β2 Adrenoceptor Functional Gene Variants, Obesity, and Blood Pressure Level Interactions in the General Population

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Hypertension 2003;42;685-692; originally published online Aug 4, 2003;
DOI: 10.1161/01.HYP.0000085648.65419.17
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 75231
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**β2 Adrenoceptor Functional Gene Variants, Obesity, and Blood Pressure Level Interactions in the General Population**

Alexandre C. Pereira, Marcilene S. Floriano, Glória F.A. Mota, Roberto S. Cunha, Fernando L. Herkenhoff, José G. Mill, José E. Krieger

**Abstract**—We investigated the association of β2 adrenoceptor functional gene variants (Arg16Gly, Gln27Glu, and Thr164Ile polymorphisms), obesity phenotypes, and blood pressure levels in a large, ethnically mixed urban population. The individuals (n=1576) were randomly selected for a cross-sectional study of cardiovascular risk factors in Vitória, Brazil. Statistically significant associations among systolic blood pressure and the Arg16Gly and Thr164Ile variants were identified in univariate analysis. The Gly16/Gly16 genotype was still associated with systolic blood pressure (SBP) in multivariate analysis adjusting for age, gender, ethnicity, total cholesterol, diabetes, and body mass index (BMI) (P=0.01). The Arg16 allele was the only genotypic variable associated with BMI, and, in a dominant model, it remained associated with an increased BMI even after adjustment for age, gender, ethnicity, triglycerides, HDL cholesterol, LDL cholesterol, diabetes, and hypertension status (P=0.02). Although the different polymorphisms did not interact in the determination of SBP, a significant interaction with BMI (P=0.02), not through linkage disequilibrium, was identified between the Gln27Glu and the Thr164Ile variants. Furthermore, a significant interaction among the Arg16Gly polymorphism and BMI (P=0.036) and waist-hip ratio (P=0.003) in determining SBP was disclosed by ANOVA factorial modeling, with SBP used as the dependent variable. An interaction between the Thr164Ile polymorphism and waist-hip ratio was also identified (P=0.018). Finally, multiple logistic regression models showed a 1.48-fold increase in the risk of hypertension in individuals harboring the Gly16/Gly16 genotype and a 1.31-fold (P=0.01) and a 1.49-fold (P=0.003) increased risk of obesity in individuals harboring the Gln27/Gln27 genotype or the presence of the Arg16 allele, respectively. Taken together, these data provide evidence for a strong but complex relation between β-adrenoceptor gene variants, hypertension, and obesity. *(Hypertension. 2003;42[part 2]:685-692.)*

**Key Words:** receptors, adrenergic beta  ■ hypertension, obesity  ■ genetics  ■ polymorphism

Both hypertension and obesity are considered complex traits.1,2 The interplay between environmental and genetic factors is a major determinant of either final phenotype. In addition, it is currently accepted that both share not only similar risk factors but possibly similar genetic determinants.3

Despite intense effort, the genetic pathways underlying hypertension or obesity remain elusive. This is largely due to the complexities circumventing these processes, which include age of onset, quantitative variability of blood pressure phenotypes, polygenic inheritance, genetic heterogeneity, incomplete penetrance, unknown mode of action of disease alleles, effect of ethnicity, age, gender, and environmental factors, such as diet, physical activity, or smoking status, to name a few.

In this scenario, the study of components of important physiological control systems that are known to contribute to the regulation of both blood pressure and fat metabolism can offer important insights into the determination of the genetic mechanisms or pathways underlying not only interindividual blood pressure or obesity variation among humans, but, most importantly, may shed light on the complex interrelations of these two common diseases.

The components of the sympathetic nervous system are of particular interest, since they influence the control of vasomotion as well as energy expenditure associated with catecholamine metabolism.4

For instance, the adrenergic receptor β2 (ADRB2) is coupled to a stimulatory G protein that activates protein kinase, which mediates a variety of responses, depending on the cell type. In vascular smooth muscle cells, ADRB2 agonists promote a rise in intracellular cAMP concentration, leading to marked vasodilation. Other potential blood pressure regulating effects of ADRB2 include their action on renal sodium handling and the control of renin release.5 In addition, the lipolytic effects of catecholamines are mediated through members of the ADRB2 family.6

Genetic polymorphisms of ADRB2 have already been associated with obesity, diabetes mellitus, and essential hypertension, with conflicting results.7

Received May 12, 2003; first decision June 9, 2003; revision accepted June 27, 2003.
From the Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), São Paulo University Medical School (A.C.P., M.S.F., G.F.A.M., J.E.K.), São Paulo, Brazil; and the Department of Physiology, Espirito Santo Federal University (R.S.C., F.L.H., J.G.M.), Vitoria, Brazil.
Correspondence to Jose E. Krieger, MD, Laboratorio de Genética e Cardiologia Molecular, InCor-Instituto do Coracao, HCFMUSP, Av. Dr. Eneas de Carvalho Aguiar, 44, 05403-000 Sao Paulo SP, Brazil. E-mail krieger@incor.usp.br
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Hypertension is available at http://www.hypertensionaha.org
DOI: 10.1161/01.HYP.0000085648.65419.17

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In the present study, we have investigated the association of these genetic variants of the ADRB2 with blood pressure–related and obesity-related phenotypes in a large sample of individuals randomly selected from an ethnically mixed urban population.

Methods

Study Population
A cross-sectional study of risk factors for cardiovascular diseases was performed in the urban population of Vitória, Brazil, following the general guidelines of the WHO-MONICA project. Ascertainment and characteristics were described previously.9

Blood Pressure Phenotype Determination
Blood pressure was measured by trained technicians, using a standard mercury sphygmomanometer on the left arm after 5 minutes’ rest with the subject in the sitting position. The first and fifth phase of Korotkoff sounds were used for systolic and diastolic pressures, respectively. Systolic and diastolic blood pressures were calculated from two readings taken by two different observers. The two measurements were obtained with a minimal interval of 10 minutes. Hypertension was defined as the mean systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥90 mm Hg.10 Pulse pressure was the difference between systolic and diastolic blood pressures.

Assessment of ADRB2 Gene Polymorphism Genotypes
Genomic DNA was extracted from leukocytes in samples of whole blood, following standard techniques. The three studied polymorphisms were detected by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), as previously described.11 Quality control for these assays was assessed by randomly selecting 50 samples to be regenotyped by two independent technicians.

Statistical Analysis
Allele and genotype frequencies among study participants were analyzed by χ² test and multivariate logistic regression, with the use of the Statistical Package StatView for Windows, version 5.0 (SAS Institute Inc). Correction for multiple comparisons was not performed in any of the analyses in the present study. To test for differences in various characteristics, the Student t test was used for continuous variables and the χ² test was used for categoric variables. In addition, univariate analysis comparing continuous phenotypes was done by using correlation or simple linear regression when appropriate.

Hardy-Weinberg equilibrium for the distribution of genotypes, linkage disequilibrium statistics, haplotype frequency estimation, and test of population differentiation were conducted with the use of Arlequin software. The odd ratios for different association models were calculated with 95% confidence intervals and 2-tailed probability values. Pairwise linkage disequilibrium analysis has shown that significant disequilibrium exists between the Arg16Gly and Thr164Ile polymorphisms. All studied loci allele and genotype frequencies were in accordance with Hardy-Weinberg equilibrium. Pairwise linkage disequilibrium analysis has shown that significant disequilibrium exists between the Arg16Gly and Gln27Glu (P<0.001) and the Arg16Gly and Thr164Ile (P<0.001) polymorphisms but not between Gln27Glu and Thr164Ile polymorphisms (P=0.06). Interestingly, we disclosed an ethnic-related genetic structure (P<0.001) through the use of the exact test of differentiation. The genotypic content related to the studied alleles of white individuals of this population was significantly different from the one of mulatto and black individuals. More specifically, this difference could be explained by a decrease in the allele frequency of both the Ile164 and Glu27 variants and an increase in the frequency of the Gln27 variant in mulatto and black individuals. Neither gender, age, or smoking status was associated with the studied variants in univariate analysis (data not shown).

Results

Demographic and Genetic Structure Data
Table 1 summarizes the demographic data. In Table 2 we present allelic, genotypic, and haplotypic data for the studied polymorphisms. All studied loci allele and genotype frequencies were in accordance with Hardy-Weinberg equilibrium.

Table 1. Demographic Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Male (female)</th>
<th>Mean age, y (range)</th>
<th>Ethnicity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1576</td>
<td>45.6 (54.4)</td>
<td>44.8 (23.1–65.5)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European descent</td>
<td>35.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mulatto</td>
<td>51.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African descent</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currently smoking, %</td>
<td>22.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>214.4 (47.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>137.6 (127.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>45.4 (12.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>142.2 (39.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>25.6 (20.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>105.0 (32.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>7.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.3 (4.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentarism, %</td>
<td>74.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist, cm</td>
<td>86.5 (12.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 (0.09)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric Acid</td>
<td>4.8 (1.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continuous variables data presented as mean (±SD). Metabolic data are expressed in mg/dL. Diabetes mellitus was defined as fasting glucose ≥125 mg/dL. BMI indicates body mass index; WHR, waist-hip ratio.
Univariate and Multivariate Analysis Regarding Blood Pressure and Obesity

Univariate analysis testing for factors associated with systolic blood pressure have shown that gender ($P=0.0007$), age ($P<0.0001$), body mass index (BMI) ($P<0.0001$), waist-hip ratio (WHR) ($P<0.0001$), total cholesterol ($P<0.0001$), tri-glycerides ($P<0.0001$), LDL cholesterol ($P<0.0001$), VLDL cholesterol ($P<0.0001$), glucose ($P<0.0001$), diabetes status ($P<0.0001$), and uric acid ($P<0.0001$) were associated with systolic blood pressure in our population. Neither smoking status, HDL cholesterol, heart rate, or physical activity was associated with systolic blood pressure in univariate analysis (data not shown).

Univariate analysis testing for factors associated with BMI have shown that gender ($P=0.004$), age ($P<0.0001$), WHR ($P<0.0001$), total cholesterol ($P<0.0001$), tri-glycerides ($P<0.0001$), LDL cholesterol ($P<0.0001$), VLDL cholesterol ($P<0.0001$), glucose ($P<0.0001$), diabetes status ($P<0.0001$), and uric acid ($P<0.0001$) were associated with BMI in our population.

The effect of the studied polymorphisms in blood pressure phenotypes in univariate analysis are shown in Table 3. Statistically significant associations between systolic blood pressure and the Arg16Gly and Thr164Ile functional variants were identified. The best-fitting genetic model for the Arg16Gly polymorphism was considering a recessive mode of action for the Gly16 allele. Regarding the Gln27Glu polymorphism, the best model was the one considering the Glu27 allele in a dominant mode of action.

We have also studied the relation between these β-adrenergic receptor functional variants and obesity-related phenotypes in our population. These data are presented in Table 4. Interestingly, a significant association was also

### Table 2. Polymorphism Allelic and Genotypic Frequencies

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allelic Frequencies (%)</th>
<th>Genotypic Frequencies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16Gly</td>
<td>Arg allele 44.4</td>
<td>Arg/Arg 21.2</td>
</tr>
<tr>
<td></td>
<td>Gly allele 55.6</td>
<td>Arg/Gly 47.3</td>
</tr>
<tr>
<td>Gln27Glu</td>
<td>Gln allele 71.8</td>
<td>Gln/Gln 128.69</td>
</tr>
<tr>
<td></td>
<td>Glu allele 28.2</td>
<td>Glu/Gln 127.49</td>
</tr>
<tr>
<td>Thr164Ile</td>
<td>Thr allele 99.99</td>
<td>Thr/Thr 127.33</td>
</tr>
</tbody>
</table>

Haplotypic frequencies, %

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16 Gln27 Ile164</td>
<td>0.001</td>
</tr>
<tr>
<td>Arg16 Gln27 Thr164</td>
<td>0.43</td>
</tr>
<tr>
<td>Arg16 Glu27 Thr164</td>
<td>0.01</td>
</tr>
<tr>
<td>Gly16 Gln27 Ile164</td>
<td>0.01</td>
</tr>
<tr>
<td>Gly16 Gln27 Thr164</td>
<td>0.27</td>
</tr>
<tr>
<td>Gly16 Glu27 Thr164</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Allele and genotype frequencies were in accordance with the Hardy-Weinberg equilibrium.

### Table 3. Blood Pressure Phenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Systolic BP mm Hg</th>
<th>Diastolic BP mm Hg</th>
<th>Pulse Pressure mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16Gly polymorphism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>126.5</td>
<td>83.17</td>
<td>43.35</td>
</tr>
<tr>
<td>Arg/Gly</td>
<td>126.57</td>
<td>82.94</td>
<td>43.62</td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>129.21</td>
<td>84.46</td>
<td>44.76</td>
</tr>
<tr>
<td>Arg/Arg vs Gly/Gly</td>
<td>0.03</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>Gln27Glu polymorphism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>128.69</td>
<td>84.78</td>
<td>43.91</td>
</tr>
<tr>
<td>Gln/Glu</td>
<td>127.49</td>
<td>83.61</td>
<td>43.89</td>
</tr>
<tr>
<td>Glu/Glu</td>
<td>125.56</td>
<td>83.46</td>
<td>42.1</td>
</tr>
<tr>
<td>Gln/Glu vs Gln/Glu vs Glu/Glu</td>
<td>0.17</td>
<td>0.09</td>
<td>0.63</td>
</tr>
<tr>
<td>Thr164Ile polymorphism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>127.33</td>
<td>84.13</td>
<td>43.71</td>
</tr>
<tr>
<td>Thr/Ile</td>
<td>136.33</td>
<td>89.53</td>
<td>46.8</td>
</tr>
</tbody>
</table>

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demonstrated between the Arg16Gly polymorphism and BMI, considering a recessive mode of action of the Gly16 allele. In addition, a tendency toward increased BMI in individuals harboring Gln27 and Ile164 was also apparent. In Figure 1, we summarize the findings regarding univariate analysis of both blood pressure–related and obesity-related phenotypes in our population.

Multiple linear regression models were constructed to show the independent relation of the studied polymorphisms and blood pressure variation in this population. Presence of the Gly/Gly genotype for the Arg16Gly polymorphism was significantly associated with systolic ($P = 0.01$) and diastolic blood pressures ($P = 0.006$), even after adjustment for age, gender, ethnicity, total cholesterol, diabetes, and BMI. Presence of the Gln/Gln genotype of the Gln27Glu polymorphism was not associated with blood pressure in our multiple linear regression model ($P = 0.30$). Presence of the Ile164 allele was marginally associated with systolic blood pressure in a model with adjustment for age, gender, ethnicity, total cholesterol, and diabetes ($P = 0.06$), but this marginal association disappeared after inclusion of BMI in the model ($P = 0.15$). This may suggest that the effect of this allele in blood pressure regulation is operant only under interaction with obesity. The same seems not to occur with the Arg16Gly polymorphism: The effect of the Gly/Gly genotype persists even after adjustment for obesity-related variables. We could not disclose any interaction between the different genetic variants studied and blood pressure (data not shown).

In addition, we have created multiple linear regression models by using BMI as the dependent variable. The only genotypic variable significantly associated with BMI in our regression model was the presence of the Arg16 allele.

### Table 4. Obesity-Related Phenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>BMI</th>
<th>WHR</th>
<th>Waist. cm</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>LDL Cholesterol</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16Gly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>26.55 (5.3)</td>
<td>1.0</td>
<td>0.88</td>
<td>1.0</td>
<td>87.67 (13.6)</td>
<td>1.0</td>
<td>212.18 (45.6)</td>
</tr>
<tr>
<td>Arg/Gly</td>
<td>26.45 (5.0)</td>
<td>0.79</td>
<td>0.86</td>
<td>0.09</td>
<td>86.07 (12.6)</td>
<td>0.14</td>
<td>215.71 (42.8)</td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>25.83 (4.6)</td>
<td>0.06</td>
<td>0.88</td>
<td>0.09</td>
<td>86.74 (12.0)</td>
<td>0.09</td>
<td>216.23 (53.8)</td>
</tr>
<tr>
<td>Arg/Arg vs Arg/Gly</td>
<td>0.29</td>
<td>0.06</td>
<td>0.08</td>
<td>0.23</td>
<td>0.8</td>
<td>0.63</td>
<td>0.59</td>
</tr>
<tr>
<td>Arg/Gly vs Gly/Gly</td>
<td>0.02</td>
<td>0.09</td>
<td>0.34</td>
<td>0.56</td>
<td>0.32</td>
<td>0.85</td>
<td>0.46</td>
</tr>
</tbody>
</table>

| Gln27Glu |       |         |           |                   |             |                 |         |
| Gln/Gln  | 26.46 (4.9) | 1.0 | 0.88 | 0.09 | 86.83 (12.8) | 1.0 | 213.90 (46.1) | 1.0 | 137.35 (120.4) | 1.0 | 141.94 (39.6) | 1.0 | 105.40 (31.1) | 1.0 |
| Gln/Glu  | 26.21 (4.8) | 0.35 | 0.87 | 0.09 | 86.29 (12.0) | 0.49 | 217.00 (50.3) | 0.22 | 138.53 (139.2) | 0.86 | 143.99 (39.4) | 0.34 | 104.74 (31.9) | 0.69 |
| Glu/Glu  | 25.56 (4.8) | 0.06 | 0.88 | 0.09 | 85.27 (11.8) | 0.25 | 206.0 (44.2) | 0.07 | 135.78 (117.7) | 0.89 | 135.96 (39.7) | 0.12 | 104.02 (37.8) | 0.65 |
| Gln/Gln vs Gln/Glu vs Glu/Glu | 0.15 | 0.18 | 0.32 | 0.61 | 0.91 | 0.73 | 0.48 |
| Gln/Gln vs Gln/Glu vs Glu/Glu | 0.08 | 0.17 | 0.3 | 0.03 | 0.85 | 0.06 | 0.71 |

| Thr164Ile Polymorphism |       |         |           |                   |             |                 |         |
| Thr/Thr  | 26.25 (4.8) | 1.0 | 0.87 | 0.09 | 86.38 (12.3) | 1.0 | 214.33 (43.9) | 1.0 | 138.19 (129.0) | 1.0 | 142.12 (38.8) | 1.0 | 104.90 (31.7) | 1.0 |
| Thr/Ile  | 27.69 (4.8) | 0.11 | 0.88 | 0.09 | 90.33 (15.7) | 0.15 | 214.47 (47.9) | 0.99 | 113.70 (60.5) | 0.3 | 148.56 (36.8) | 0.37 | 110.50 (44.9) | 0.34 |

with adjustment for age, gender, ethnicity, total cholesterol, and diabetes ($P = 0.06$), but this marginal association disappeared after inclusion of BMI in the model ($P = 0.15$). This may suggest that the effect of this allele in blood pressure regulation is operant only under interaction with obesity. The same seems not to occur with the Arg16Gly polymorphism: The effect of the Gly/Gly genotype persists even after adjustment for obesity-related variables. We could not dislose any interaction between the different genetic variants studied and blood pressure (data not shown).

In addition, we have created multiple linear regression models by using BMI as the dependent variable. The only genotypic variable significantly associated with BMI in our regression model was the presence of the Arg16 allele. Presence of this allele in a dominant model was still significantly associated with increased BMI even after adjustment for age, gender, ethnicity, triglycerides, HDL cholesterol, LDL -cholesterol, diabetes status, and hypertension status.

![Figure 1](https://hyper.ahajournals.org/)

**Figure 1.** Mean values for blood pressure and obesity phenotypes against Arg16Gly, Gln27Glu, and Thr164Ile ADRB2 polymorphisms. Data are presented as mean values with SEM. A, Systolic blood pressure; B, BMI; C, WHR.
Interestingly, although no interaction was found between the Arg16Gly polymorphism and any of the other studied variants, a significant interaction was present between the Gln27Glu and the Thr164Ile polymorphism in determining BMI \( (P = 0.02) \). This interaction is graphically presented in Figure 2A. In the absence of the Ile164 allele, the Gln27/Gln27 genotype has no effect on BMI. On the other hand, when in the presence of the Ile164 allele, there is a statistically significant association between the Gln/Gln genotype and increased BMI.

Interaction Effects Between ADRB2 Polymorphism, Blood Pressure, and Obesity

To better understand the complex relations between \( \beta \)-adrenoceptor gene polymorphism, blood pressure, and obesity phenotypes, we have also constructed ANOVA factorial modeling by using systolic blood pressure as the dependent variable and the best-fitting genetic model for each of the studied polymorphisms, BMI, and WHR. The Arg16Gly polymorphism significantly interacted with BMI \( (P = 0.036) \) and WHR \( (P = 0.003) \) to determine systolic blood pressure. As expected, a significant interaction between the Thr164Ile polymorphism and WHR was also disclosed \( (P = 0.018) \). No interaction between blood pressure and obesity phenotypes was present for the Gln27Glu polymorphism. These interactions are graphically presented in Figure 3.

Because of linkage disequilibrium existence between the Arg16Gly and the Thr164Ile polymorphisms in our population, we have also studied whether one of these particular alleles could be associated with blood pressure because of linkage disequilibrium. The relation between different haplotypes of the Arg16Gly and the Thr164Ile polymorphisms and systolic blood pressure in our population is shown in Figure 2B. As can be seen, the presence of the Ile164 allele increases blood pressure both in the presence of the Arg/Arg genotype or the Gly/Gly genotype, suggesting an independent role in modulating blood pressure in this population. The same can be noticed for the Gly16 allele.

Finally, we have conducted univariate analysis by using dichotomous variables for these phenotypes: hypertension and obesity. Hypertension was significantly associated with the Gly/Gly genotype \( (P = 0.015) \) and marginally with the Ile164 allele \( (P = 0.06) \) but not with the Gln/Gln genotype. Obesity, on the other hand, was associated with the Arg16 allele \( (P = 0.02) \) and with the Gln/Gln genotype \( (P = 0.01) \) but not with the Ile164 allele. To better characterize such associations, we have constructed multiple logistic regression models, studying the relation between the studied polymorphisms and other risk factors for the development of hypertension and obesity. The only studied genetic variable that remained statistically associated with hypertension after multiple adjustment was the presence of the Gly16/Gly16 genotype. This genotype was associated with a 1.48-fold increase in the risk of presenting hypertension, even after adjustment for ethnicity, age, gender, diabetes, total cholesterol, LDL cholesterol, HDL cholesterol, and BMI.
adrenergic receptor, leading to depressed receptor degrada-
cytes. The receptor with the Glu27 allele does not appear to
activity to catecholamine-induced lipolysis in human adipo-
human bronchial smooth muscle and 5-fold
regulation of the receptor in transfected fibroblasts and
has been ascribed a greater degree of agonist-induced down-
relevant. The Gly16 allele of the Arg16Gly polymorphism
of the studied polymorphisms is known to be functionally
as candidates to predispose to hypertension and obesity. Each
This study investigated 3 genetic variants of the ADRB2 gene
1.49-increased risk of obesity in the same model (P
triglycerides, total cholesterol, and hypertension status
in our multiple logistic regression model for
can be expressed in the fully mature form on Western blots, and
in vitro observations have shown that the Gln27Glu substi-
tution reduces agonist-mediated downregulation of the β2
adrenergic receptor, leading to depressed receptor degrada-
tion during agonist exposure and presumably resulting in
sustained vasodilation. Finally, the 164Ile variant of the
ADRB2 is associated with a decreased native adipocyte
receptor function.

Although several previous reports have described associa-
tions between these polymorphisms and hypertension or
obesity, few studies have taken into account both vari-
bles in the same design. To our knowledge, this is the most
extensive work done analyzing these polymorphisms and
blood pressure and obesity phenotypes both as continuous
and dichotomous variables in the same population. In addi-
tion, the design of our study was aimed at reducing selection
bias through the enrollment of individuals who are truly
representative of the general population, following a
MONICA type design.

We show a significant association between the Gly16/
Gly16 genotype and blood pressure phenotypes either mea-
sured as quantitative or qualitative variables. Presence of this
genotype was associated with systolic and diastolic blood
pressures in multiple regression models adjusted for con-
founding variables and increased the chance of hypertension
in 48% in a multiple logistic regression model also adjusting
for confounding variables. Interestingly, the other allele of
this locus, the Arg16 allele, was also significantly associated
with obesity phenotypes, both quantitative (BMI) and qualita-
tive (obesity) in multiple regression models. Although not
associated with blood pressure, the Gln27 allele was signifi-
cantly associated with obesity (OR, 1.31 for the Gln/Gln
genotype). The same allele was also associated with BMI.
Interestingly, this association is operant through an interac-
tion with the Ile164 allele.

Association reports of β2 adrenoceptor variants have been
discordant regarding both hypertension and obesity. The
relations between the polymorphisms of the ADRB2 gene
and hypertension phenotypes have been assessed in Europe-
an, American, Japanese, and African Caribbean popula-
tions, and the apparent lack of consistency in the
results among these studies may be, at least partially,
attributable to ethnic differences. Another possible explanation for
the discordant data regarding association of these alleles with
hypertension is a population-specific role for a particular
allele. These could be explained, for example, through
different gene-gene or gene-environment interactions. A third
possibility is that no causal relation exists between the
Arg16Gly polymorphism and hypertension, and the different
associations reported are due to linkage disequilibrium with
another unstudied, nearby genetic variant.

Our study design can contemplate some of these criticisms.
Indeed, our analysis has shown the existence of significant
population structure in our ethnically mixed individuals.
Although the population structure in our ADRB2 gene
study is a population-specific role for a particular
allele. These could be explained, for example, through
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ure was higher in individuals with the Gly/Gly genotype in all ethnic subgroups of our population (white, 124.3×
126.2 mm Hg; African descent, 128.4×131.3 mm Hg; and
other ethnicities, 120.2×129.7 mm Hg). This observation
gives support to the idea that the Arg16Gly variant, as shown
in in vitro and in vivo intermediate phenotypes, can directly
influence blood pressure regulation. Still, particular gene-
environmental interactions may be operant and may explain
some of the discordant findings in the literature.

The association between ADRB2 polymorphisms and obe-
sity has also been contradictory. The Glu/Glu genotype was
associated with obesity in Swedish women\textsuperscript{11} and obesity and fat distribution in Japanese\textsuperscript{16,17}; however, the Gln/Gln geno-
type was associated with obesity in French.\textsuperscript{21} Again, the same
possible scenarios already pointed out for blood pressure may
explain data on obesity. It is reassuring that the Arg16 allele,
not previously shown to be linked to obesity, has been shown
to have a 5-fold decreased sensitivity to catecholamine-
induced lipolysis in human adipocytes and, in our data, is
associated with a continuous variable and a dichotomous
obesity-related variable. Although linkage disequilibrium
with the Gln27Glu could be modulating this association,
little disequilibrium data between these two alleles is only
of marginal statistical significance ($P=0.06$). In addition,
ethnicity does not appear to influence these results, since it
was adjusted in all multivariate models and an increased BMI
was associated with the Arg16 allele despite ethnic subgroup
(26.0×25.6 for whites; 26.8×25.9 for African descent). Taken
together, our data suggest an association between the
Arg16 allele and increased BMI and a higher risk of obesity.

It should also be noted that the effect of this locus was
discordant with the known relation between obesity and
hypertension. Although the presence of the Gly16 allele is
associated with increased blood pressure levels, the presence
of the other variant, the Arg16 allele, is associated with
increased obesity. It is tempting to suggest that these discord-
unt findings are due to the power of previous, unadjusted studies to identify a relation
with blood pressure. This is also an example of how under-
standing of the pleiotropic actions of a particular gene/allele
may shed light on aspects of a complex pathophysiological
process.

Concordant with previous findings but discordant with
others, we report an increased risk of obesity in individuals
with the Gln27/Gln27 genotype. Interestingly, the association
between Gln27/Gln27 genotype was significantly influenced
by the Thr164Ile variant. This interaction has not been
previously described and empowers the hypothesis that
haplotype-based analyses may help to explain literature-
discordant results for these polymorphisms.

Potential sources of bias when studying different single
nucleotide polymorphisms (SNPs) of the same gene, espe-
cially when they can all potentially be functionally relevant,
are the presence of linkage disequilibrium between SNPs and
the different functional effects of the haplotypes created by
these SNPs. Unfortunately, we could not define the chromo-
somal phase of our alleles in every participant to conduct
individual haplotype analysis. Nevertheless, we have taken
these potential limitations into account by using information
of linkage disequilibrium derived from our own population
and by testing for interactions between the studied alleles and
the phenotypes of interest. This approach was only made
possible because of the considerable number of individuals
investigated in our study. As a result, we were able to disclose
a significant interaction between the Gln27Glu and the
Thr164Ile polymorphisms and BMI (Figure 2B) and analyze
the association of both the Arg16Gly and the Thr164Ile allele
and SBP in the context of significant linkage disequilibrium
between these two genetic variants (Figure 2A). Our data
generate an interesting hypothesis to be tested not only in
human populations but also in the context of computer
modeling and in vitro assays.

Finally, our data made it possible to investigate another
unstudied aspect of this complex system: whether these
genetic variants were not only influencing the interindividual
variability of blood pressure and obesity but also if they could
be modulating the relation between obesity and blood pres-
sure in the general population. Interestingly, significant in-
teractions could be disclosed between the Arg16Gly poly-
morphism and BMI and WHR and between the Thr164Ile
polymorphism and WHR. These data can have important
implications in the creation of algorithms to predict hyper-
tension, based on known risk factors such as obesity.

The current paradigm for the understanding of complex
biological systems implies the existence of multilevel inter-
actions between genetic and environmental variables. Genetic
association studies, although exploratory regarding causal
relations, may have an important role in the generation of
hypothosis to be tested in more controlled studies. However,
they must take into account not only statistical issues such as
class and selection bias but also the possible pleiotropic
effect of the studied genes. Our findings add information to
the controversy regarding ADRB2 genetic variants, hyperten-
sion, and obesity. The design of our study has made possible
the study of blood pressure and obesity phenotypes both as
quantitative and qualitative variables. As a result, the associ-
ation of different cardiovascular phenotypes with the studied
polymorphisms has disclosed previously undescribed associ-
ations and interactions in this multivariable system. Taken
together, our data provide further evidence for a role, possibly
direct, for the ADRB2 alleles in the homeostatic control of
both blood pressure and fat metabolism.

Acknowledgments

This work was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP 01-00009-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 520696 and 522733), and Prefeitura de Vitória/Facitec.

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