Essential Hypertension and $\beta_2$-Adrenergic Receptor Gene
Linkage and Association Analysis


Abstract—A region on human chromosome 5 (5q31.1-qter) contains several genes that encode important blood pressure regulators and thus is a good candidate for analysis of linkage and association with hypertension. We recruited 638 individuals from 212 Polish pedigrees with clustering of essential hypertension. These subjects were genotyped for 11 microsatellite markers that span this region to test for linkage to essential hypertension and systolic and diastolic blood pressures. The segment of this region of $\approx 7$ cM delineated by D5S1480 and D5S500 markers was linked to blood pressures in multipoint analysis. In 2-point analysis, D5S1480—the marker in close proximity to $\beta_2$-adrenergic receptor gene—reached the maximal linkage to essential hypertension and adjusted systolic and diastolic blood pressures, implicating this gene as a positional candidate for further association studies. Arg16Gly, Gln27Glu, and Thr164Ile—3 functional single nucleotide polymorphisms within the $\beta_2$-adrenergic receptor gene—were tested for association with essential hypertension. None of these polymorphisms showed a significant association with essential hypertension, separately or in the haplotype analysis. This study provided evidence of linkage of 5q31.1-5qter region to essential hypertension in the European population. Moreover, it implicated the chromosomal segment in close proximity to D5S1480 and D5S500. The detailed analysis of 3 single nucleotide polymorphisms does not support the role of the $\beta_2$-adrenergic receptor gene as a major causative gene for the detected linkage. (Hypertension. 2002;40:635-641.)

Key Words: chromosomes ■ adrenergic receptors ■ genes ■ linkage ■ blood pressure

Essential hypertension is a multifactorial complex trait with a strong hereditary component. Apart from genome-wide scans and candidate gene approach (principal methods used in pursuit of genetic loci that may determine predisposition to essential hypertension), a target chromosomal region approach combining the rationale of 2 major strategies has been postulated. Selection of a small chromosomal region implicated by genome-wide searches and containing several candidate genes pathophysiologically related to the investigated phenotypes allows for denser saturation with microsatellite markers and may be followed by subsequent positional analysis. The distal segment of the long arm of chromosome 5 (5q31.1-qter) is an outstanding target chromosomal region for studies on essential hypertension, having been linked to both systolic and postexercise diastolic blood pressure in genome-wide scans performed in white populations. Furthermore, this region contains a cluster of genes coding for proteins known as important blood pressure regulators ($\beta_2$-adrenergic receptor gene [ADRB2], $\alpha_1B$-adrenergic receptor, dopamine D4 receptor, annexin VI) and implicated as possible contributors to the pathogenesis of several cardiovascular disorders (platelet-derived growth factor receptor, glutathione peroxidase).

We performed a linkage analysis of this region using 3 related phenotypes: a diagnosis of essential hypertension (a qualitative trait) and 2 quantitative phenotypes—systolic and diastolic blood pressure. We searched for a linkage indicating positional loci for further association analyses. One of the loci in the implicated portion of the target region, ADRB2, was subsequently analyzed in association studies.

Methods

Subjects
The participants in this project (Silesian Hypertension Study) were recruited between 1999 to 2000 in Silesia, a region in the south of Poland with a high prevalence of cardiovascular morbidity and mortality. The study was designed to investigate for genetic predisposition to several cardiovascular phenotypes and was based on collecting probands with diagnosed essential hypertension along with their available parents and/or siblings. The project was approved by the local bioethical committee, and informed consent was obtained from each participant. We recruited 638 white individuals from 212 families with clustering of essential hypertension. Com-
Multilocus linkage analysis of systolic blood pressure to 5q31.1-qter chromosomal region. D5S494 through D5S211 represent microsatellite markers within examined region. Distances are shown in centiMorgans.

Phenotyping
Phenotyping included clinical history obtained by standardized questionnaires, physical examination, and laboratory tests according to the recommendations of the World Health Organization. Hypertension was defined as systolic and/or diastolic blood pressure >140/90 mm Hg on 3 separate occasions and/or remaining on antihypertensive treatment. Subjects with secondary forms of hypertension were excluded from the study. Complete phenotypic information was obtained from 635 subjects representing 210 families. Six other individuals from 3 families were excluded because of the inconsistencies in Mendelian segregation.

Identification and Localization of Genetic Loci
Within the Candidate Chromosomal Region
Eight microsatellite markers (DSS1480, DSS636, DSS820, DSS2093, DSS1471, DSS1456, DSS462, DSS211) spanning the 35-cM region on the distal portion of long arm of chromosome 5 (5q31.1-qter) were initially selected for molecular analysis. A set of 3 additional markers (DSS500, DSS642, DSS494) located proximally from DSS1480 and covering the distance of ~20 cM was chosen at a later stage to define the linkage region more accurately (Figure).
of the DNA fragment, including 3 functional SNPs within the ADRB2 gene, was performed in 15 randomly selected unrelated individuals to confirm the results of restriction fragment–length polymorphisms.

Statistical Analysis
Verification of genotypes for inconsistencies in Mendelian segregation was performed by means of PEDCHECK program. Multiple methods were used for linkage and association analysis of both qualitative and quantitative traits because this provides greater reliability of final result. Haseman-Elston regression analysis, based on regressing the siblings’ squared phenotype difference on their genetic similarity (defined as alleles shared identical by descent [IBD]) was used to test for 2-point linkage in case of microsatellite markers and the investigated phenotypes. Another IBD sib-pair test, SPLINK (Unix version 1.08), was applied to investigate for linkage of microsatellite markers to essential hypertension. Confirmatory 2-point linkage analysis based on estimation of alleles identical by state (IBS) at a microsatellite marker compared with the random distribution of alleles was performed for hypertension as a binary trait, by means of IBS χ² test. The Haseman-Elston and IBS χ² 2-point linkage tests were completed with SIB-PAIR program. For further confirmation of the results obtained in 2-point linkage analysis, multipoint nonparametric Z-score rank test was performed with quantitative phenotypes using MAPMAKER/SIBS.

The subsequent strategy, testing for association of essential hypertension with the ADRB2 gene as a positional candidate, was performed using family-based association tests. The transmission disequilibrium test (TDT) assessing the number of transmitted versus non-transmitted alleles from heterozygous parents to affected (hypertensive) probands (compared with expected 50%/50% transmission/nontransmission ratio) was used to test for association of essential hypertension with the SNPs of the ADRB2. The results of the TDTs were then verified by means of the empirical variance-family based association test (EV-FBAT) method, determining the value of an association test under the null hypothesis of linkage but no association by use of an empirical variance-covariance estimator. Unlike other family-based tests, this method is not affected by pedigree configurations and can be used in case of binary, quantitative, or time-to-onset traits, as well as multi- and biallelic markers.

Clayton’s modified TDT was performed in case of haplotype combinations, using the program TRANSMIT. This test calculates a score vector that is equalized over all possible combinations of parental haplotypes and transmissions, in concordance with the observed data, and deals with the problem of partially unknown parental genotypes and haplotype phase uncertainty. Binary logistic regression analysis was performed in the parental generation to test for association between essential hypertension and each genetic variant of the ADRB2 in the presence of other covariates, including age, gender, and body mass index.

Results
Clinical Characteristics
There were 629 individuals (age, 45.8±15.7 years) from 207 families, with 313 (49%) men and 316 (51%) women included in the final analysis. Of these, 401 (63.7%) subjects were hypertensive, and 270 (67.3%) of the hypertensive subjects remained on treatment. The demographic and clinical data of all individuals divided into probands, parents, and siblings are shown in Table 1.

Linkage Studies on 5q31.1-pter
All the microsatellite markers were highly informative, with the number of alleles from 8 (D5S820, D5S462, D5S211) to 15 (D5S949), and heterozygosity from 60% (D5S462) to 82% (D5S900).

In the first set of 8 markers, there was a significant linkage of the D5S1480 microsatellite marker to essential hypertension in 2-point linkage analysis (Table 2). The other markers did not show statistically significant linkage to this phenotype. Two-point linkage analysis of systolic and diastolic blood pressures (adjusted nonparametrically for treatment effect) showed statistical significance of the same marker (Table 2).

In view of the marginal location of the significant marker assigning the linkage within the candidate region, we also performed similar 2-point linkage studies for a set of 3 markers located proximally from D5S1480. Two of these markers (D5S500 and D5S1492) were significantly linked to essential hypertension, reaching linkage values of t=2.45 (P=0.008) and t=1.96 (P=0.03), respectively. Consistently, Haseman-Elston regression analysis of adjusted systolic and diastolic blood pressure revealed that the marker most proximal to DSS1480–DSS500 reached a borderline significance level in 2-point linkage analysis with diastolic blood pressure (P=0.06) and systolic blood pressure (P=0.09).

A joint multipoint analysis of all 11 microsatellite markers indicated that a region of ≈7 cM in close proximity to DSS1480 and DSS500 is linked to systolic blood pressure (Figure). The same tendency was evident in multipoint linkage analysis of adjusted diastolic blood pressure, with the maximal Z-score of 1.8 at the position of the DSS1480 marker.

<p>| TABLE 1. Demographic and Clinical Characteristics of the Individuals in Silesian Hypertension Study |</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probands (n=207)</th>
<th>Mothers (n=144)</th>
<th>Fathers (n=130)</th>
<th>Siblings (n=148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F</td>
<td>119/88</td>
<td>0/144</td>
<td>130/0</td>
<td>64/84</td>
</tr>
<tr>
<td>Age, y</td>
<td>36.2±15.6</td>
<td>54.2±10.8</td>
<td>55.8±10.0</td>
<td>42.4±14.8</td>
</tr>
<tr>
<td>Hypertensive subjects, n (%)</td>
<td>207 (100)</td>
<td>85 (59)</td>
<td>61 (47)</td>
<td>48 (33)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>142.6±14.0</td>
<td>139.4±23.3</td>
<td>138.6±19.8</td>
<td>131.6±21.8</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>91.2±10.6</td>
<td>88.7±13.6</td>
<td>87.2±11.6</td>
<td>83.7±11.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6±4.6</td>
<td>27.5±5.1</td>
<td>27.6±4.1</td>
<td>25.7±4.6</td>
</tr>
</tbody>
</table>

Values are mean±SD. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.
Association Studies of the Positional Candidate, ADRB2 Gene

Arg16Gly, Gln27Glu, and Thr164Ile polymorphisms were not associated with essential hypertension in the TDT (Table 3). This lack of association was confirmed by EV-FBAT test (Arg16Gly, \( P = 0.67 \); Gln27Glu, \( P = 0.55 \)). Testing for association of essential hypertension with Thr164Ile polymorphism using EV-FBAT could not be performed because of a rarity of Ile allele (only 17 individuals in the study were carriers of this allele).

Among 7 observed haplotypes (denoted A through G), B, D, and F represented the most common variants, comprising 97.4% of the total haplotypes (Table 4). The number of transmitted haplotypes from parents to hypertensive offspring was not significantly different from the expected number of transmissions (Table 4).

In the binary regression model, including age, gender, and body mass index as potential cofounders, none of the ADRB2 polymorphisms were associated with hypertension. The odds ratio for hypertension in subjects homozygous for a wild variant compared with heterozygous individuals and homozygous for a mutant allele was 0.85 (95% CI, 0.3 to 2.2; \( P = 0.74 \)) and 1.46 (95% CI, 0.5 to 4.2; \( P = 0.49 \)) for Arg16Gly, 1.33 (95% CI, 0.6 to 3.1; \( P = 0.5 \)) and 1.54 (95% CI, 0.5 to 4.4; \( P = 0.43 \)) for Gln27Glu, and 0.7 (95% CI, 0.1 to 4.9; \( P = 0.72 \)) for Thr164Ile, respectively.

### Discussion

In the present study, the maximal linkage was detected both in 2-point and multipoint analysis at the same chromosomal position corresponding to the D5S1480 microsatellite marker. In contrast, the linkage analysis of systolic blood pressure performed by Krushkal et al.\(^2\) on the same chromosomal region implicated different markers located proximally to the telomere. This discrepancy is not surprising and may reflect several differences in ethnic (European versus American origin), demographic (age), and clinical (normotension versus hypertension) profile of the subjects between these studies.

To avoid a potential bias that may arise from a linkage analysis of a dichotomous trait based on arbitrary categorization (hypertension), we performed additional studies of systolic and diastolic blood pressures, detecting consistent linkage in the proximal segment of 5q31.1-qter chromosomal region for both qualitative and quantitative traits.

A qualitative-quantitative joint analysis has been postulated to increase the evidence for linkage, especially in case of multifactorial diseases,\(^15\) and it has been widely implemented in studies aiming to dissect genetic predisposition to atopic complex disorders.\(^16,17\)

Consistent linkage signal obtained in 2-point and multipoint analysis narrowed down searches for candidate genes to the chromosomal segment assigned by D5S1480 and D5S500. Among several candidates for further positional analyses, we selected the ADRB2, the gene located in close proximity to the marker of the highest linkage. The priority was given to this candidate also in light of the well-documented role of the ADRB2 in blood pressure regulation and its essential contribution to the development of several cardiovascular and metabolic phenotypes related to hypertension.\(^18\)

To test for the relationships between essential hypertension and the ADRB2, we performed association studies of 3

### Table 3

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>Transmissions n (%)</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16Gly</td>
<td>Arg</td>
<td>54 (54.5)</td>
<td>0.8</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Gly</td>
<td>45 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln27Glu</td>
<td>Gln</td>
<td>62 (49.2)</td>
<td>&lt;0.1</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>64 (50.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr164Ile</td>
<td>Thr</td>
<td>8 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ile</td>
<td>1 (20)</td>
<td>1.8</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The numbers of informative parents were 99, 126, and 5 for Arg16Thr, Gln27Glu, and Thr164Ile polymorphisms, respectively.

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**TABLE 3.** Arg16Gly, Gln27Glu, and Thr164Ile Polymorphisms in TDT Test-Transmissions of Alleles From Heterozygous Parents to Offspring With Essential Hypertension

<table>
<thead>
<tr>
<th>SNP</th>
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</tr>
</tbody>
</table>

HT indicates essential hypertension; H-E, Haseman-Elston regression analysis; \( t \), Haseman-Elston \( t \) statistics value; \( \chi^2 \), IBS \( \chi^2 \) statistics.
TABLE 4. Haplotype TDT Test for 3 Functional SNPs of the ADRB2

<table>
<thead>
<tr>
<th>H</th>
<th>Allele</th>
<th>Gln27Glu Allele</th>
<th>Thr164Ile Allele</th>
<th>Estimated Frequency</th>
<th>Observed Transmissions</th>
<th>Expected Transmissions</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Gly</td>
<td>Glu</td>
<td>Thr</td>
<td>42.2%</td>
<td>217</td>
<td>211</td>
<td>0.52</td>
<td>0.47</td>
</tr>
<tr>
<td>D</td>
<td>Arg</td>
<td>Gln</td>
<td>Thr</td>
<td>34.8%</td>
<td>178</td>
<td>174</td>
<td>0.59</td>
<td>0.44</td>
</tr>
<tr>
<td>F</td>
<td>Gly</td>
<td>Gln</td>
<td>Thr</td>
<td>20.4%</td>
<td>95</td>
<td>103</td>
<td>2.03</td>
<td>0.15</td>
</tr>
</tbody>
</table>

H indicates haplotype; and B, D, and F are the symbols corresponding to the most frequent haplotypes.

Functional SNPs within the coding region of this gene. Arg16Gly, Gln27Glu, and Thr164Ile were selected for further analysis in light of the data regarding their influence on agonist-mediated receptor downregulation and affinity in vitro, as well as vascular desensitization in vivo. The lack of association of the ADRB2 polymorphisms with essential hypertension was evident in the TDT and verified by less conservative EV-FBAT test. Furthermore, these results were confirmed by the haplotype analysis. For the 2 common SNPs, our study had 86% power to detect association (P<0.05), with an odds ratio of 1.6.

The relationships between the polymorphisms of the ADRB2 gene and cardiovascular phenotypes have been assessed in European, American, Japanese, and African Caribbean populations, and the apparent lack of consistency in the results among these studies may be, at least partially, attributable to ethnic differences. Our results, analyzed in context of other European studies, are in agreement with the data obtained from English, French, and non-Aboriginal populations. It should be noted that all investigations on European normotensive subjects suggest the association of the ADRB2 with blood pressure, whereas the European studies involving hypertensive individuals are negative. One of the possible explanations for this discrepancy could be a pleiotropic effect exerted by the ADRB2. Its contribution to several cardiovascular and metabolic phenotypes (insulin resistance, obesity, heart failure) is of significantly different prevalence among hypertensive and normotensive individuals is well documented and cannot be excluded as a factor confounding the relationship of the ADRB2 with blood pressure. Whether the suggested different role of the ADRB2 in normotensive and hypertensive populations may be caused by its synergistic influence on multiple cardiovascular/metabolic phenotypes (acting as potential cofounders) or represents a distinct genetic background of high and low blood pressure remains to be elucidated.

The question that remains to be answered is which locus within our candidate region may be responsible for the observed linkage. Several genes coding for proteins involved in blood pressure regulation and hypertensive complications located in close proximity to D5S1480 and D5S500—such as annexin VI, glutathione peroxidase 3 gene, platelet-derived growth factor receptor-\( \beta \) gene, fibroblast growth factor-1, and glucocorticoid receptor gene—seem the most obvious positional candidates for further studies.

**Perspectives**

Our study implicates a short 7-cM segment on the long arm of the chromosome 5 as harboring a gene or genes for human essential hypertension. Furthermore, detailed haplotype analysis of three functional SNPs excluded ADRB2 as a causative gene. Further studies will focus on the remaining positional candidate genes, thus bringing closer the dissection of complex cardiovascular traits.

**Acknowledgments**

We are grateful to physicians for their involvement in the recruitment of the families, Miroslawa Kasprzak and Danuta Pupar for their excellent assistance in DNA extraction, and Dr Wai Kwong Lee for advice on molecular analysis. This study was supported by International Society of Hypertension Clinical Research Fellowship (to M.T.) and by a British Heart Foundation Program grant (PG20000023) and the European Commission EURNETGEN QLG1–2000–01137 Program within EU Framework 5 (to A.F.D). F.J.C. is supported by a Wellcome Trust Traveling Fellowship.

**References**


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