Effects of polymorphisms in $\beta_1$-adrenoceptor and $\alpha$-subunit of G protein on heart rate and blood pressure during exercise test. The Finnish Cardiovascular Study

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THE REGULATION OF CARDIOVASCULAR responses, i.e., blood pressure and heart rate (HR), during physical stress is influenced by several environmental and genetic factors (4). Previous studies have tested several candidate genes in relation to these indexes of hemodynamics and hypertension (2, 8, 9, 19, 23). However, the majority of these results have been published based on the resting state, and knowledge on the effects of these genetic factors during exercise is scarce. As a part of the ongoing Finnish Cardiovascular Study (the FINCAVAS study) on the genetic factors underlying the variation in response to physical stress, we focused on genes predisposing to these differences.

One such genetic candidate is the gene $ADRB1$ in chromosome 10 (10q24-q26). $ADRB1$ encodes the $\beta_1$-adrenergic receptor, which is a major mediator of the sympathetic inotropic and chronotropic effects in the heart (5, 17). Two nonsynonymous single nucleotide polymorphisms (SNP) have been identified in $ADRB1$: Arg389Gly, which causes a substitution of arginine by glycine at amino acid position 389, and Ser494Gly, which replaces serine with glycine at position 49 (13, 24).

In vitro, the wild-type Arg389 form (73% frequency in Caucasians) of the $ADRB1$ Gly389Gly polymorphism produces increased high-affinity agonist binding and enhanced adenyly cyclase activities compared with the form Gly389 (15). The ex vivo experiments on human atrial preparations have yielded varying results: the Arg389Gly polymorphism has either not exerted any effects (16, 22) or the Arg389 variant has demonstrated greater inotropic and cAMP responses to norepinephrine than the Gly389 variant (21). In clinical studies, a link between the Arg389Gly polymorphism and resting HR, as well as diastolic arterial pressure (DAP), has been reported (2, 8), but HR response during exercise has not depended on the Arg389Gly alleles (6, 11, 26). Exercise responses in blood pressure have not been reported.

The Gly49 allele (15% frequency in Caucasians) is associated with greater agonist-promoted downregulation and altered glycosylation of the $\beta_1$-receptor in hamster fibroblasts compared with the Ser49 allele (12, 20). The SNP has not had any effects in the experiments with human atrial preparations (22), but Gly49 homozygotes have been reported to have lower basal HR than serine carriers (19). The impact of this SNP on HR, systolic arterial pressure (SAP), and DAP during exercise is unknown.

The stimulatory G protein subtype $G_s$ is a trimeric transmembrane protein that mediates the signals from $\beta_1$-adrenergic receptors to the adenyl cyclase, which catalyzes production of the second-messenger cAMP. The three subunits of these proteins have several SNPs, most of which do not induce.

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physical stress. reports on its influence on cardiovascular function during shown to be associated with hypertension (9), but there are no hypothesis that the above-mentioned polymorphisms modulate FINCAVAS study, was designed and carried out to test the effects of these genes during exercise is scarce or lacking examined in the resting state, while the knowledge of the function of exercise parameters has previously been reviewed in signal transduction. The GNAS1 gene in chromosome 20 (20q13.2) encodes the α-subunit that couples β1-adrenergic receptors with the adenylyl cyclase. There is a silent SNP in that gene at base locus 393 in which thymine is replaced by a cytosine (T393C). This polymorphism has been shown to be associated with hypertension (9), but there are no reports on its influence on cardiovascular function during physical stress.

The functional importance of these two SNPs in the β1-adrenergic receptor gene and the T393C polymorphism in Gs protein for cardiovascular parameters has previously been examined in the resting state, while the knowledge of the effects of these genes during exercise is scarce or lacking completely. The present study, a part of the ongoing FINCAVAS study, was designed and carried out to test the hypothesis that the above-mentioned polymorphisms modulate the responses in HR and blood pressure during a clinical exercise stress test.

METHODS

Study Cohort

The FINCAVAS study is an ongoing follow-up study focusing on the genetic background of exercise test responses and coronary heart disease (CHD). The present participant pool consisted of the patients undergoing exercise stress tests at the Tampere University Hospital between October 2001 and January 2003. All of the consecutive patients coming to take an exercise stress test and willing to participate in the study were recruited. The 890 patients with technically successful exercise tests and acquired genotypic samples were included in this study (563 men and 327 women, mean age 58.1 ± 12.6 yr). The participant characteristics are given in Table 1. The study protocol was approved by the Ethical Committee of the Hospital District of Pirkanmaa, Finland, and all patients gave informed consent before the study initiation, as stipulated in the Declaration of Helsinki.

Study Design

Data on demographics [i.e., weight, height, body mass index (BMI), waist-to-hip ratio], classical cardiovascular risk factors (i.e., smoking, hypertension, diabetes, occurrence of hyperlipidemia, and family history), lifestyles, medications, and medical history were gathered by using a computer-based questionnaire after the patients had signed the informed consent but before the exercise test. Blood samples were drawn for DNA analyses.

Exercise Test Protocol

Before the exercise stress test, the subject lay down in the supine position for 10 min, and the resting ECG was digitally recorded. The exercise test was performed by using a bicycle ergometer with electrical brakes. During the test, HR was registered every minute with ECG, whereas SAP and DAP were measured with a brachial cuff every second minute. Ventricular extrasystoles were automatically detected by the ECG analyzer Case Workstation (GE Medical Systems, Waukesha, WI). The following three values of each of the parameters were taken for analysis: resting, maximal during the exercise, and 4 min after the test (recuperation).

DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit and Qiagen BioRobot M48 Workstation, according to the manufacturer’s instructions (Qiagen, Hilden, Germany). DNA samples were genotyped by employing the 5′ nuclelease assay and fluorogenic allele-specific TaqMan MGB probes (14), using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The nucleotide sequences of primers and probes used in the PCR were deduced from published sequences deposited in the GenBank and Celera databases and synthesized by Applied Biosystems. PCR reaction containing genomic DNA, 1× Universal PCR Master Mix, 900 nM of each primer, and 200 nM of each probe was performed in 384-well plates by using the standard protocol in a total volume of 5 μl. End-point fluorescence was measured and genotype calling was carried out by the allelic discrimination analysis module after the PCR resulted in clear identification of the Ser49Gly and Arg389Gly polymorphisms of ADRB1 and the T393C polymorphism of GNAS1. Negative and positive controls (known genotypes) and random duplicates were used as quality control.

Statistical Analysis

All statistical analyses were performed with the SPSS release 12.0.1 for Windows (SPSS, Chicago, IL). The HR, SAP, and DAP values were compared between the genders and the different status of β-blockade with Student’s t-test for independent samples, *P < 0.05 and †P < 0.01.

Table 1. Patient characteristics for men and women

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 563)</th>
<th></th>
<th></th>
<th>Women (n = 327)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Min</td>
<td>Max</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.8†</td>
<td>14.1</td>
<td>50</td>
<td>134</td>
<td>71.3</td>
<td>13.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.0†</td>
<td>6.5</td>
<td>159</td>
<td>196</td>
<td>162.0</td>
<td>6.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3</td>
<td>4.4</td>
<td>17</td>
<td>45</td>
<td>27.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Age, yr</td>
<td>58.1</td>
<td>12.6</td>
<td>17</td>
<td>82</td>
<td>58.7</td>
<td>13.1</td>
</tr>
<tr>
<td>HR at rest, beats/min</td>
<td>62†</td>
<td>12</td>
<td>35</td>
<td>123</td>
<td>64</td>
<td>12</td>
</tr>
<tr>
<td>SAP at rest, mmHg</td>
<td>134†</td>
<td>18</td>
<td>82</td>
<td>218</td>
<td>140</td>
<td>21</td>
</tr>
<tr>
<td>DAP at rest, mmHg</td>
<td>79</td>
<td>10</td>
<td>54</td>
<td>108</td>
<td>79</td>
<td>10</td>
</tr>
</tbody>
</table>

Mean, SD, minimum (Min), and maximum (Max) values are given. n, No. of subjects. BMI, body mass index; HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure. Statistics: t-test for independent samples, *P < 0.05 and †P < 0.01.
covariate in the analyses. Also, the interaction between these covariates and polymorphisms studied was calculated. A $P < 0.05$ was considered statistically significant, and 95% confidence intervals (CI) were calculated when applicable.

Categorical variables were compared by using the $\chi^2$ test (Hardy-Weinberg equilibrium), and odds ratios (OR) with 95% CIs for acute myocardial infarction (AMI), CHD, occurrence of hypertension, and extrasystoles for the different genotype groups were calculated with multinomial regression analysis.

RESULTS

Descriptive Data and the Effect of $\beta$-Blocking Agents, Gender, and Age on HR, SAP, and DAP Values

The descriptive data in women and men, respectively, are shown in Table 1 and the genotype frequencies in Table 2. All genotype distributions adhered to the Hardy-Weinberg equilibrium.

Effect of $\beta$-blocking agents. The patients taking $\beta$-blocking agents had a lower HR than those not taking $\beta$-blockers at all of the three points in time during the exercise test: at rest (95% CI of difference: $-3.1$ to $-6.0$ beats/min), maximal load (CI: $-26.8$ to $-32.7$ beats/min), and recovery (CI: $-14$ to $-18$ beats/min). Those on $\beta$-blockers had lower SAP (CI: $-11$ to $-19$ mmHg) and tended to have lower DAP (CI: $0$ to $-3$ mmHg) at maximal load.

Effect of gender. Men had a lower HR than women at rest (CI: $-0.3$ to $-3.2$ beats/min) and during maximal exercise ($-0.7$ to $-7.8$ beats/min); they also had lower SAP at rest (CI: $-4$ to $-9$ mmHg) but not at exercise (CI: $0$ to $7$ mmHg). DAP did not differ between the genders.

Effect of age. The higher the age group, the lower the HR at all three phases. At rest and recovery, the SAP values increased in line with increasing age: the higher the age group, the higher the SAP ($P < 0.001$ for both time points, ANOVA). However, during exercise, the middle-aged subjects had the highest SAP ($P < 0.01$, ANOVA). DAP was higher in the middle-aged than the younger and older age groups at rest as well as during exercise ($P < 0.01$, ANOVA), and the two oldest groups had higher DAP than the youngest (<40 yr) at recovery.

In further statistical analyses, gender, age as a continuous variable, or, alternatively, as age group (<40 yr, 40–60 yr, and >60 yr), and the use of $\beta$-adrenergic antagonism (yes/no) were taken as covariates.

Effects of Genotypes on HR, SAP, and DAP and Their Changes

In all subjects, and in men and women separately, no statistically significant genotype $\times$ time interaction was found for any of the genotypes studied in relation to HR, SAP, or DAP responses over the three study phases (RANOVA, $P > 0.10$ for interaction in all analyses where age, BMI, and $\beta$-adrenergic antagonism were used as covariates), with one exception showing that HR response depended on the GNAS1 genotype ($P = 0.04$, Fig. 1).

In all subjects at rest, the Ser49Gly polymorphism of ADRB1 tended ($P = 0.06$, ANOVA) to differentiate HR (Fig. 2A), even though the HR values for each genotype did not differ from each other in pairwise post hoc analysis.

Arg389Gly polymorphism of ADRB1 tended ($P = 0.07$, ANOVA) to differentiate basal HR among the patients without $\beta$-adrenergic antagonists, but the group of Gly-Gly homozygotes having lower HR than the other two groups consisted of only 15 patients. The female Gly homozygotes of Arg389Gly had lower maximal HR during exercise than the two other genotype groups, but, again, the number of Gly homozygotes was low ($n = 21$, $P = 0.04$, ANOVA). Arg389Gly polymorphism of ADRB1 affected maximal SAP during exercise ($P = 0.04$, ANOVA, Fig. 2B) and the change in SAP from rest to maximal ($P = 0.03$, ANOVA, Fig. 2C).

T393C polymorphism of GNAS1 had subtle but statistically significant (ANOVA, $P < 0.05$) influence on blood pressures: at rest, SAP values for the three genotypes (CC, CT, TT) were $137 \pm 20$, $137 \pm 20$, and $136 \pm 21$ mmHg, and DAP values were $79 \pm 10$, $79 \pm 10$, and $80 \pm 11$ mmHg, respectively. The maximal SAP values were $194 \pm 30$, $191 \pm 30$, and $194 \pm 32$ mmHg, and DAP values were $92 \pm 13$, $92 \pm 13$, and $93 \pm 14$ mmHg, respectively.

Effects of Genotypes on AMI, Occurrence of CHD, Hypertension, and Ventricular Extrasystoles

In multinomial regression analysis, none of the ADRB1 and GNAS1 alleles presented elevated risk for AMI or CHD (i.e., OR = 1 was within the range of 95% CI, data not shown). However, among all subjects, Gly389 homozygotes tended to be more likely to have hypertension (OR = $1.69$, 95% CI $1.00$–$2.86$, $P = 0.050$) compared with Arg389 carriers. Arg389 homozygotes, particularly men, were less likely to have ventricular extrasystoles during the exercise (OR = 0.68, 95% CI $0.51$–$0.91$, $P = 0.009$ and OR = 0.60, 95% CI $0.42$–$0.86$, $P = 0.006$, respectively) than Gly389 carriers.

DISCUSSION

We studied the effects of two SNPs in ADRB1 and one SNP in GNAS1 on HR and blood pressure responses in a large, unsellected patient cohort attending an exercise stress test at a university clinic. The Ser49Gly polymorphism of ADRB1 tended to differentiate baseline HR independent of gender, age group, BMI, and treatment with $\beta$-blockers: Ser-Ser homozygotes had highest mean HR, and Gly-Gly homozygotes lowest (Fig. 2A). This finding is supported by a large cohort of hypertensive patients among whom the Gly49 homozygotes

Table 2. $\beta_1$-Adrenoceptor (ADRB1) and $\alpha$-subunit of G protein (GNAS1) genotype frequencies in the whole study population

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>ADRB1</td>
<td>Ser49Gly*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ser-Ser</td>
<td>585</td>
<td>67.8</td>
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<tr>
<td></td>
<td>Ser-Gly</td>
<td>255</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>Gly-Gly</td>
<td>23</td>
<td>2.7</td>
</tr>
<tr>
<td>ADRB1</td>
<td>Arg389Gly</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arg-Arg</td>
<td>523</td>
<td>58.8</td>
</tr>
<tr>
<td></td>
<td>Arg-Gly</td>
<td>307</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td>Gly-Gly</td>
<td>60</td>
<td>6.7</td>
</tr>
<tr>
<td>GNAS1</td>
<td>T131C‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T-T</td>
<td>338</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>T-C</td>
<td>432</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>C-C</td>
<td>111</td>
<td>12.6</td>
</tr>
</tbody>
</table>

$n$, No. of subjects. ADRB1, $\beta_1$-adrenergic receptor gene; GNAS1, G protein $\alpha$-subunit gene. Data are missing from *27 and ‡9 subjects.
were found to have a 5 beats/min lower resting HR than the Ser49 carriers (19). A recent report with a relatively small number of untreated hypertensive patients \((n = 11005)\) showed a trend toward a higher blood pressure response with a \(\beta\)-antagonist in Ser49 homozygotes compared with Gly49 carriers (10), but it was not observed in our larger study. The Gly389 homozygotes had higher maximal SAP during exercise than those with at least one Arg389 allele (Fig. 2B); consequently, the change of SAP from the resting state to the maximal had the same pattern (Fig. 2C). However, this polymorphism did not differentiate the blood pressure response during \(\beta\)-blocker treatment, being in agreement with a study on untreated hypertensive patients (18) \((n = 147)\) but not with other studies on either healthy or untreated hypertensive participants (10, 25) \((n = 34 – 40)\). The largest available study populations, therefore, endorse the negative finding, but they also support the view that the \(ADRB1\) Arg389Gly polymorphism may modulate hemodynamics in certain situations.

The \(GNAS1\) T393C polymorphism was the only SNP examined that differentiated the HR response over the three phases of the exercise test: rest, maximal load, and recovery (Fig. 1). However, the difference between the allele combinations seemed rather small to possess any clinical impact, and the same applies to the diminutive differences in SAP and DAP values between the \(GNAS1\) alleles. This SNP was not associated with the blood pressure response during \(\beta\)-blocker therapy, even though the \(T393\) carriers have been classified as good responders to \(\beta\)-blockade in a previous study on 268 untreated hypertension patients (9). On the other hand, the present cross-sectional study was not specifically designed for the detection of modulatory influences during drug treatment, and, therefore, the present findings might underestimate the role of these SNPs during \(\beta\)-blocker treatment.

The SNPs in \(ADRB1\) and \(GNAS1\) studied are most likely not disease-causing genes, but rather risk modifiers, and might thus also influence the progression of cardiovascular diseases (11). In line with this, Arg389 homozygotes have been reported to be at increased risk of developing hypertension (2). Similarly, the \(GNAS1\) T393C polymorphism has been shown to associate with hypertension (9) and to modulate the probability of hypertension, depending on alcohol consumption and smoking status (1, 7). \(ADRB1\) Gly49 homozygotes with congestive heart failure have had a decreased 5-yr mortality risk compared with Ser49 carriers (3). However, previous (11) and current evi-

**Fig. 1.** Heart rate (HR) (means ± SD) at three phases of the exercise test by the \(\alpha\)-subunit of G-protein \((GNAS1)\) T393C genotypes: cytosine homozygotes \((C/C, n = 334)\), thymine-cytosine heterozygotes \((T/C, n = 425)\), and thymine homozygotes \((T/T, n = 106)\). In repeated analysis of variance, the response curves differed from each other \((P = 0.04\) for interaction).
dence suggests that there are no associations between these two β-adrenergic receptor SNPs and CHD or risk of AMI. The fact that the present Arg389 homozygotes were less prone to extrasystoles than Gly389 carriers is a novel finding deserving further study.

In the present trial, all of the SNPs studied were selected according to their association with adrenergic cascade. Even though these polymorphisms do not seem to induce marked effects on the measured cardiovascular parameters, they may have a linkage effect with some functionally more important polymorphisms. Due to the homogeneity of the Finnish population, linkage disequilibrium within the current population is probably high. We aim to further expand the patient material and genotype of other polymorphisms known or suspected to alter cardiovascular function or diseases (the FINCAVAS study). The independent and combined effects of the present genotypic variations studied on cardiovascular morbidity and mortality will also be assessed prospectively with a long-term follow-up.

In conclusion, the three polymorphisms examined in the genes ADRB1 and GNAS1 seem to have a modulating role in the regulation of hemodynamics, both at rest and during exercise. However, the impact of the polymorphisms in absolute numbers appears to be minimal and thus possibly clinically insignificant.

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