerous cardiovascular disorders. Moreover, a wide variety of drugs that are effective in the treatment of cardiovascular disorders target GPCRs. Regulators of G protein signaling (RGS) proteins negatively regulate GPCR signaling by accelerating the inactivation of G proteins by stimulation of their GTPase-activating protein (GAP) activity. From a conceptual standpoint, RGS proteins have the potential to be important in cardiovascular physiology because of their modulation of GPCR signaling. Indeed, RGS proteins have emerged as important components of the cardiovascular system. Among the >30 known RGS proteins, ≥20 are expressed in the heart and vasculature.

Recently, numerous reports have demonstrated an important role for RGS2 in cardiovascular regulation. Specifically, the data provide evidence that (1) RGS2 is required for normal vascular function and blood pressure; (2) RGS2-deficient mice exhibit a hypertensive (HT) phenotype; (3) RGS2 is involved in NO/cGMP-mediated vascular relaxation; and (4) both peripheral vascular mechanisms, as well as a central nervous system mechanism, may be involved. Heximer et al have demonstrated that both rgs2−/− and rgs2+/- mice are HT, suggesting that genetic variation in RGS2 function or expression may be a risk factor for the development of hypertension in humans. In the present study, we set out to test the hypothesis that genetic variation in RGS2 is associated with human hypertension.

Methods

Subjects and DNA Samples
A cohort of 250 HT and normotensive (NT) subjects was recruited for this study by advertisement and referral from a general population in the San Diego area, as well as a hypertension specialty clinic. Seated blood pressure (BP) was measured in triplicate with a calibrated oscillometric device (Dinamap, Critikon). NT subjects were healthy, based on laboratory tests and clinical history. HT patients were ascertained from outpatient clinics of University of California San Diego/Veteran’s Administration, either hypertension specialty clinic or primary care (general internal medicine) clinic. All of the patients had a diagnosis of hypertension based on repeated BP measurements in the outpatient clinic, in the seated position, using
PCR and Sequencing Reactions

PCR reactions were performed in MJ Research Dyad thermal cyclers. Four sets of oligonucleotide primers were designed to generate fragments of RGS2 (Genbank: NT_004671.15) sequence: (1) 5'-CACCAGAAGCTTCATCGGCTG-3' and 5'-GGTTAATCTGGGTGTATGTC-3'; (2) 5'-AGTTCTGGTCTGGCTGT-3' and 5'-GAACACGACACCAATACTACCC-3'; (3) 5'-CTTGCACTTCACACTG-3' and 5'-CTTTATTTCCCA-3'; and (4) 5'-AGACCAATCGACATTTAGG-3' and 5'-GGATGGAATACGAGCCGAT-3'.

Twenty-five-μL PCR reactions were performed using 25 ng of DNA (or H2O for negative controls), 25 mM of MgCl2, 10 mM of 2-deoxyxynucleoside 5'-triphosphates, 20 μmol/L of primer, and 0.5 U reaction of AmpliTaq Gold TaqDNA Polymerase (Applied Biosystems). Taq polymerase was heatactivated by incubation at 95°C for 15 minutes. The reaction was cycled 40 times with a denaturation step of 95°C for 30 seconds, an annealing step of 65.8°C for 1 minute, an elongation step of 72°C for 1 minute, and a final elongation step of 72°C for 4 minutes. Verification of the location of each variant. Fourteen SNPs and 2 two-base in/dels were identified (Table 2). All of the coding sequence in/dels had a minor allelic frequency <5%. In the coding region (see Figure 1) we detected 1 nonsynonymous and 2 synonymous SNPs. Three black subjects, 2 of whom were HT, carried the Glu50Lys nonsynonymous SNP of exon 2. At Ala144(GCT) of exon 4, 2 subjects carried a T→C transition, and at Phe189 (TTC) of exon 5, 1 subject carried a C→T transition. The remaining 14 variants were found in noncoding regions with the majority located upstream of the ATG start codon. In white subjects, 4 variants had frequencies ≥1%: −43 (17.5%), 1892 (22.1%), 2138 (22.1%), and 2297 (19.2%). The black subjects exhibited 7 variants with a frequency ≥4%: −161 (4.9%), −43 (4.8%), −38 (10.1%), −3 (4.4%), 1891 to 1892 (24.8%), 2138 to 2139 (24.8%), and 2297 (4.1%). χ2 analysis revealed significant ethnic differences in SNP frequencies for SNPs at positions −161, −43, −3, 1892 (T→C), and 2297 (Table 2). Association studies of genotype with BP status revealed that the TC and AA in/dels, at positions 1891 to 1892CT and 2138 to 2139AA in/del, were significantly associated with higher BP (∙ P<0.05) in black subjects but not in white subjects (Figure 2). Other RGS2 variants were not associated with BP status or BP phenotypes.

Pairwise linkage disequilibrium (LD) calculation was performed among the 4 most common variants (−43 A→T, 1891 to 1892 TC in/del, 2138 to 2139 AA in/del, and 2297 A→G). The results revealed high levels of LD among these variants with the position 1891 to 1892CT and 2138 to 2139 AA in/del in complete LD in both ethnic groups (D' = 1.0, D = 1.0; P<0.0001). Additionally, in both ethnic groups, SNPs located at −43 and 2297 were observed to be in near-absolute LD (D' = 0.956, D = 0.894; P<0.0001). Therefore, either one of the in/del or SNPs can serve as a marker for its nearby in/del or SNP in black and white populations.

Haplotype analysis revealed 11 distinct haplotypes (Table 3), all of which exhibited either the presence or absence of both 1891 to 1892CT and 2138 to 2139 AA in/del. In addition,
SNPs -43 and 2297 were in strong LD: >98% of white and black subjects had the -43A/2297A or -43T/2297G genotype. Haplotype analysis revealed ethnic specificity that was not seen in the assessment of individual allelic frequencies. Although we found no ethnic difference for individual 1891 to 1892TC and 2138 to 2139AA in/dels, when analyzed in combination with the -43 and 2297 variants, substantial ethnic diversity was observed. Thus, haplotype C was much more common in white subjects, whereas haplotype D was more common in black subjects.

Figure 1. RGS2 sequence with labeled variants. Coding regions are shown in bold and underlined. SNPs and in/dels are depicted in bold.

**TABLE 2. RGS2 Variants and Allelic Frequency in White and Black Subjects**

<table>
<thead>
<tr>
<th>No.</th>
<th>Position</th>
<th>Reference SNP (RefSNP ID)</th>
<th>White (n=128), %</th>
<th>Black (n=122), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-213 G&gt;A</td>
<td>rs11002</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>-162 C&gt;T</td>
<td>rs11002</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>-161 G&gt;T</td>
<td>rs16834859</td>
<td>0.4</td>
<td>4.9*</td>
</tr>
<tr>
<td>4</td>
<td>-69 C&gt;T</td>
<td>rs11002</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>-65 G&gt;A</td>
<td>rs11002</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>-43 A&gt;T</td>
<td>rs12130714</td>
<td>17.5</td>
<td>4.8†</td>
</tr>
<tr>
<td>7</td>
<td>-38 C&gt;T</td>
<td>rs11002</td>
<td>0.4</td>
<td>10.1‡</td>
</tr>
<tr>
<td>8</td>
<td>-3 A&gt;T</td>
<td>rs11002</td>
<td>0</td>
<td>4.4§</td>
</tr>
<tr>
<td>9</td>
<td>160 A&gt;T</td>
<td>rs11002</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>180 C&gt;G</td>
<td>rs11002</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>1132 C&gt;A  (G50K)</td>
<td>rs11002</td>
<td>0%</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Position indicates nucleotide position with respect to ATG start codon. Variant notation denotes major allele>minor allele. Variants with an allele frequency >4% are bolded. Variants exhibiting a significant difference between ethnicities: *χ²=4.55, P<0.05; †χ²=9.35, P<0.01; ‡χ²=11.24, P<0.001; §χ²=5.38, P=0.025; ±χ²=13.06, P<0.001.
subject, is likely derived from a recombination event in haplotypes E and G. The other rare haplotypes are likely derived from recombination events of haplotypes A and C.

**Discussion**

Over the past decade since their discovery, RGS proteins have emerged to become potentially important disease and drug targets, including the possibility that their genetic variation may contribute to disease. GPCRs have long been known to regulate numerous physiological processes, and, as knowledge of GPCRs grows, it is apparent that these proteins are likely to be critical regulators of human physiology.

RGS2, one of the best-studied RGS proteins, is found in many tissues, including those involved in cardiovascular regulation, and as a quantitative trait locus that modulates anxiety in mice. RGS2 is unique in that it may be the most potent GAP for Gs, with selectivity for that Gs. This is likely to be physiologically important, because a wide variety of key neurotransmitters and hormones that are active in the cardiovascular system (e.g., acetylcholine, norepinephrine, angiotensin II, thromboxane A2, thrombin, and endothelin 1) are agonists for GPCRs that signal via Gs. In addition to GAP activity, RGS2 can bind and inhibit adenylyl cyclase and interact with Gs. RGS2 and other components involved in GPCR signaling.

Studies with RGS2-deficient mice have demonstrated that both homozygous and heterozygous knockout mice exhibit an HT phenotype. This suggests that genetic alterations in RGS2 that produce a decrease in function and/or protein expression levels may contribute to the development (or exacerbation) of hypertension. Moreover, patients with Bartter’s/Gitelman’s syndrome, a hypotensive disorder, have enhanced expression of RGS2, consistent with a role of RGS2 in vascular regulation in humans.

In this study, we identify 16 genetic variants in the RGS2 gene obtained from a population of 250 individuals of white and black ethnicities. Many of these variants occur at low frequencies (<4%) with only 3 in coding regions, 2 of which are silent polymorphisms. We identified 1 nonsynonymous SNP, which converts a glutamine to a lysine (Gln50Lys) that was expressed in a heterozygous manner in 2 HT and 1 NT black subjects. Because its allelic frequency is only 1.3%, a large population would be required to determine whether this nonsynonymous variant is unlikely to be a major contributor to the HT phenotype.

By contrast, various RGS2 SNPs are found at a frequency >4% (SNPs –161, -43, -38, -3, 1891 to 1892, 2138 to 2139, and 2297), several of which show a statistically significant ethnic (black versus white) specificity (SNPs –161, -43, -38, -3, 1891 to 1892, 2138 to 2139, and 2297), several of which show a statistically significant ethnic (black versus white) specificity.

**TABLE 3. RGS2 Haplotype Distribution**

<table>
<thead>
<tr>
<th>No.</th>
<th>−161</th>
<th>−43</th>
<th>−38</th>
<th>−3</th>
<th>1891 to 1892</th>
<th>2138 to 2139</th>
<th>2297</th>
<th>NT (n=146), %</th>
<th>HT (n=108), %</th>
<th>NT (n=116), %</th>
<th>HT (n=118), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>A</td>
<td>59.3</td>
<td>56.5</td>
<td>73.3</td>
<td>57.6*</td>
</tr>
<tr>
<td>B.</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>D</td>
<td>D</td>
<td>A</td>
<td>21.3</td>
<td>23.1</td>
<td>7.8</td>
<td>16.9†</td>
</tr>
<tr>
<td>C‡</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>G</td>
<td>15.3</td>
<td>19.4</td>
<td>3.4</td>
<td>4.2</td>
</tr>
<tr>
<td>D§</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>D</td>
<td>D</td>
<td>A</td>
<td>…</td>
<td>0.9</td>
<td>6.9</td>
<td>9.3</td>
</tr>
<tr>
<td>E.</td>
<td>T</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>A</td>
<td>0.7</td>
<td>…</td>
<td>1.7</td>
<td>5.9</td>
</tr>
<tr>
<td>F.</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>G</td>
<td>2.7</td>
<td>…</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>G.</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>G</td>
<td>I</td>
<td>I</td>
<td>A</td>
<td>…</td>
<td>…</td>
<td>0.9</td>
<td>2.5</td>
</tr>
<tr>
<td>H.</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>G</td>
<td>D</td>
<td>D</td>
<td>A</td>
<td>…</td>
<td>…</td>
<td>0.9</td>
<td>2.5</td>
</tr>
<tr>
<td>I.</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>A</td>
<td>…</td>
<td>…</td>
<td>2.6</td>
<td>…</td>
</tr>
<tr>
<td>J.</td>
<td>G</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>A</td>
<td>0.7</td>
<td>…</td>
<td>1.7</td>
<td>…</td>
</tr>
<tr>
<td>K.</td>
<td>T</td>
<td>A</td>
<td>C</td>
<td>G</td>
<td>I</td>
<td>I</td>
<td>A</td>
<td>…</td>
<td>…</td>
<td>0.9</td>
<td>0.8</td>
</tr>
</tbody>
</table>

n represents the No. of chromosomes sequenced; D, deletion; I, insertion. Haplotypes exhibiting a significant difference between NT and HT groups: *χ²=6.33, P<0.025; †χ²=4.55, P<0.05. Haplotypes exhibiting significant difference between Whites and Blacks: ‡χ²=22.27, P<0.001; §χ²=18.81; P<0.001.
Two of the 16 variants, the 1891 to 1892TC and 2138 to 2139AA in/del, which are in complete LD, are more common, each occurring 20% to 25% in both whites and blacks. These variants appear to be associated with hypertension in the black, but not the white, population. It would be of interest to determine whether the 1891 to 1892TC and 2138 to 2139AA in/del variants alter RGS2 activity or expression, although both variants are in intronic regions. The 1891 to 1892TC in/del occurs in 17 to 18 nucleotides before the start of exon 4, at a location that may affect splicing.

Haplotypic analysis revealed additional associations between genetic variation and hypertension. The frequencies of haplotypes A and B are significantly different among the NT and HT black populations (Table 3) suggesting that a combination of variants in RGS2 may be required for the promotion of the HT phenotype.

While this article was being written, Yang et al32 described genetic variations of RGS2 in a Japanese population. Only 6 of the 16 variants that we identified were found in that population, suggesting ethnic variation in the RGS2 gene. Neither we nor Yang et al32 observed a contribution of these 6 variants (−161, −43, 1891 to 1892, 2067, 2297, and 2456) to HT status or BP in white Americans, blacks, or Japanese. Interestingly, Yang et al32 found that the 1891 to 1892 TC in/del was significantly associated with HT in Japanese women. We also observed an association with HT for this variant, albeit only in our black population with no apparent gender specificity. Approximately 40% of Japanese individuals in the sample of Yang et al32 had the 1891 to 1892 TC in/del, a slightly higher frequency than we noted, but the 2138 to 2139 AA in/del was not found in their population, although we found it in complete LD with the 1891 to 1892 TC in/del in both whites and blacks. Perhaps the regions of DNA sequenced by Yang et al32 did not include the 2138 to 2139 nucleotides.

In summary, our results indicate that RGS2 is a relatively conserved gene with little genetic variation, in particular in its coding sequence. This may be indicative of the importance of this molecule in regulating human physiology. However, the intronic 1891 to 1892TC and 2138 to 2139AA in/del, as well as haplotype B, are associated with hypertension in blacks and, thus, should be considered potential ethnic-specific genetic contributors to this disorder.

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References


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