Genetic Variation in the Renin-Angiotensin System and Autonomic Nervous System Function in Young Healthy Japanese Subjects

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Context: The renin-angiotensin system (RAS) interacts with the autonomic nervous system (ANS) in the regulation of blood pressure and cardiovascular function. Several genetic polymorphisms in the RAS have been identified and have been implicated as a cause of hypertension and cardiovascular disease.

Objective: The aim of the present study was to evaluate the relation between genetic polymorphisms of the RAS (M235T of AGT gene, insertion/deletion of ACE gene, A1166C of AT1R gene, and A1675G of AT2R gene) and ANS function.

Subjects: One hundred forty-nine young healthy Japanese males were genotyped for each RAS polymorphism.

Main Outcome Measures: ANS function was evaluated by power spectral analysis of heart rate variability (HRV) during supine rest and in a standing position.

Results: In a supine position, subjects homozygous for the AGT 235T allele had a higher HRV sympathetic index than 235M allele carriers, whereas the orthostatic change in this index was relatively blunted in AGT 235TT carriers. In the analysis of gene-gene interaction, these effects of the AGT 235T homozygotes on HRV sympathetic index were more apparent in the presence of the ACE D allele. Meanwhile, the AT1R 1166C allele was significantly associated with higher HRV low-frequency power and sympathetic index in a standing position. These data suggest that the ACT M235T polymorphism is associated with sympathetic predominance at rest, and AT1R 1166C allele carriers have potentially increased sympathetic response.

Conclusions: Cardiac autonomic function can be modulated by genetic variation in the RAS even in young and healthy states.

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Abbreviations: ACE, Angiotensin I-converting enzyme; ANS, autonomic nervous system; AT1R, angiotensin type 1 receptor; BP, blood pressure; CVD, cardiovascular disease; ECG, electrocardiogram; HF, high frequency; HRV, heart rate variability; I/D, insertion/deletion; LF, low frequency; PNS, parasympathetic nervous system; RAS, renin-angiotensin system; SNS, sympathetic nervous system.

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has generally shown two major distinct regions of periodicity in electrocardiogram (ECG) R-R intervals: a high-frequency (HF) component (>0.15 Hz) and a low-frequency (LF) component (<0.15 Hz). Previous studies have shown that the HF component is mediated solely by parasympathetic nervous system (PNS) activity and that the LF component arises from both sympathetic nervous system (SNS) and PNS activities (12, 14). In addition, the ratio of LF to HF (SNS index) has been considered an index of sympathovagal balance or sympathetic-orthostatic activity (12, 14).

**Subjects and Methods**

**Study population**

One hundred forty-nine young healthy Japanese males, recruited at random from Kyoto University, participated in each examination after written informed consent was obtained. The study population consisted of 31 yr from 18–31 yr (21.3 ± 0.2 yr, mean ± sem). All subjects were normotensive [causal supine blood pressure (BP) < 140/90 mm Hg] and nonobese [body mass index (BMI) < 30 kg/m²]. It was determined by interview that they were not taking any medication and had no history of organic diseases such as CVD, metabolic disorder, renal disease, or neuropathy. BMI, BP (systolic and diastolic), and heart rate (supine rest/standing) were measured as baseline characteristics, and family history (including whether the subjects had relatives within the third degree who had hypertension, diabetes, or obesity) was investigated through interviews. All subjects underwent ECG recording and power spectral analysis of HRV. However, HRV in the standing position could not be determined for eight subjects.

The study protocol was reviewed by the appropriate institutional review committee of Kyoto University School of Medicine, and the guidelines of the Declaration of Helsinki were followed.

**Determination of genetic polymorphisms in the RAS**

Genomic DNA was extracted from whole blood (DNA Extractor WB Kit; Wako, Osaka, Japan). Techniques based on PCR-restriction fragment length polymorphism were applied to genotype the study subjects for M235T polymorphism in the AGT gene (17), A1166C polymorphism of the AT1R gene (18), and A1675G polymorphism of the AT2R gene (11), as previously described. I/D polymorphism of the ACE gene was detected using the technique described by Mayer et al. (19). The genotype of the ACE I/D polymorphism of one subject could not be determined because of an inadequate blood sample.

**ECG R-R interval power spectral analysis**

For details of the HRV analysis methodology, we refer to previous studies (12–14). Each subject was studied in a quiet room with an ambient temperature of 25°C. They rested supine for at least 20 min before ECG recording. The CM5-lead ECG was continuously recorded during supine rest and postural change to a standing position. After 10 min of supine rest, the subjects stood up by the bedside and remained at standing rest for another 10 min. During the test, the respiratory rate was controlled at 0.25 Hz (15 breaths/min) by means of an electric metronome to reduce significant variations in HRV spectral power from individual variation in breathing frequency and to avoid interference with the LF component by the parasympathetic component (20). The R-R interval power spectral analysis procedures have been described previously (21–23). Briefly, the ECG R-R interval data obtained from the CM5 lead was digitized at 1000 Hz, and the derived R-R interval time series were then aligned in 2-Hz sequence for power spectral analysis. The DC component and linear trend were completely eliminated by digital filtering for band-pass between 0.007 and 0.5 Hz. After passing through the Hamming-type data window, power spectral analysis was performed by means of a fast Fourier transform on the consecutive 480-sec time series of R-R interval data obtained during the tests. We evaluated very low frequency (VLF) (0.007–0.035 Hz), LF (0.035–0.15 Hz), HF (0.15–0.5 Hz), and total power (TP) (0.007–0.5 Hz) by integrating the spectrum for the respective band width. SNS index and percent HF were calculated as the ratios of (VLF + LF)/HF and HF/TP, respectively. The average heart rate in beats per minute in each position (supine rest/standing) was derived from the R waves of the ECG.

**Statistical analysis**

Hardy-Weinberg equilibrium was verified by comparison of the observed and expected genotype frequency using the χ² test. Following previous reports (15, 16, 21, 24, 25), a natural logarithmic transformation was used to normalize the distribution of HRV power spectral indices because these data showed a distribution skewed to the right. Differences in BMI, BP, and log-transformed values (ln) of HRV indices were evaluated by one-way ANOVA. These data are expressed as mean ± sem. The χ² test was performed for analysis of the relationship of genotype distributions to family history of hypertension, diabetes, or obesity. Correlations between HRV indices were assessed with Pearson’s correlation coefficients. To assess the genotype-posture interaction effects on HRV indices, repeated-measures ANOVA was used. A nonparametric test (Mann-Whitney U test or Kruskal-Wallis, as appropriate) was performed to evaluate differences among genotypes in the orthostatic change rate in SNS index that were not distributed normally, even after transformation of the variables. This index is therefore indicated with median and interquartile ranges in the Results and is represented in the figures as a box and whisker plot presenting the median and quartiles. Statistical analysis was performed using the Statview Statistical Package (SAS Institute Inc., Cary, NC). Significant differences were considered to be present at P < 0.05.

**Results**

**Characteristics of the study subjects**

The general clinical characteristics of the study subjects are shown in Table 1. The participants of the present study were all in good health with almost ideal BP and BMI. Table 2 shows genotype and allele frequency for each RAS polymorphism. There was no detectable deviation from the Hardy-Weinberg equilibrium for AGT M235T (χ² = 1.28; P = 0.26), ACE I/D (χ² = 1.72; P = 0.19), or AT1R A1166C (χ² = 0.15; P = 0.70) polymorphisms. Because the AT1R gene is mapped on the X-chromosome, we here show only the allele frequency of the AT1R A1675G polymorphism.

Table 3 shows the clinical characteristics for each RAS polymorphism according to the genotype. Because the number of subjects homozygous for the AT1R A1166C allele or AGT 235M allele was limited, statistical comparison was performed according to the presence or absence of the AT1R A1166C allele or the AGT 235M allele. There were no significant differences in any of the clinical characteristics for the AGT, ACE, and AT1R polymorphisms. Subjects with the AT1R A1166C allele showed a significantly lower heart rate at supine rest, but no such difference was observed in a standing position.

**TABLE 1. Clinical characteristics of study subjects (n = 149)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean value ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21.3 ± 0.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3 ± 0.2</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>114.8 ± 0.9</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>65.4 ± 0.8</td>
</tr>
<tr>
<td>Heart rate (supine rest, bpm)</td>
<td>61.6 ± 0.8</td>
</tr>
<tr>
<td>Heart rate (standing, bpm)</td>
<td>79.8 ± 0.9</td>
</tr>
<tr>
<td>Family history of hypertension, diabetes, or obesity (%)</td>
<td>28.9</td>
</tr>
</tbody>
</table>
TABLE 2. Genotype and allele frequencies of the studied polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype, no. (%)</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT (M235T)</td>
<td>MM, 3 (2.0)</td>
<td>M, 0.185</td>
</tr>
<tr>
<td></td>
<td>MT, 49 (32.9)</td>
<td>T, 0.815</td>
</tr>
<tr>
<td>ACE (intron 16 I/D)</td>
<td>II, 56 (37.8)</td>
<td>I, 0.635</td>
</tr>
<tr>
<td></td>
<td>ID, 76 (51.4)</td>
<td>D, 0.365</td>
</tr>
<tr>
<td>AT1R (A1166C)</td>
<td>AA, 121 (81.2)</td>
<td>A, 0.903</td>
</tr>
<tr>
<td></td>
<td>AC, 27 (18.1)</td>
<td>C, 0.097</td>
</tr>
<tr>
<td>AT1R (A1675G)</td>
<td></td>
<td>A, 0.611</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G, 0.389</td>
</tr>
</tbody>
</table>

Association of ANS with RAS gene polymorphisms

Pearson’s correlation coefficients between power spectral parameters of HRV derived from 8-min ECG R-R interval data in the supine rest position are presented in Table 4. As expected, these HRV indices were highly to moderately correlated with each other. TP was highly correlated with VLF, LF, and HF, whereas VLF and PNS index were highly correlated with LF and SNS index, respectively. On the basis of these correlations, three HRV indices, LF, HF, and SNS index, were selected as representative indices for this study.

Figure 1 shows the ECG R-R interval power spectral parameters according to AGT M235T or AT1R A1166C polymorphisms. At supine rest, AGT TT carriers had a significantly higher SNS index than MM/MT carriers [SNS index (geometric mean); TT 850.9%; MM 395.6% (131.2–673.9%); P = 0.041; mean ± sem (geometric mean); TT vs. MM + MT]. In addition, an interaction effect between the AGT genotypes and posture on SNS index was statistically significant (P = 0.010 by repeated-measures ANOVA), and the SNS index of AGT TT carriers showed significantly lower responses to orthostatic change, compared with those of MM/MT carriers. These correlations, three HRV indices, LF, HF, and SNS index, were selected as representative indices for this study.

In the present study, we found a significant interaction of the ACD I/D polymorphism with HRV spectral parameters (data not shown). Meanwhile, a significant three-way interaction effect, a genotype-genotype-posture interaction, was found between the AGT and ACE polymorphisms (Fig. 2; P = 0.029 by repeated-measures ANOVA). A higher SNS index in a supine position and a lower orthostatic change in AGT TT carriers as compared with MM/MT carriers were, furthermore, significant in the simultaneous presence of the ACE D allele (Fig. 2). However, there was no independent association of the ACD I/D polymorphism with HRV spectral parameters (data not shown).

No significant difference was observed in any of the ECG R-R interval power spectral parameters for the AT1R polymorphism.

Discussion

In the present study, we found, using HRV power spectral analysis, that the AGT M235T polymorphism and the AT1R A1166C polymorphism were significantly associated with ANS function in young and healthy Japanese men. In supine rest, a higher SNS index was observed in subjects homozygous for the AGT 235T allele than in carriers of the MT and MM genotypes, which suggested that the AGT 235TT genotype was associated with shift in sympathovagal valance toward sympathetic predominance. In addition, the rate of orthostatic change in SNS index was lower among AGT 235TT carriers than among noncarriers, indicating that sympathetic responsiveness to postural perturbation was blunted in AGT 235TT subjects. These effects of the AGT polymorphism were significantly modulated by the ACE I/D polymorphisms. The differences in SNS index among the AGT genotypes were more apparent in the simultaneous presence of the ACE D allele. In a standing position, the AT1R A1166C allele was associated with higher LF power and SNS index. This observation suggests that carriers of the AT1R A1166C allele have a potentially higher sympathoexcitatory response to states of sympathetic activation such as orthostatic change.

It should be noted that the subjects in the present study were young and healthy, with almost ideal BP and BMI, and that there were no significant differences in basic clinical features among the genotypes of each RAS polymorphism, except for a lower supine heart rate in carriers of the AT1R A1166C allele. Therefore, the observed results suggest that the AGT and AT1R polymorphisms contribute to variability in sympathovagal modulation, which is not through a preexisting disease and may be an early subclinical effect preceding manifestation of pathological phenotypes.

In the current study, sympathetic predominance and blunted autonomic response were observed in AGT 235TT carriers. Higher SNS index or lower parasympathetic parameters as reflected in HRV are presented in some types of hypertension and cardiac disease (25–27), and it has been suggested that sympathetic predominance can also contribute to prospective pathogenesis of hypertension and CVD (15, 16). In addition, poor responses to orthostatic change in both sympathetic and parasympathetic activation have been reported in hypertensive subjects (26, 28) and in patients with symptoms of chronic psychosocial stress that predispose to CVD (29). The association of the M235T polymorphism of the AGT gene with hypertension or CVD has been described in many previous reports (8, 9). The AGT M235T polymorphism is related to circulating AGT level, and the 235TT genotype has the highest plasma AGT concentration (30, 31). AGT is the precursor of angiotensin II, which stimulates norepinephrine release from peripheral sympathetic terminals and increases central sympathetic activation (1–4). Thus, it can be speculated that the AGT M235T polymorphism may be associated with an increased incidence of cardiovascular abnormalities, through an impairment of autonomic function, although a follow-up study is needed.

In the present study, we found a significant interaction between AGT M235T and ACE I/D genotypes in resting
sympathetic activity and its orthostatic response. These interative effects were in line with previous reports that showed an epistatic interaction of the AGT M235T polymorphism with ACE I/D polymorphism on cardiac hypertrophy or hypertension (32, 33). However, gene-gene interactions were not observed among other RAS polymorphisms, and larger sample sizes might be needed to detect its possible interactive contributions to ANS function.

In addition, we found that the sympathetic parameters of HRV were higher in AT1R 1166C allele carriers in a standing position, in which both the sympathetic system and RAS could be activated. Although a molecular functional role for this point mutation in the 3' untranslated region of the AT1R gene has not been demonstrated so far, it can be hypothesized that the AT1R A1166C polymorphism may be in linkage disequilibrium with other functional genetic variants affecting the expression or properties of AT1R. Some reports have indicated that the AT1R A1166C allele is associated with increased humoral and hemodynamic sensitivity to angiotensin II (34, 35), and a significant relationship between this polymorphism and hypertension, CVD, myocardial infarction, vascular stiffness, and left ventricular hypertrophy has also been reported (8, 9). Through AT1R, angiotensin II enhances the sympathetic system in both central and peripheral tissues and is up-regulated in pathological conditions such as idiopathic dilated cardiomyopathy and after myocardial infarction (37). We found no positive associations between the AT1R A1166C polymorphism and HRV spectral parameters, which suggests that the effects of this polymorphism on cardiac ANS function may be obscured, at least in healthy states, although the detailed (patho)physiological roles of this polymorphism are largely unknown.

The present study has potential limitations. Because the study subjects comprise only male Japanese, our conclusions cannot be generalized to other populations. In this context,
the distribution patterns of genotypes of RAS polymorphisms differ among various races (9). Therefore, the putative associations between RAS polymorphisms and ANS function should be investigated in other population samples. In addition, the study subjects were young and healthy, which could minimize possible alterations in ANS and RAS activities resulting from preexisting diseases such as hypertension, obesity, and CVD. The present observations should be considered cautiously in relation to the pathology of these diseases and could not be applied to the phenotypes of patients with hypertension or CVD. A follow-up study is needed to elucidate the long-term effects of RAS polymorphisms on autonomic function and to evaluate the prevalence of these diseases.

In conclusion, AGT and AT\(_1\)R genetic polymorphisms influence HRV spectral parameters in young and healthy subjects. The AGT 235TT genotype was associated with the sympathetic predominance and a blunted autonomic response to orthostatic change, and relatively higher sympathetic activity on standing was detected in AT\(_1\)R 1166C allele carriers. It is hypothesized that these marginal but crucial variations in autonomic activity or sympathovagal balance resulting from RAS polymorphisms may be early potential phenotypes that are precursors of future pathological episodes of various ANS dysfunction-related diseases. Additional research is necessary to explore the precise pathophysiological and etiological roles of the present associations with respect to hypertension and CVD in the general population.

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