Epistatic Interaction Between $\beta_2$-Adrenergic Receptor and Neuropeptide Y Genes Influences LDL-Cholesterol in Hypertension

Maciej Tomaszewski, Fadi J. Charchar, Beata Lacka, Ullamari Pesonen, William Y.S. Wang, Ewa Zukowska-Szczechowska, Wladyslaw Grzeszczak, Anna F. Dominiczak

Abstract—$\beta_2$-Adrenergic receptor gene and neuropeptide Y gene may potentially influence lipid metabolism and overall energy balance. Therefore, we examined associations of these genes with lipid fractions and obesity-related phenotypes in hypertensive subjects. A total of 638 white individuals from 212 Polish families with clustering of essential hypertension were genotyped for cardiovascular risk determinants. Each subject was genotyped for functional polymorphisms of $\beta_2$-adrenergic receptor gene (Arg16Gly and Gln27Glu) and neuropeptide Y (Leu7Pro). Of 3 common haplotypes of $\beta_2$-adrenergic receptor gene, Arg16Gln27 was overtransmitted to offspring with elevated levels of total cholesterol ($Z=2.2; P=0.026$) and LDL-cholesterol ($Z=3.2; P=0.002$). Individually, Leu7Pro was not associated with any of the metabolic phenotypes in family-based tests or case-control analyses. However, in the presence of Arg allele of Arg16Gly and Gln allele of Gln27Glu, homozygosity for Leu variant of the Leu7Pro polymorphism was associated with 2.1-increased odds ratio (confidence interval, 1.10 to 3.81; $P=0.024$) of elevated LDL in hypertensive subjects, independent of age, gender, body mass index, adjusted blood pressures, antihypertensive therapy, and use of nonselective $\beta$-blockers and diuretics. Consistently, there was a significant multilocus association among variants of Arg16Gly, Gln27Glu, and Leu7Pro in hypertensive probands with elevated LDL (cases; $P=0.028$) but not in hypertensive subjects with normal LDL (controls). This study revealed an association of LDL-cholesterol with $\beta_2$-adrenergic receptor gene haplotype and provided evidence for epistatic interaction between $\beta_2$-adrenergic receptor gene and neuropeptide Y gene in determination of LDL-cholesterol in patients with essential hypertension. (Hypertension. 2004;44:1-6.)

Key Words: receptors, adrenergic ■ neuropeptide Y ■ genes ■ cholesterol

$\beta_2$-Adrenergic receptor ($\beta_2$AR) and neuropeptide Y are coexpressed within adipose tissue, liver, and pancreas, acting together in regulation of several fundamental metabolic functions. Secreted from sympathetic fibers innervating adipocytes, neuropeptide Y controls lipolysis, affecting overall energy balance, lipid mobilization, and circulating concentrations of several lipid fractions. Moreover, localized within the hepatic parenchyma, $\beta_2$AR and neuropeptide Y have been suggested to affect metabolic functions of the liver. Finally, both proteins can influence metabolic profile by regulation of insulin secretion in the pancreatic islets.

It is not clear whether the metabolic effects of $\beta_2$AR and neuropeptide Y are dependent on the genetic variation within their genes ($\beta_2$-adrenergic receptor gene [ADRB2] and NPY, respectively).

Some evidence supporting this hypothesis comes from a recent genome-wide scan, indicating a suggestive linkage of ADRB2 to an LDL-related phenotype. Confirmatory association studies using a family-based approach are lacking, and few cross-sectional case-control investigations focusing on single ADRB2 or NPY polymorphisms suggested associations of these genes with several lipid fractions.

Because ADRB2 and NPY are pathophysiologically relevant candidates in genetic studies on human metabolism, we sought to examine individual and joint associations of both genes with lipids and obesity-related phenotypes in patients with essential hypertension. Instead of studying single polymorphisms only, we extended our family-based investigations to a haplotype strategy and also tested for interactions between ADRB2 and NPY by use of family-based association test (FBAT). The FBAT is a powerful method of...
analysis that investigates potential association between single or multiple genetic variants and a phenotype treated either as a qualitative or quantitative trait using data from nuclear families. The FBAT uses conditioning on trait information and genotypes from available parents or the genotype combination of offspring (if the parental data are missing) in deriving the distribution for the S-statistic. This conditional approach is related to the original transmission disequilibrium test that compares transmission and nontransmission of alleles to affected offspring. Most important, FBAT is robust to population admixture, phenotype distribution specification, and ascertainment based on phenotypes.

Methods

Subjects
Polish families with clustering of essential hypertension from Silesian Hypertension Study (SHS) were included in this analysis. Among 212 recruited families, there were 127 families with both parents and hypertensive proband (with or without siblings), 19 families with hypertensive proband along with 1 parent and the sibling(s) of proband, and 66 sib-ships consisted of hypertensive proband and additional sibling(s).

Details of the SHS study design were described previously. Of 638 subjects recruited from 212 families, 9 individuals (5 families) were excluded from the study because of incomplete phenotyping or Mendelian inconsistencies in genotyping, as reported previously. Two subjects remaining on lipid-lowering medication were not included in the current analysis because of a well-known effect of this treatment on outcome variables. Data from 627 individuals representing 207 families were used in FBATs. Moreover, for the purpose of additional case-control analyses, all 197 available hypertensive index probands (each representing 1 family) were selected from the offspring generation and divided (based on their LDL level) into 2 groups: 113 cases (hypertensive subjects with elevated circulating concentrations of LDL; above a cut-off value of 4.1 mmol/L) and 84 controls (hypertensive individuals whose LDL levels were below the cut-off value). Ten hypertensive subjects were excluded from this case-control analysis because of unavailability of lipid phenotypes (missing serum samples). None of the SHS subjects included in the current analysis had any diseases (chronic renal failure, hypothyroidism, chronic liver disease, or diabetes mellitus) or were on treatment that may potentially affect lipid profile (excluding nonselective β-blockers and diuretics in 67 subjects).

To eliminate a potential confounding influence of this medication on lipid phenotypes, adjustment for therapy with nonselective β-blockers and diuretics was performed in multivariate analysis. Clinical history, height, weight, and blood pressure measurements were obtained according to the standard protocol, as described previously. Each subject underwent a venipuncture. Serum was separated from blood by centrifugation and stored at −70°C until biochemical analysis. All analyses were performed on fasting samples that had not been thawed previously.

Fasting lipid profile (total cholesterol [TC], HDL-cholesterol, and triglycerides [TGs]) was determined by enzymatic methods, as described previously. Concentrations of LDL-cholesterol were calculated using the Friedewald equation.

For the purpose of family-based haplotype analysis, lipid parameters were categorized into dichotomous (affected/unaffected) phenotypes based on the cut-off values suggested by the guidelines of the National Cholesterol Education Program Adult Treatment Panel III (TC > 5.2 mmol/L; HDL < 1.0 mmol/L; LDL > 4.1 mmol/L; TGs > 2.3 mmol/L). Similarly, each individual was classified as normal or overweight based on the cut-off value of body mass index (BMI; 25 kg/m²).

Genetic Markers
Genomic DNA was extracted from peripheral blood as described previously. Each subject was genotyped for 2 single-nucleotide polymorphisms (SNPs; Arg16Gly and Gln27Glu) within ADRB2 gene and a Leu7Pro polymorphism within NPY by use of polymerase chain reaction (PCR)–restriction fragment length polymorphism method, as described in detail previously.

Statistical Analysis
Each of the SNPs was tested for association with qualitative metabolic phenotypes using the FBAT. Modification of the single marker FBAT (haplotype FBAT) was used to test for association of the metabolic phenotypes with ADRB2 haplotypes. Power of the FBAT statistic to detect an association between a dichotomous phenotype and each SNP was computed based on the FBAT (interactive software package that provides tools for the design and the data analysis of family-based association studies). At significance level of 0.05, optimal offset based on disease prevalence and penetrance of 0.7, 0.5, 0.1 for AA, AB, and BB genotype combinations, our study had ~99.6%, 99.3%, and 61.6% power to detect associations between the dichotomous lipid phenotype and Arg16Gly, Gln27Glu, and Leu7Pro, respectively.

Testing for interactions between ADRB2 and NPY was based on complementary strategies: case-control evaluation of intergenic allelic associations among 3 tested polymorphisms, as well as multivariate binary logistic regression analysis along with its quantitative verification (comparison of differences in LDL according to ADRB2 before and after stratification according to NPY Leu7Pro polymorphism). All gene–gene interaction analyses were performed with regard to LDL fraction.

The first strategy (case-control evaluation of intergenic allelic associations among 3 tested polymorphisms) is based on the concept of interactions between pathophysiologically related genes located in distant regions of the human genome that lead to certain allelic associations apparent in affected subjects but not in healthy controls. Detection of such allelic associations has turned out possible by use of linkage disequilibrium (LD) test. Because assessment of LD is intuitively reserved for tightly linked loci on the same chromosome, testing of interactions between genes located on different chromosomes was termed a test of intergenic associations. Assessment of multilocus intergenic allelic associations among SNPs of ADRB2 and NPY was based on heterozygote excess calculation and was performed independently in unrelated hypertensive probands with elevated (cases) and normal (controls) LDL levels. Multilocus analyses were performed by genetic data analysis program. This software was also used in evaluation of Hardy–Weinberg equilibrium.

The second strategy (binary logistic regression analysis used in our previous studies on genetic interactions) was performed with LDL as an outcome variable, and age, gender, BMI, adjusted blood pressures, antihypertensive medication, use of nonselective β-blockers and diuretics, as well as Arg16Gly, Gln27Glu, and Leu7Pro polymorphisms as independent predictors in 197 unrelated hypertensive index patients with elevated (cases) and normal (controls) LDL levels.

Mann–Whitney test was used to evaluate LDL according to ADRB2 genotypes before and after stratification by NPY Leu7Pro polymorphism.

Results

Metabolic Characteristics

The metabolic characteristics of 197 hypertensive probands divided into cases (subjects with elevated LDL) and controls (subjects with normal LDL) are presented in Table 1.

ADRB2 and NPY: Genotype Distribution and Allele Frequencies

Distribution of genotypes and allele frequencies was computed in 274 biologically unrelated subjects representing the
parental generation (Table 2). Observed frequencies of genotypes were in Hardy–Weinberg equilibrium.

TABLE 1. Metabolic Characteristics of the Cases (Hypertensive Subjects With Elevated LDL) and Controls (Hypertensive Subjects With LDL Within Normal Limits)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cases (n=113)</th>
<th>Controls (n=84)</th>
<th>P  Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL (mmol/L)</td>
<td>5.04±0.78</td>
<td>3.27±0.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>6.28±0.67</td>
<td>4.40±0.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.99±0.30</td>
<td>0.98±0.29</td>
<td>0.944</td>
</tr>
<tr>
<td>TGs (mmol/L)</td>
<td>1.69±0.78</td>
<td>1.51±0.99</td>
<td>0.164</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.89±4.70</td>
<td>26.0±4.65</td>
<td>0.190</td>
</tr>
</tbody>
</table>

ADRB2, NPY, and Metabolic Phenotypes: Single-Locus FBATs
In the single-locus FBAT analysis, Arg16Gly polymorphism was associated with TC and more significantly with LDL; Arg allele was transmitted to affected offspring more frequently than expected by chance (Table 3). Gln variant of Gln27Glu polymorphism was over-represented in offspring with elevated concentrations of LDL, although this association was of borderline statistical significance (Table 3).

None of these 2 functional polymorphisms were associated with HDL, TGs, or overweight status in the single-locus FBAT (data not shown).

TC, LDL, HDL, TGs, overweight status, and hypertension were not associated with Leu7Pro polymorphism in the single-locus FBAT analysis (Table 3; data not shown).

ADRB2 and Metabolic Phenotypes: Haplotype Family-Based Association Analysis
Of 3 common ADRB2 haplotypes, Arg16Gly (H2) was over-represented in offspring with elevated levels of TC and, more strikingly, LDL (Table 4). There was a borderline underrtransmission of Gly16Glu27 haplotype (H1) to offspring with increased circulating concentrations of LDL (Table 4). HDL, TGs, and overweight status were not associated with ADRB2 in haplotype FBAT.

ADRB2, NPY, and LDL: Case–Control Evaluation of Intergenic Allelic Associations Among Arg16Gly, Gln27Glu, and Leu7Pro
Case-control analysis of multilocus allelic associations among polymorphisms of ADRB2 and NPY is shown in Table 5. There was a statistically significant intergenic allelic association between Gln27Glu polymorphism of ADRB2 and Leu7Pro SNP of NPY among cases (subjects with essential hypertension and elevated LDL levels) but not among controls (hypertensive probands with LDL within normal limits).

TABLE 2. SNPs Within ADRB2 and NPY: Distribution of Genotypes and Allele Frequencies in the Parental Generation of SHA

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype Distribution</th>
<th>Allele</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRB2–Arg16Gly</td>
<td>Arg16Gly Arg16Arg</td>
<td>Arg</td>
<td>36.7%</td>
</tr>
<tr>
<td></td>
<td>Arg16Gly</td>
<td>Arg</td>
<td>42.7%</td>
</tr>
<tr>
<td></td>
<td>Gly16Gly Gly16Gly</td>
<td>Gly</td>
<td>42%</td>
</tr>
<tr>
<td>ADRB2–Gln27Glu</td>
<td>Gln27Glu Gln27Gln</td>
<td>Gln</td>
<td>56%</td>
</tr>
<tr>
<td></td>
<td>Gln27Glu Gly27Glu</td>
<td>Gln</td>
<td>52.2%</td>
</tr>
<tr>
<td></td>
<td>Gly27Glu Gly27Glu</td>
<td>Gly</td>
<td>17.9%</td>
</tr>
<tr>
<td>NPY–Leu7Pro</td>
<td>Leu7Leu Leu7Leu</td>
<td>Leu</td>
<td>94.5%</td>
</tr>
<tr>
<td></td>
<td>Leu7Pro Leu7Pro</td>
<td>Pro</td>
<td>10.9%</td>
</tr>
</tbody>
</table>

TABLE 3. LDL, TC, and Polymorphisms Within ADRB2 and NPY: Single-Locus Family-Based Association Analysis

<table>
<thead>
<tr>
<th>Gene/SNP</th>
<th>Allele</th>
<th>S</th>
<th>E(S)</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>Arg16Gly Arg</td>
<td>71.0</td>
<td>56.5</td>
<td>3.1</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Gly</td>
<td>53.0</td>
<td>67.5</td>
<td>-3.1</td>
<td>0.002</td>
</tr>
<tr>
<td>ADRB2</td>
<td>Gln27Glu Gln</td>
<td>85.0</td>
<td>75.3</td>
<td>1.9</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>Glu</td>
<td>49.0</td>
<td>58.7</td>
<td>-1.9</td>
<td>0.002</td>
</tr>
<tr>
<td>NPY</td>
<td>Leu</td>
<td>21.0</td>
<td>19.3</td>
<td>1.0</td>
<td>0.302</td>
</tr>
<tr>
<td></td>
<td>Pro</td>
<td>5.0</td>
<td>6.7</td>
<td>-1.0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

| TC       | Arg16Gly Arg | 66.0 | 56.5 | 2.1 | 0.033 |
|          | Gly    | 58.0 | 67.5 | -2.1| 0.002 |
| ADRB2    | Gln27Glu Gln | 77.0 | 70.0 | 1.6 | 0.116 |
|          | Glu    | 49.0 | 56.0 | -1.6| 0.002 |
| NPY      | Leu    | 24.0 | 21.2 | 1.5 | 0.128 |
|          | Pro    | 4.0  | 6.8  | -1.5| 0.002 |

S indicates the observed value of S-statistic; E(S), the expected value of S-statistic; Z, test statistic (normalized value of S); P, level of significance corresponding to Z.

TABLE 4. LDL, TC, and Haplotypes of ADRB2: Family-Based Association Analysis

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Allele of Arg16Gly</th>
<th>Allele of Gln27Glu</th>
<th>S</th>
<th>E(S)</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>H1 Gly Gly27Glu</td>
<td>61.8</td>
<td>70.2</td>
<td>-1.8</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2 Arg Gln27Glu</td>
<td>74.8</td>
<td>60.5</td>
<td>1.2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H3 Gly Gln27Glu</td>
<td>34.2</td>
<td>39.5</td>
<td>-1.3</td>
<td>0.200</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>H1 Gly Gly27Glu</td>
<td>51.8</td>
<td>57.6</td>
<td>-1.4</td>
<td>0.173</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2 Arg Gln27Glu</td>
<td>63.8</td>
<td>53.6</td>
<td>2.2</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H3 Gly Gln27Glu</td>
<td>24.2</td>
<td>28.2</td>
<td>-1.1</td>
<td>0.274</td>
<td></td>
</tr>
</tbody>
</table>

S indicates the observed value of S-statistic; E(S), the expected value of S-statistic; Z, test statistic (normalized value of S); P, level of significance corresponding to Z.
Consequently, there was a significant multilocus association among allelic variants of 3 tested polymorphisms (Arg16Gly, Gln27Glu, and Leu7Pro) in the cases but not in the control group (Table 5).

**Combinations of Arg16Gly, Gln27Glu, and Leu7Pro and the Risk of Elevated LDL: Crude and Multivariate Logistic Regression Analysis**

The Hosmer and Lemeshow goodness-of-fit test confirmed that the multivariate logistic regression models (including age, sex, BMI, adjusted blood pressures, antihypertensive therapy, use of nonselective β-blockers and diuretics, as well as Arg16Gly, Gln27Glu, and Leu7Pro polymorphisms as predictors of elevated LDL) fit the observed data accurately ($P=0.37$ to 0.76). Medication that could potentially affect lipid metabolism (nonselective β-blockers and diuretics) was not associated with the outcome variable, either in the univariate or multivariate analysis ($P=0.45$ to 0.71).

Coeexistence of Arg allele and Gln allele at the ADRB2 locus showed a borderline association with the increased risk of elevated LDL among hypertensive probands (Table 6). Absence of the Pro allele at Leu7Pro locus on its own did not increase the risk of elevated LDL in hypertensive subjects ($P=0.13$). However, in combination with Gln allele at Gln27Glu ($P=0.046$), Arg allele at Arg16Gly ($P=0.036$), or most importantly, Arg16–Gln27 ADRB2 configuration ($P=0.024$), detrimental genotype at NPY Leu7Pro doubled the risk of elevated LDL in hypertensive subjects (Table 6). The results of a crude comparison of LDL according to ADRB2 genotypes before and after stratification by NPY Leu7Pro are presented in Table 7. NPY Leu7Pro polymorphism was not associated on its own with LDL in this analysis. Consistent with the data from the family-based association analysis, carriers of Gln at Gln27Glu had higher levels of circulating LDL, although this comparison reached $P=0.09$. This association became more apparent when ADRB2 and NPY loci were analyzed together (Table 7).

**Discussion**

This study revealed an association of LDL-cholesterol with ADRB2 haplotype and provided evidence for epistatic interaction between the ADRB2 and NPY in determination of LDL-cholesterol in patients with essential hypertension.

In the present analysis, associations of ADRB2 SNPs with metabolic phenotypes were investigated simultaneously. There is a pathophysiological and genetic rationale for such an approach. First, Arg16Gly and Gln27Glu have been shown to affect agonist-mediated receptor downregulation, thus both SNPs may potentially influence cardiovascular and metabolic phenotypes. Second, as confirmed in this study, Arg16Gly and Gln27Glu are in significant LD (their alleles do not segregate independently during meiosis) and therefore cannot be examined in isolation. Previous studies on ADRB2 and metabolic phenotypes used mostly nonhaplotype individual analysis of Arg16Gly or Gln27Glu of ADRB2, and this may be a reason of conflicting results from these investigations. In addition, most of the former studies were based on cross-sectional case-control approaches, and thus, a potential bias attributable to population stratification cannot be fully excluded. Our study controls for both potential confounding factors: population stratification (by use of family-based approach) and LD (by use of haplotype strategy). Consequently, any bias in the current analysis is highly unlikely.

The relationship between ADRB2 and LDL shown in the present study is particularly important from a genetic and clinical point of view. First, the data contributed supplementary association confirmation for the previous genome-wide linkage analysis implicating ADRB2 as linked to one of LDL-related phenotypes. Moreover, this analysis sheds new light on previous observations regarding associations of ADRB2 with cardiovascular and metabolic disorders. Although not associated with essential hypertension directly, ADRB2 may influence lipid disturbances in hypertensive patients. Finally, these results reinforced utility of genetic markers in the evaluation of metabolic risk in hypertensive patients whose cardiovascular morbidity and mortality are closely related to lipid disturbances.

Our data support the hypothesis of epistatic interaction between ADRB2 and NPY in regulation of LDL levels in hypertensive subjects. The effect of NPY locus appears to be altered by ADRB2. Specifically, Leu7Leu genotype within NPY SNP was associated with increased concentrations of LDL only in the presence of Arg16Gln27 combination of

---

**Table 5. Evaluation of Multilocus Intergenic Allelic Associations Among Polymorphisms of ADRB2 and NPY in Hypertensive Index Patients With Elevated (Cases) and Normal (Controls) LDL**

<table>
<thead>
<tr>
<th>Multilocus Combination</th>
<th>Cases (n=113)</th>
<th>Controls (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRB2-Arg16Gly/ADRB2-Gln27Glu/ADRB2-Leu7Pro</td>
<td>P=0.472</td>
<td>P=0.508</td>
</tr>
<tr>
<td>ADRB2-Gln27Glu/ADRB2-Leu7Pro</td>
<td>P=0.040</td>
<td>P=0.187</td>
</tr>
<tr>
<td>ADRB2-Arg16Gly/ADRB2-Gln27Glu/NPY-Leu7Pro</td>
<td>P=0.028</td>
<td>P=0.431</td>
</tr>
</tbody>
</table>

$P$ indicates level of significance corresponding to an intergenic association among allelic variants computed for each of 2-locus and multilocus combinations.

**Table 6. Genetic Predictors of Elevated LDL in Hypertensive Subjects (Adjusted Odds Ratios From Multivariate Logistic Regression Analysis)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg16 (M)</td>
<td>0.121</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gln27 (M)</td>
<td>0.315</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Leu7 (M)</td>
<td>0.13</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Arg16-Gln27 (M)</td>
<td>0.074</td>
<td>1.78</td>
<td>0.95–3.33</td>
</tr>
<tr>
<td>Gln27-Leu7 (M)</td>
<td>0.046</td>
<td>2.06</td>
<td>1.01–4.18</td>
</tr>
<tr>
<td>Arg16-Leu7 (M)</td>
<td>0.036</td>
<td>1.94</td>
<td>1.05–3.60</td>
</tr>
<tr>
<td>Arg16-Gln27-Leu7 (M)</td>
<td>0.024</td>
<td>2.05</td>
<td>1.10–3.81</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; M, predictors included in the multivariate logistic regression model (age, sex, body mass index, adjusted blood pressures, antihypertensive medication, use of nonselective β-blockers and diuretics).

Arg16, Gln27, and Leu7 refer to the combination of detrimental genotypes (presence of Arg allele, presence of Gln allele, absence of Pro allele at Arg16Gly, Gln27Glu and Leu7Pro polymorphisms, respectively). Nondetrimental allelic combinations of each polymorphism were used as reference in estimation of the odds ratios.
TABLE 7. LDL in Hypertensive Index Probands According to Individual and Joint ADRB2 Arg16Gly and Gln27Glu Genotypes Before and After Stratification by NPY Leu7Pro Polymorphism

<table>
<thead>
<tr>
<th>Stratification by Leu7Pro</th>
<th>Arg16Gly</th>
<th>Gln27Glu</th>
<th>Arg16Gly + Gln27Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arg16</td>
<td>Gly16</td>
<td>P</td>
</tr>
<tr>
<td>—</td>
<td>4.31±1.12 (122)</td>
<td>4.25±1.10 (75)</td>
<td>0.49</td>
</tr>
<tr>
<td>Leu-7</td>
<td>4.37±1.11 (107)</td>
<td>4.39±1.14 (149)</td>
<td>P</td>
</tr>
<tr>
<td>Pro-7</td>
<td>3.89±1.18 (15)</td>
<td>3.86±1.49 (19)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Arg16 refers to the presence of Arg allele at Arg16Gly locus; Gln27, presence of Gln allele at Gln27Glu locus; P, level of significance in the Mann–Whitney test.

Comparison of LDL between genetic variants of ADRB2 gene is presented in the top part of the table (reads horizontally). Stratification of the ADRB2 genotypes according to the Leu7Pro polymorphism within NPY is presented in the bottom part of the table (reads vertically). Comparison of LDL in Gly16, Gln27, and Gly16-Glu27 subgroups after stratification by Leu7Pro could not be performed because of a low number of subjects with Pro-7 variant.

ADRB2 polymorphisms. Thus, ADRB2 seems to have an unmasking effect on Leu7Pro polymorphism. Although the definition of epistasis is controversial, it is generally agreed that such unmasking effects are epistatic.31 In addition, gene–gene interaction analysis has implicated Leu7Pro polymorphism of Leu7Pro SNP as a variant increasing LDL levels. In contrast, single-locus association studies performed so far have shown that different genetic variants (Leu7Pro versus Leu7Leu) were implicated by single-locus and multilocus analyses, respectively. This is in agreement with current views on cardiovascular diseases as polygenic disorders and was recently reinforced by implication of ACE I allele (known as cardioprotective) as a prophylactic variant in the presence of certain haplotypes of angiotensinogen gene.26

Although a positive association analysis never proves causation, a substantial body of evidence from clinical and experimental data provides several mechanistic explanations for possible effects of ADRB2–NPY interplay. Because polymorphic variations within both genes have been suggested to affect functions of β2AR30 and secretion of neuropeptide Y,32 the effect of ADRB2–NPY interaction on LDL may be translated into cross-talks between the proteins, either within adipocytes, hepatocytes, or pancreatic islets.

The detected epistatic interaction between ADRB2–NPY sheds new light on the previous observations suggesting coexistence of abnormalities in NPY expression and β2AR activity in experimental model of genetic hypertension.53 In view of this observation, our selection of essentially hypertensive patients for the current analysis seems justified. Nonetheless, confirmation of these results in the general population is warranted.

Given that NPY and ADRB2 contain more SNPs than analyzed in this study, one cannot exclude that other SNPs remaining in LD with the tested polymorphisms influence the associations with LDL. Functional significance of the polymorphisms used in the current study make this presumption less likely.

Perspectives

This study has provided further evidence that several functional polymorphisms within candidate genes act together in determining LDL-cholesterol. Therefore, future investigations should focus on haplotypes/haplogroups instead of single polymorphisms to facilitate the dissection of human metabolic syndrome. One can also speculate that the haplotype combination at ADRB2 locus might be useful as a genetic marker of metabolic risk in hypertensive patients.

Acknowledgments

This study was supported by the British Heart Foundation Programme Grant (RG/002/012), the European Commission EURNETGEN QLG1-2000-01137 Programme within European Union Framework 5, the Wellcome Trust Cardiovascular Functional Genomics Grant (066780/Z/01/Z, A.F.D.), the Wellcome Trust International Research Development Award (067827/Z/02/Z), and the Award of the Foundation for Polish Science (M.T.).

References

Epistatic Interaction Between β2-Adrenergic Receptor and Neuropeptide Y Genes Influences LDL-Cholesterol in Hypertension

Maciej Tomaszewski, Fadi J. Charchar, Beata Lacka, Ullamari Pesonen, William Y.S. Wang, Ewa Zukowska-Szczechowska, Wladyslaw Grzeszczak and Anna F. Dominiczak

Hypertension. published online September 13, 2004;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2004/09/13/01.HYP.0000143844.81979.61.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/