β2-Adrenergic Receptor Gene Variation and Hypertension in Subjects With Type 2 Diabetes

Kristina Bengtsson, Marju Orho-Melander, Olle Melander, Ulf Lindblad, Jonas Ranstam, Lennart Råstam, Leif Groop

**Abstract**—The aim of this study was to investigate whether polymorphisms in the β2-adrenergic receptor gene (5′LC-Arg19Cys, Arg16Gly, Gln27Glu) are associated with hypertension in patients with or without type 2 diabetes and with the blood pressure levels in normotensive sib pairs. The association study included 291 hypertensive patients without type 2 diabetes, 124 hypertensive patients with type 2 diabetes, and 265 healthy control subjects from Sweden. In addition, normotensive sib pairs that were discordant for the Arg16Gly (72 pairs) and Gln27Glu (40 pairs) polymorphisms were identified in type 2 diabetes families from Finland. Genotyping was performed using polymerase chain reaction–restriction fragment-length polymorphism analysis. Homozygous carriers of the Arg16 allele had a significantly increased odds ratio (OR) for hypertension in patients with type 2 diabetes (OR 2.14; 95% confidence interval [CI], 1.05 to 4.33), particularly among lean (body mass index<27 kg/m²) patients (OR 3.47; 95% CI, 1.06 to 11.33). The Gln27 allele showed a weaker association to hypertension (OR 1.55; 95% CI, 1.00 to 2.41) and was found to be in linkage disequilibrium with the Cys19 allele of the 5′LC-Arg19Cys polymorphism. In the paired-sibling analysis, siblings with at least 1 copy of the Arg16 allele had higher systolic blood pressure (P=0.049), and non-diabetic siblings had a higher body mass index (P=0.026) than siblings homozygous for the Gly16 allele. These results indicate that the Arg16 allele of the β2-adrenergic receptor gene confers a greater increased risk for hypertension in subjects with type 2 diabetes and is associated with higher blood pressure levels and higher body mass index in sib pairs who are discordant for the polymorphism. (Hypertension. 2001;37:1303-1308.)

**Key Words:** genetics ■ hypertension, genetic ■ blood pressure ■ diabetes ■ receptors, adrenergic, beta ■ sibling

The β2-adrenergic receptors (B2AR) are G-protein coupled receptors, which are widely distributed in the body, and they mediate vasodilatation, bronchial dilatation, and lipolysis. Three common polymorphisms in the B2AR gene on chromosome 5q31 to 32 have been reported: Arg16Gly, Gln27Glu, and 5′LC-Arg19Cys. Several studies have investigated the role of these polymorphisms in asthma, obesity, and hypertension. Whereas the findings in asthma have been consistent, the relationship between these polymorphisms and hypertension and obesity is unclear.

The Gly16 allele of the Arg16Gly polymorphism has been associated with increased expression of the receptor 18 and could therefore influence the susceptibility to hypertension, obesity, and type 2 diabetes. Although the Gln27Glu polymorphism has been associated with obesity, 6,7 type 2 diabetes, and hypertriglyceridemia, these findings have not been replicated in other studies. The Gln27Glu polymorphism is in linkage disequilibrium with the 5′LC-Arg19Cys polymorphism in the 5′-leader cistron (5′LC). The 5′LC codes for a polypeptide that seems to affect the expression of the receptor 18 and could therefore influence the susceptibility to hypertension, obesity, and type 2 diabetes.

The aim of this study was to investigate the role of the Arg16Gly, Gln27Glu, and 5′LC-Arg19Cys polymorphisms in the B2AR gene in hypertension and type 2 diabetes in a case-control association study and in genotype-discordant sibling pairs.

**Methods**

For the case-control association study, we enrolled 291 unrelated hypertensive patients without type 2 diabetes and with age at onset of ≤60 years and 265 unrelated healthy control subjects without medication, personal history of hypertension, first-degree family history of hypertension, or type 2 diabetes from southern Sweden.
The subjects identified in the Skaraborg Hypertension and Diabetes Project consisted of 2 cohorts. One cohort included 1149 patients with hypertension and/or diabetes and the other cohort included an age-stratified, random sample (1400 invited, 1109 participating) from the population 40 years of age and older. Hypertensive patients and normotensive spouses from 250 families were recruited from healthcare centers in the Scania region. In addition, 124 unrelated hypertensive patients with type 2 diabetes were ascertained from the Skaraborg Hypertension and Diabetes project.24 Three subjects were excluded because of genotyping failure and misclassification. Hypertension was defined as 3 consecutive blood pressure measurements ≥160 mm Hg (systolic blood pressure) and/or ≥90 mm Hg (diastolic blood pressure) or ongoing antihypertensive treatment. The control subjects had systolic and diastolic blood pressures ≤150 mm Hg and ≤80 mm Hg, respectively. The diagnosis of type 2 diabetes was based on criteria from the World Health Organization.22 The participants were categorized as follows:1 all hypertensives are patients with hypertension, regardless of whether they had type 2 diabetes or not (n=415);2 hypertensives without type 2 diabetes (n=291);3 hypertensives with type 2 diabetes (n=124); and4 healthy controls (n=265) (Table 1).

For the genotype-discordant sib pair study, 494 normotensive siblings were ascertained from 118 families with type 2 diabetes in Botnia, Finland,23 and they were genotyped for the B2AR gene Arg16Gly and Gln27Glu polymorphisms to identify genotype discordant pairs. In total, we identified 72 sib pairs in which 1 of the siblings was homozygous for the Gly16 allele and the other sibling carried either 1 or 2 Arg16 alleles. Similarly, we identified 40 sib pairs in which 1 of the siblings was homozygous for the Glu27 allele whereas the other sibling had 1 or 2 Gln27 alleles. The 72 sib pairs who were discordant for Arg16Gly polymorphism originated from 46 sibships that comprised 118 individuals, and the 40 sib pairs who were discordant for the Gln27Glu polymorphism originated from 26 sibships and included 64 individuals (Table 1). The ethics committees of the Gothenburg and Lund University approved the study. Informed consent was obtained from all the participants.

Blood pressure was measured with a sphygmomanometer after 5 minutes of rest in the supine position in the Swedish subjects and twice within 10 minutes after 15 minutes of rest in the sitting position in the Finnish subjects, the values given are the averages of the 2 measurements. Body weight and height were measured and body mass index (BMI) was calculated. BMI ≥27 kg/m² was considered as overweight. Total genomic DNA was extracted from the whole blood by standard methods.24 The Arg16Gly and Gln27Glu polymorphisms were genotyped by polymerase chain reaction and restriction fragment-length polymorphism methods. The Arg16Gly polymorphism was amplified in a genomic 203-bp fragment using primers β1 to 16-MM-F (5'-CGCCCATTTTGCTGCACCAGAAT) and β2 to 16/27-R (5'-CCATGGAAGTGTAAGTGAAGTT), of which the former contains a nucleotide mismatch (underlined) to create a BsrDI recognition site in case of the Gly16 allele. The Gln27Glu polymorphism was amplified in a genomic 169-bp fragment with primers β1 to 27-MM-F (5'-CCGGACCACGACGTCACCAG) and β2 to 16/27-R,
TABLE 2. OR for All HT and for HT With and Without Type 2 Diabetes for the Arg16Gly and Gln27Glu Polymorphisms in B2AR Gene

<table>
<thead>
<tr>
<th>B2AR gene</th>
<th>Arg16Gly</th>
<th>Gly/Gly</th>
<th>Arg/Gly</th>
<th>CI</th>
<th>OR</th>
<th>Arg/Arg</th>
<th>CI</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All HT, n=415</td>
<td>1.0</td>
<td>1.26</td>
<td>0.90–1.76</td>
<td>1.60</td>
<td>0.96–2.65</td>
<td>0.069</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT without type 2 diabetes, n=291</td>
<td>1.0</td>
<td>1.20</td>
<td>0.84–1.71</td>
<td>1.38</td>
<td>0.80–2.39</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT with type 2 diabetes, n=124</td>
<td>1.0</td>
<td>1.38</td>
<td>0.84–2.29</td>
<td>2.14</td>
<td>1.05–4.33</td>
<td>0.035</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The genotype frequencies of the Arg16Gly polymorphism were for controls Arg/Arg 10.9% (n=29), Arg/Gly 44.2% (n=117), and Gly/Gly 44.9% (n=119); for hypertensives, 13.4% (n=39), 46.7% (n=136), and 39.9% (n=116); and for hypertensives with type 2 diabetes, 18.5% (n=23), 46.0% (n=57), and 35.5% (n=44), respectively. The genotype frequencies of the Gln27Glu polymorphism for controls were Gln/Gln 26.8% (n=71), Gln/Glu 48.3% (n=128), and Glu/Glu 24.9% (n=66); for hypertensives, 30.2% (n=88), 49.8% (n=145), and 19.9% (n=58); and for hypertensives with type 2 diabetes, 37.1% (n=46), 46.0% (n=57), and 16.9% (n=21), respectively. HT indicates hypertension.

Results

In the Swedish population, the genotype frequencies for the Arg16Gly polymorphism in the B2AR gene were 13.4% for Arg16Arg, 45.6% for Arg16Gly, and 41.0% for the Gly16Gly genotype, and the genotype frequencies for the Gln27Glu polymorphism were 30.1% for Gln27Gln, 48.5% for Gln27Glu, and 21.3% for the Glu27Glu genotype. This is in accordance with the genotype frequencies reported in other European populations. The observed genotype frequencies in the different study groups were in Hardy-Weinberg equilibrium. The codon 16 and 27 polymorphisms were in linkage disequilibrium in the Swedish population: 100% of the Arg16-carrying chromosomes had Gln at position 27, whereas 28% of the Gly16-carrying chromosomes had Gln and 72% had Glu in position 27. The 5’LC-Arg19Cys polymorphism was in tight linkage disequilibrium with the Gln27Glu polymorphism; the Cys19 allele was associated with the Gln27 allele in all but 1 (n=1112) chromosome.

There was a significant increase in the OR (OR 2.14; 95% CI 1.05 to 4.33, P=0.035) for hypertension in the Arg16Arg genotype carriers with type 2 diabetes (Table 2). Similarly, the hypertension was analyzed by logistic regression and expressed as odds ratio (OR) with 95% confidence interval (CI) and with the Gly16Gly and Gln27Glu genotypes as reference. Data from the genotype-discordant sib pairs were analyzed using a modified permutation test for paired replicates. The 2-tailed probability value was estimated using a large (10^7) random sample from all possible permutations. If the observed sum of differences entered into the 5% region of rejection, the difference between pairs was considered statistically significant. For sib pairs discordant for the Arg16Gly polymorphism, the differences in phenotypic variables were computed as the values in the sibling with the Arg16Arg or Gly16Arg genotype minus the value in the sibling with the Gly16Gly genotype. For sib pairs discordant for the Gln27Glu polymorphism, the differences were computed as the values in the sibling with the Gln27Gln or Gln27Glu genotype minus the value in the sibling with the Gln27Glu genotype. Analyses, except for the permutation tests, were performed using BMDP Statistical Software version 1.12 and an SPSS statistical program (SPSS/PC+ for the IBM-PC/XT/AT, 1986). All statistical tests were 2-sided, and P<0.05 was considered statistically significant.

Statistical Methods

Results are presented as mean±SD. Differences between group means were tested by ANCOVA. Association between genotypes and hypertension was analyzed by logistic regression and expressed as odds ratio (OR) with 95% confidence interval (CI) and with the Gly16Gly and Gln27Glu genotypes as reference. Data from the genotype-discordant sib pairs were analyzed using a modified permutation test for paired replicates. The 2-tailed probability value was estimated using a large (10^7) random sample from all possible permutations. If the observed sum of differences entered into the 5% region of rejection, the difference between pairs was considered statistically significant. For sib pairs discordant for the Arg16Gly polymorphism, the differences in phenotypic variables were computed as the values in the sibling with the Arg16Arg or Gly16Arg genotype minus the value in the sibling with the Gly16Gly genotype. For sib pairs discordant for the Gln27Glu polymorphism, the differences were computed as the values in the sibling with the Gln27Gln or Gln27Glu genotype minus the value in the sibling with the Gln27Glu genotype. Analyses, except for the permutation tests, were performed using BMDP Statistical Software version 1.12 and an SPSS statistical program (SPSS/PC+ for the IBM-PC/XT/AT, 1986). All statistical tests were 2-sided, and P<0.05 was considered statistically significant.

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There was a significant increase in the OR (OR 2.14; 95% CI 1.05 to 4.33, P=0.035) for hypertension in the Arg16Arg genotype carriers with type 2 diabetes (Table 2). Similarly,
there was an increase in the OR for hypertension in the Gln27Gln genotype carriers (OR 1.55; 95% CI, 1.00 to 2.41, \( P = 0.050 \)). Neither the Arg16 nor the Gln27 allele conferred an increased risk for hypertension in subjects without type 2 diabetes. The carriers of the different genotypes showed no statistically significant difference between the serum creatinine values and the occurrence of proteinuria. The Arg16Arg genotype was associated with hypertension, especially in the nonobese (BMI < 27 kg/m²) subgroup of patients with hypertension and type 2 diabetes (OR 3.47; 95% CI, 1.06 to 11.33, \( P = 0.040 \)).

Among the sib pairs discordant for the Arg16Gly polymorphism, those siblings carrying 1 or 2 Arg16 alleles had higher systolic blood pressure compared with siblings homozygous for Gly16 allele (\( P = 0.049 \), Table 3). Among the nondiabetic pairs (n = 54), siblings with 1 or 2 Arg16 alleles had a higher BMI than siblings homozygous for Gly16 allele (\( P = 0.026 \)). This was due to the male siblings (11 pairs), 25.9 kg/m² versus 24.4 kg/m² (\( P = 0.018 \)). Figure 1 shows the difference in blood pressure and BMI of the individuals in the sib pair combinations used in the permutation test. There was a significant difference in systolic blood pressure between the siblings and in BMI in nondiabetic siblings homozygous for the Gly16 allele and carriers of 1 or more Arg16 alleles.

There were no significant differences in blood pressure levels or BMI between siblings discordant for the Gln27Glu polymorphism.

### Discussion

The key finding of the present study was an increased risk for hypertension in type 2 diabetic carriers of the Arg16 and Gln27 alleles of the B2AR gene. This increased risk was not due to differences in nephropathy between the carriers. We also found that normotensive siblings from families with type 2 diabetes carrying 1 or 2 Arg16 alleles had a higher systolic blood pressure and a higher BMI compared with siblings homozygous for Gly16 allele. Because the B2AR regulates blood pressure as well as glucose and fat metabolism, we tested the B2AR polymorphisms for association with hypertension as well as diabetes and obesity. Some caution in interpretation of the data is warranted because we performed more than 1 test. The observed association between hypertension and the Arg16 allele is in accordance with a previous report in Scandinavians.9 In addition, a recent study in German twins showed that the Arg16 allele was associated with higher systolic blood pressure levels.10 On the other hand, Gratze et al found that young normotensive white male subjects homozygous for the Gly16 allele had higher blood pressure and decreased peripheral vasodilatation in response to salbutamol infusion.13 In addition, an association between the Gly16 allele and hypertension has been reported in African Caribbeans.8 Salt-sensitive individuals show a greater downregulation of the B2AR in response to high-salt intake.27 Black Africans are known to be more salt sensitive than

### TABLE 3. Differences in Clinical Characteristics of Siblings Discordant for the Arg16Gly and Gln27Glu Polymorphisms Analyzed by the Permutation Test

| Variables | Arg16Gly | | | Gln27Glu | | |
|-----------|---------|-----------|-----------|---------|-----------|
|          | OSD     | \( P \)  | OSD       | \( P \)  | OSD       | \( P \)  |
| All, n=72 | SBP, mm Hg | +319.00 | 0.049 | +190.00 | 0.11 | +120.00 | 0.26 |
| All, n=40 | DBP, mm Hg | +126.00 | 0.17 | +120.00 | 0.15 | +89.00 | 0.27 |
| All, n=72 | BMI, kg/m² | +55.80 | 0.17 | +73.02 | 0.026 | +1.77 | 0.96 |
| All, n=40 |              |         |         |         |         | +18.46 | 0.52 |

Differences were calculated as the value in sibling with Arg16Arg or Arg16Gly genotypes minus the value in sibling with Gly16Gly genotype and as the value in the sibling with Gln27Gln or Gln27Glu genotypes minus the value in sibling with the Glu27Glu genotype. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; and OSD, observed sum of differences.
whites,28 which has been proposed to explain the association between hypertension and the Gly16 allele in African Caribbeans.8

There are several other polymorphisms in the vicinity of the Arg16Gly polymorphism, such as the 5′LC-Arg19Cys, Gln27Glu, and Thr164Ile. Unfortunately, previous studies have not taken linkage disequilibrium between these polymorphisms into account. In vitro the 5′LC-Cys19 allele has been associated with an increase in B2AR gene expression, the Gln27 allele has been associated with a resistance to downregulation, the Ile164 allele has been associated with an impaired G-protein coupling,12 and the Arg16 allele has been associated with decreased receptor density on the surface of cultured human fibroblasts.29 Liggett and coworkers reported that the Gly16 allele is associated with increased agonist-induced downregulation of the B2AR in Chinese hamster fibroblasts and that the effect of the Gly16 allele dominates when combined with the Gln27 allele.12 Interestingly, the Gln27 allele has otherwise been shown to be resistant to agonist-induced downregulation.30

In this study we found that the haplotype LC-Cys19-Arg16-Gln27 was associated with increased risk for hypertension in the case-control association study and with elevated systolic blood pressure and higher BMI in genotype-discordant sib pairs. The allele frequencies of the polymorphisms in the B2AR gene differ between different populations.31 The findings that the Arg16 allele is associated with hypertension and higher blood pressure levels in Scandinavians, whereas the Gly16 allele is associated with hypertension in other populations,8,13 may suggest that it is not the 

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