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

Tuomo Nieminen,1 Terho Lehtimäki,2,3 Jarno Laiho,2,3 Riikka Rontu,2,3 Kari Niemelä,4 Tiit Kööbi,5 Rami Lehtinen,5,6 Jari Viik,7 Väinö Turjanmaa,5 and Mika Kähönen5

1Department of Pharmacological Sciences, Medical School, University of Tampere, 2Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Tampere University Hospital, 3Centre for Laboratory Medicine, Medical School, University of Tampere, 4Heart Centre, Department of Cardiology, Tampere University Hospital, 5Department of Clinical Physiology, Tampere University Hospital and Medical School, University of Tampere, and 6Tampere Polytechnic, and 7Ragnar Granit Institute, Tampere University of Technology, Tampere, Finland

Submitted 26 July 2005; accepted in final form 4 October 2005


The effects of polymorphisms in $\beta_1$-adrenoceptor and $\alpha$-subunit of G protein influence heart rate and blood pressure during exercise. The Finnish Cardiovascular Study investigated the effects of genetic polymorphisms on heart rate and blood pressure responses during exercise. The study population comprised 890 participants (563 men and 327 women, mean age 58.1 ± 12.6 yr) from the Finnish Cardiovascular Study. Their heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured at rest and during exercise. The study 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 Ser49Gly, which replaces serine with glycine at position 49 (13, 24). In vitro, the wild-type Arg389 form (73% frequency in Caucasians) of the $ADRB1$ polymorphism produces increased high-affinity agonist binding and enhanced adenylyl cyclase activities compared with the form Gly389 (15). The experiments conducted 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 $G_\alpha\beta\delta$ is a trimeric transmembrane protein that mediates the signals from $\beta_1$-adrenergic receptors to the adenylyl cyclase, which catalyzes production of the second-messenger cAMP. The three subunits of these proteins have several SNPs, most of which do not induce changes in the regulation of cardiovascular responses, i.e., blood pressure and heart rate (HR), during physical stress.
physical stress. Reports on its influence on cardiovascular function during exercise have been shown to be associated with hypertension, but there are no studies that have completely examined its role in cardiovascular function during physical stress.

The functional importance of these two SNPs in the β-adrenergic receptor gene and the T393C polymorphism in the GNAS1 gene for cardiovascular parameters has previously been reported in several studies. There are no reports on its influence on cardiovascular function during physical stress.

The FINCAVAS study, a part of the ongoing FINCAVAS study, was designed and carried out to test the hypothesis that the above-mentioned polymorphisms modulate the response of cardiovascular parameters to exercise. The study included 890 patients coming to take an exercise stress test and willing to participate in the study. All of the consecutive patients coming to take an exercise stress test were recruited. The study was approved by the Ethical Committee of the Tampere University Hospital. 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. The patient characteristics are given in Table 1.

| 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: *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>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Women (n = 327)</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td></td>
<td>84.8</td>
<td>14.1</td>
<td>50</td>
<td>134</td>
<td></td>
<td>71.3</td>
<td>13.7</td>
<td>44</td>
<td>126</td>
</tr>
<tr>
<td>Height, cm</td>
<td></td>
<td>176.0</td>
<td>6.5</td>
<td>159</td>
<td>196</td>
<td></td>
<td>162.0</td>
<td>6.1</td>
<td>144</td>
<td>183</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td>27.5</td>
<td>4.4</td>
<td>17</td>
<td>45</td>
<td></td>
<td>27.2</td>
<td>5.0</td>
<td>17</td>
<td>46</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
<td>58.1</td>
<td>12.6</td>
<td>17</td>
<td>82</td>
<td></td>
<td>58.7</td>
<td>13.1</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>HR at rest, beats/min</td>
<td></td>
<td>62*</td>
<td>12</td>
<td>35</td>
<td>123</td>
<td></td>
<td>64</td>
<td>12</td>
<td>34</td>
<td>110</td>
</tr>
<tr>
<td>SAP at rest, mmHg</td>
<td></td>
<td>134†</td>
<td>18</td>
<td>82</td>
<td>218</td>
<td></td>
<td>140</td>
<td>21</td>
<td>88</td>
<td>220</td>
</tr>
<tr>
<td>DAP at rest, mmHg</td>
<td></td>
<td>79</td>
<td>10</td>
<td>54</td>
<td>108</td>
<td></td>
<td>79</td>
<td>10</td>
<td>46</td>
<td>112</td>
</tr>
</tbody>
</table>

**DNA Extraction and Genotyping**

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (Qiagen, Hilden, Germany). DNA samples were genotyped by employing the direct nucleic acid assay and the allelic discrimination module of the PCR. 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 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. The corresponding values in different age groups were compared with ANOVA. The subjects with a pause of at least 3 days in taking β-blocker medication before the exercise stress test were classified as being to the nonuser group.

The longitudinal exercise stress test data were analyzed by repeated-measurement analyses of variance (ANOVA) using the genotypes as categorical factors and the HR, SAP, and DAP values measured at different points in time (resting, exercise, and recovery) as dependent repeated variables. The corresponding genotype differences at each of the three phases were evaluated separately with ANOVA. Well-known modulators of HR, age, and β-adrenergic antagonism (yes/no) were taken as covariates in the ANOVA and RANOVA analyses. Age was used either as a continuous variable or as age groups (<40 yr, 40–60 yr, and >60 yr). The study population was analyzed as a whole and divided based on gender. Since BMI is often an explaining factor for the different results in men and women, BMI was used as a
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 χ² 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 β-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 β-blocking agents. The patients taking β-blocking agents had a lower HR than those not taking β-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 β-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: 0–7 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 β-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 × 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 β-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 β-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 ± 20, 137 ± 20, and 136 ± 21 mmHg, and DAP values were 97 ± 10, 70 ± 10, and 80 ± 11 mmHg, respectively. The maximal SAP values were 194 ± 30, 191 ± 30, and 194 ± 32 mmHg, and DAP values were 92 ± 13, 92 ± 13, and 93 ± 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, unselected 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 β-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...
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 \(\text{GNAS1} T393C\) 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 \(\text{GNAS1}\) 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 \(\text{GNAS1}\) 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 \(\text{GNAS1} T393C\) 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 α-subunit of G-protein (\(\text{GNAS1} T393C\)) 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).](image)

![Fig. 2. Effects of the \(\beta\)-adrenoceptor gene (\(ADRB1\)) polymorphisms Ser49Gly and Arg389Gly in analysis of variance. A: in all subjects at rest, the Ser49Gly polymorphism tended \((P = 0.06)\) to differentiate HR. Arg389Gly polymorphism affected maximal systolic arterial pressure (SAP) during exercise \((P = 0.04)\) (B) and the change in SAP from rest to maximal \((P = 0.03)\) (C). \(n\), No. of subjects.](image)
ence 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.

ACKNOWLEDGMENTS

The authors thank Marita Koli and Nina Peltonen for skilful technical assistance and the staff of the Department of Clinical Physiology for collecting the exercise test data.

GRANTS

Financial support was received from the Medical Research Fund of Tampere University Hospital, the Finnish Foundation for Cardiovascular Research, the Academy of Finland (Grant 104821), and the Emil Aaltonen Foundation, Finland.

REFERENCES


