A Functional Variant in the \( \alpha_{2B} \) Adrenergic Receptor Gene, a Positional Candidate on Chromosome 2, Associates With Hypertension

Fredrik von Wowern, Kristina Bengtsson, Ulf Lindblad, Lennart Råstam, Olle Melander

Abstract—In a genome-wide scan in Scandinavians, we found suggestive linkage between early-onset primary hypertension and a region on chromosome 2. The \( \alpha_{2B} \)-adrenergic receptor gene, a candidate gene within this region, harbors a functional insertion/deletion (I/D) polymorphism of three glutamate residues. The aim of this study was to investigate if the DD genotype is associated with hypertension in Swedes. We performed an association study between the I/D polymorphism of the \( \alpha_{2B} \)-adrenergic receptor and hypertension in the Skaraborg population. The material consists of all known patients with primary hypertension in Skara (n=772 nondiabetic subjects; n=171 normoalbuminuric type 2 diabetic subjects) and 817 population control subjects. We first compared genotype frequencies between patients with early-onset hypertension (aged 50 years or younger at onset) and subjects with normotension (blood pressure <120/80 mm Hg). Thereafter, the polymorphism was tested for association with hypertension at the population level. When comparing patients with early-onset hypertension and normotensive subjects, the DD versus II genotype was associated with early-onset hypertension when diabetic subjects were excluded from the analysis (OR=2.0; 95% CI=1.2 to 3.5) or when they were not excluded (OR=1.8; 95% CI=1.0 to 3.1). At the population level, the DD versus II genotype was weakly associated with nondiabetic hypertension (OR=1.4; 95% CI=1.0 to 1.8). Our data suggest that carriers of the DD versus II genotype of the \( \alpha_{2B} \)-adrenergic receptor are at increased risk for hypertension. The genotypic effect is most evident when comparing groups corresponding to the upper and lower tails of the blood pressure distribution in the population; however, in nondiabetic hypertensive subjects it is weakly detectable even at the population level.

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One common variant of the human $\alpha_{2B}$-adrenergic receptor gene encodes a protein with a deletion (D) of 3 consecutive glutamate residues at amino acid positions 301 to 303. Absence of the D is referred to as the insertion (I) variant. 8,9

The third intracellular loop of the $\alpha_{2B}$-adrenergic receptor, which harbors the I/D polymorphism, has been shown to be instrumental for the function of the receptor. 10 The functional importance of the I/D polymorphism has been shown by in vitro studies revealing an impaired agonist-induced desensitization of the D-variant of the receptor. 11

Based on the findings of our own 4 and several other recent genome-wide scans 3–5 showing linkage between a locus on chromosome 2 containing the $\alpha_{2B}$-adrenergic receptor gene and blood pressure phenotypes, together with previous evidence from human, 12,13 animal, 7 and in vitro studies suggesting a central role of the $\alpha_{2B}$-adrenergic receptor gene and its I/D polymorphism in control of sympathetic nervous system activity, we explored the possibility that the I/D polymorphism of the $\alpha_{2B}$-adrenergic receptor gene is involved in the cause of primary hypertension. To do this, we studied the Skaraborg material. 14 The community of Skara in the south of Sweden formed the basis for one of the major recruiting centers of our genome-wide scan. Thus, the material in the present study closely resembles the material used in our previous genome-wide scan 2 in terms of population genetics.

The aim of the present study was 2-fold: to test the hypothesis that the frequency of the DD versus II genotype of the $\alpha_{2B}$-adrenergic receptor gene is increased in patients with EOHT aged 50 years or younger at diagnosis, i.e., the same phenotype as in our previous genome-wide scan 2 when compared with subjects with normotension (NT) as defined by the Joint National Committee 7 (JNC7) (blood pressure $<120$ mm Hg), 15 and to test the hypothesis that the DD genotype of the $\alpha_{2B}$-adrenergic receptor gene is associated with primary hypertension at the population level by comparing genotype frequencies between all patients with primary hypertension in the population, except those with signs of diabetic nephropathy, with those in a set of population controls.

### Methods

#### Subjects

All participants gave written informed consent and the study was approved by the local ethical committee. The procedures were in accordance with institutional guidelines.

#### The Skaraborg Material

The Skaraborg material consists of two collections of individuals from the primary health care center in Skara in southern Sweden. The material has been described in detail previously. 14 From 1992 to 1993, all patients in Skara with primary hypertension and type 2 diabetes were surveyed, including blood sampling for DNA. From 1993 to 1994, a corresponding survey was conducted on an age-stratified sample of the Skara population aged 40 years and older (participation rate 80%). For the present study, DNA samples were analyzed from 772 patients, of whom 51 had microalbuminuria (n = 772). Thus, the material used in the present study consisted of 943 patients with primary hypertension (of whom 772 patients did not have diabetes and 171 had normoalbuminuric type 2 diabetes) and 817 population controls (Table 1).

#### Patients with EOHT versus NT

In an attempt to maximize the difference in genetic hypertension susceptibility variants between cases and controls, and thereby to increase the power, we first compared genotype frequencies between a subset of the patients described having onset of primary hypertension at age 50 years or younger (EOHT) (n = 311, of whom n = 51 had normoalbuminuric type 2 diabetes), i.e., the same phenotype as in our previous genome scan, 2 and a subset of the population controls described having NT as defined by JNC7 (systolic blood pressure $<120$ mm Hg and diastolic blood pressure $<80$ mm Hg) (n = 261). The clinical characteristics of the EOHT and NT groups, which correspond to the upper and lower tails of the population distribution of blood pressure, are given in Table 2.

#### Phenotyping

Blood pressure was measured in the supine position after 10 minutes rest using a mercury sphygmomanometer by specially trained nurses. The diagnosis of essential hypertension was based on 3 consecutive measurements of supine right brachial artery blood pressure above 160 mm Hg systolic blood pressure and/or 90 mm Hg diastolic blood pressure on different occasions, according to the National Guidelines at that time, 16 or the presence of antihypertensive medication. The diagnosis of type 2 diabetes mellitus was based on WHO criteria. 17

### Table 1. Clinical Characteristics of Hypertensive Patients and Population Controls

<table>
<thead>
<tr>
<th></th>
<th>Population Controls (n = 817)</th>
<th>Hypertensive Patients Without Type 2 Diabetes (n = 772)</th>
<th>Hypertensive Patients With or Without Type 2 Diabetes (n = 943)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD) $P$ Value</td>
<td>Mean (SD) $P$ Value</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>60.4 (12.8)</td>
<td>65.7 (12.2) $&lt; 0.00001$</td>
<td>65.5 (11.3) $&lt; 0.00001$</td>
</tr>
<tr>
<td><strong>Age at onset (y)</strong></td>
<td>NA</td>
<td>53.9 (11.7) $&lt; 0.0001^*$</td>
<td>54.1 (11.3) $&lt; 0.00001^*$</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.1 (4.0)</td>
<td>27.7 (4.6) $&lt; 0.00001$</td>
<td>28 (4.6) $&lt; 0.00001$</td>
</tr>
<tr>
<td><strong>Sex (% male)</strong></td>
<td>49</td>
<td>NA</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>SBP (mm Hg)</strong></td>
<td>132 (19.2)</td>
<td>155 (19.1) $&lt; 0.00001$</td>
<td>157 (19.7) $&lt; 0.00001$</td>
</tr>
<tr>
<td><strong>DBP (mm Hg)</strong></td>
<td>75 (9.6)</td>
<td>84 (9.3) $&lt; 0.00001$</td>
<td>84.5 (9.3) $&lt; 0.00001$</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; AHM, antihypertensive medication; SBP, systolic blood pressure; DBP, diastolic blood pressure; NA, not analyzed.

$P$ values refer to the difference between patients and population controls.

*Difference between age at onset for patients and age of population controls.
None had type 1 diabetes. Body mass index (BMI) was calculated as the ratio of the weight in kilograms to the square of the height in meters (kg/m²). Urine was collected during 24 hours and tested for microalbuminuria using the MICRAL test.¹⁸

Genotyping
Standard methods were used to extract genomic DNA from whole blood. A forward primer-5'-AGGTTTGTGGGGCATCT and reverse primer-5'-CAAGCTGAGGCCGGAGACACT were used in the following PCR program: 94°C for 5 minutes, 33 thermocycles with 94°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds, and final extension at 72°C for 10 minutes. The 9 base-pair difference between the D and I alleles was detected by UV light after DNA electrophoresis on a 3% agarose gel with ethidium bromide. The authenticity of the genotypes was checked by rerunning a random set of 200 samples and comparing them against the original genotypes. The genotyping success rate was 100%. All genotypes were read by two independent investigators who were unaware of the phenotypic status of the study subjects.

Statistics
Data were analyzed with NCSS statistical software (version 6.021, Statistical Solutions Limited, Cork, Ireland). Frequency differences were analyzed by χ² test and differences in continuous variables by t test and ANOVA or Mann-Whitney and Kruskal-Wallis test, depending on whether the variable was normally distributed. The genotypic effect of the α₂B-adrenergic receptor I/D polymorphism on the risk of hypertension was analyzed by multiple logistic regression and expressed as odds ratio (OR) for hypertension with 95% confidence intervals (95% CI). The II genotype was defined as reference (OR=1.0) and compared with the ID and DD genotypes. Crude as well as age-, sex-, and BMI-adjusted ORs were calculated. All statistical tests were two-sided and P<0.05 was considered statistically significant.

Results
Impact of the DD Genotype on the Risk of EOHT Versus NT
The genotype frequencies of the α₂B-adrenergic receptor gene I/D polymorphism in the EOHT and NT groups are shown in Table 3. Carriers of the DD genotype had a significantly increased OR for EOHT whether normoalbuminuric type 2 diabetic subjects were included in the EOHT group in crude and age-, sex-, and BMI-adjusted analyses (Figure 1). The point estimate of the OR for EOHT in carriers of the DD genotype was intermediate between that of DD and II carriers but did not differ significantly (Figure 1).

Impact of the DD Genotype on the Risk of Primary Hypertension at the Population Level
To investigate if the genotypic effect of the DD genotype on the risk of primary hypertension that was detected in the analysis of EOHT and NT was present at the population level, we compared genotype frequencies between all patients with primary hypertension regardless of age at onset (with or without normoalbuminuric type 2 diabetic subjects) and population controls. The genotype frequencies of the α₂B-adrenergic receptor gene I/D polymorphism in the hypertensive and population control groups are shown in Table 4. In the crude analysis comparing nondiabetic patients with primary hypertension and population controls, carriers of the DD genotype had a significantly increased OR for primary hypertension (Figure 2). After adjustment for age, sex, and BMI, this association was of borderline significance (Figure 2). When the normoalbuminuric type 2 diabetic subjects were included in the primary hypertension group, no significant effect of the DD genotype could be detected, neither in the crude nor in the age-, sex-, and BMI-adjusted analysis (Figure 2). The point estimate of the OR for primary hypertension (with or without normoalbuminuric type 2 diabetic subjects) in carriers of the ID genotype was intermediate between that of DD and II carriers but did not differ significantly (Figure 2).

TABLE 3. Genotype Frequencies of the EOHT and NT Groups

<table>
<thead>
<tr>
<th></th>
<th>NT (n=261)</th>
<th>EOHT Without Type 2 Diabetes (n=260)</th>
<th>EOHT With or Without Type 2 Diabetes (n=311)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>II</td>
<td>98 (37.5)</td>
<td>79 (30.4)</td>
<td>103 (33.1)</td>
</tr>
<tr>
<td>ID</td>
<td>127 (48.7)</td>
<td>125 (48.1)</td>
<td>145 (46.6)</td>
</tr>
<tr>
<td>DD</td>
<td>36 (13.8)</td>
<td>56 (21.5)</td>
<td>63 (20.3)</td>
</tr>
</tbody>
</table>

EOHT indicates early-onset primary hypertension; NT, normotensive. None of the genotype frequencies in any of the groups deviated from the Hardy-Weinberg equilibrium.
Analysis of the I/D Polymorphism in Relation to Blood Pressure Levels

We also performed an exploratory analysis comparing blood pressure levels in carriers of the DD and II genotypes in the population controls, who were all free of antihypertensive medication. However, we found no significant difference between carriers of the DD genotype (n = 130) and the II genotype (n = 295) in systolic (134 ± 19.4 mm Hg versus 133 ± 20.1 mm Hg, P = 0.89) or diastolic (75.1 ± 8.9 mm Hg versus 76.3 ± 9.8 mm Hg, P = 0.25) blood pressure.

Discussion

This is the first study to our knowledge that is primarily designed to detect association between the I/D variant and hypertension. We found that the DD versus II genotype is clearly associated with EOHT, in particular with nondiabetic EOHT, when NT subjects are used as the control group. At the population level, the DD versus II genotype was weakly associated with nondiabetic primary hypertension but the association was not significant if normoalbuminuric type 2 diabetic subjects with primary hypertension were included in the analysis.

Two previously published studies have failed to support a role of the I/D variant in the development of essential hypertension. However, in one of these studies, hyper-tension was a secondary phenotype making the results exposed to greater uncertainty as compared with a study primarily designed for studying hypertension. In addition, only 206 patients with hypertension were included and, in contrast to our study, age at onset, diabetic state, and albuminuria were not taken into account in the analysis. The other study was designed to locate linkage in a sibling pair material. Because the frequency of the DD genotype among the 155 sibling pairs concordant for hypertension was very low (n = 3), it is highly unlikely that this study harbors sufficient power to detect linkage to hypertension.

Contrasting EOHT and NT Groups

The present study was performed in two stages. In the first stage, we aimed at maximizing the power to detect a genetic effect of the DD genotype by comparing two contrasting groups corresponding to the upper (EOHT) and lower (NT) tails of the population blood pressure distribution. There were 3 rationales for this design. First, the fact that previous studies have indicated that genetic factors are more important for the disease pathogenesis in EOHT than in late-onset primary hypertension suggests that EOHT patients have clustering of hypertension susceptibility gene variants. Second, inclusion of a control group with low normal blood pressure (the NT group) should further increase the power to detect a genetic effect, because these subjects are likely to have a reduced frequency of hypertension susceptibility variants compared with the general population. This should be true as long as the age of the NT group is not inappropriately low, because there could be a substantial risk of many of them having hypertension when they reach the same age as the patients. Importantly, this was not the case. In fact, the mean age of the NT group was significantly higher than the mean age at onset of the EOHT group (Table 2). Third, the phenotype of EOHT was mimicked from the one used in our previous genome-wide scan, the results of which guided us to the α2B-adrenergic receptor locus.

To further reduce the heterogeneity of the EOHT phenotype, we did not include hypertensive type 2 diabetic patients with microalbuminuria or macroalbuminuria, because the pathogenesis of hypertension in patients with diabetic kidney lesions is likely to be different from that in “pure” primary
hypertension. Furthermore, because hypertension is present in as much as 50% of patients with type 2 diabetes, it is possible that the pathogenesis of hypertension differs between nondiabetic subjects and type 2 diabetic subjects, despite absence of clinically detectable incipient diabetic nephropathy. We therefore performed analyses with and without normoalbuminuric type 2 diabetic subjects in the EOHT group. In accordance with the discussion, although still significant, the genotypic effect of the DD genotype on the risk of EOHT seemed to be diluted by inclusion of hypertensive normoalbuminuric type 2 diabetic subjects (Figure 1).

Primary Hypertension at the Population Level

In the second stage we examined whether the genetic effect is seen also at the population level by comparing genotype frequencies in all patients with primary hypertension in the Skara population (except hypertensive patients with microalbuminuria or macroalbuminuria) and those in the population controls. There was a weak effect of the DD genotype on the risk of nondiabetic primary hypertension (significant in crude analysis, borderline significant in adjusted analysis), whereas there was no effect of the DD genotype when normoalbuminuric type 2 diabetic subjects were included in the primary hypertension group (Figure 2). This suggests that in all patients with primary hypertension, as opposed to EOHT, the genotypic effect of the DD genotype is diluted mainly by environmental factors. In addition, an increased load of blood pressure elevating environmental and genetic factors, including a higher frequency of the DD genotype, are likely to be responsible for the higher level of blood pressure in the population controls as opposed to the NT group, thereby further obscuring the effect of the DD genotype. Finally, as in the analysis of the EOHT versus NT groups, the diabetic phenotype seems to add complexity to the phenotype of primary hypertension.

The rather weak association between the DD genotype and nondiabetic primary hypertension at the population level, in contrast to the stronger association to EOHT, is by no means surprising. The genetic component of primary hypertension is expected to be a composition of the sum of many genes, of which the individual effect is likely to be small to moderate.

We were unable to detect any effect of the I/D polymorphism on blood pressure in the untreated population controls. In this context, it is important to stress that blood pressure recordings taken on a single occasion are subjected to a substantial intra-individual variability, which together with the modest effect on blood pressure of the I/D polymorphism may explain the lack of association with blood pressure.

There are a number of potential mechanisms that could explain the role of the α2B-adrenergic receptor in the development of hypertension. The hypertensive effect mediated by the α2B-adrenergic receptor in mice has been shown to be especially important for salt-sensitive hypertension. This is highly relevant for humans because salt sensitivity is associated with a positive family history of primary hypertension, and it is also a characteristic of a large proportion of patients with primary hypertension. There is also evidence that the α2B-adrenergic receptor mediates peripheral vasoconstriction in mice. Human in vivo studies have shown that the DD genotype of the receptor is associated with reduced flow dilatation of the brachial artery, reduced coronary blood flow, and increased peripheral resistance on adrenaline infusion.

In conclusion, our data suggest that the DD versus II genotype of the α2B-adrenergic receptor gene contributes to the risk of primary hypertension. The genotypic effect is most evident when comparing the EOHT and NT groups, which correspond to the upper and lower tails of the population blood pressure distribution. For nondiabetic primary hypertension, the effect is weakly detectable even at the population level.

Perspectives

Small genetic effects present a major obstacle to defeat when trying to elucidate the cause of complex diseases. To overcome this, we suggest that studying contrasting phenotypes such as EOHT and NT may be fruitful in detecting small single gene effects. Once a number of these small single gene effects have been discovered, gene–gene and gene–environment interactions can be tested in population-based samples. Hopefully, this may result in a battery of interacting risk genotypes, which significantly affect the risk of primary hypertension also at the population level, and thus can be applied in clinical practice. Furthermore, additional studies are called for to verify the findings of this study and the usage of contrasting phenotypes as a mean for detecting small single gene effects.

Acknowledgments

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References


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