Leptin Is Associated With Blood Pressure and Hypertension in Women From the National Heart, Lung, and Blood Institute Family Heart Study

Duanduan Ma, Mary F. Feitosa, Jemma B. Wilk, Jason M. Laramie, Kai Yu, Catherine Leiendecker-Foster, Richard H. Myers, Michael A. Province, Ingrid B. Borecki

Abstract—Leptin is a key neuroendocrine hormone regulating food intake, metabolism, and fat accumulation, and it may also affect blood pressure and contribute to hypertension through sympathetic activation in the vasculature or at the renal level. Although previous studies have shown that the distribution of leptin is significantly different between males and females, as is the risk of hypertension between males and females, results regarding the role of leptin in the gender-specific regulation of blood pressure are controversial. Thus, we performed family-based association analyses in the National Heart, Lung, and Blood Institute Family Heart Study to test the hypothesis that LEPTIN gene variants and the plasma leptin level influence variability in blood pressure and the risk of hypertension differently by gender. We identified significant associations between LEPTIN single nucleotide polymorphisms with blood pressure and hypertension, but in postmenopausal women only. We also identified significant associations between plasma leptin levels and both blood pressure and hypertension in women. The current study supports a role for LEPTIN and plasma leptin levels in blood pressure regulation in women. It also provides insight into the gender differences in hypertension, as well as the differential distribution and activity of leptin in men and women. (Hypertension. 2009;53:473-479.)

Key Words: leptin ■ blood pressure ■ hypertension ■ association ■ gender

In developed and developing countries, hypertension has been identified as an expanding health crisis, and there are apparent gender differences in the mechanisms involved in the relationship of obesity and hypertension in men and women. Although numerous studies have been performed to identify the genetic background underlying hypertension and to seek quantitative trait loci controlling blood pressure, there is a paucity of reports addressing genes and their products that are involved in sex-specific regulation of blood pressure and sex-specific onset of hypertension. Understanding how such factors differentially influence the regulation of blood pressure in men and women can provide insight into the contributing pathways and may provide clues for treatment strategies targeted toward appropriate populations.

The LEPTIN gene (LEP) was originally identified as a murine obesity gene, and its human homologue is a neuroendocrine hormone functioning as an important regulator of food intake, neuroendocrine outflow, metabolism, and fat accumulation. As shown in experimental studies, leptin may also affect blood pressure and contribute to the occurrence of hypertension through sympathetic activation in the circulatory district or at the renal level, and microinjections of leptin have indicated that higher intracerebroventricular level of leptin lead to higher effects toward sympathetic activation. It is widely recognized that the leptin levels are several times higher in women than men, which suggests the possibility of greater effects on the sympathetic nervous system (SNS) in women. Although many studies have previously examined the association between leptin levels and blood pressure, the gender-specific effects of genetic variants in LEP as well as leptin levels in the regulation of blood pressure are still unsettled. Some previous studies report associations in gender-specific manners that are different from each other, whereas other studies do not detect gender differences. In the current study, we aim to evaluate whether DNA sequence variability in the upstream LEP promoter region, and within the LEP gene itself, as well as plasma leptin levels influence blood pressure and hypertension differentially by gender in a large, multicenter study: the National Heart, Lung, and Blood Institute Family Heart Study (NHLBI-FHS).

Methods

Subjects

The current association studies were based on the same data set used in a previous evaluation of LEP variants with body mass index...
However, we further adjusted for the effects of BMI in addition to hereafter referred to as regular adjustment in the current study. The measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were performed in sitting position by random-zero sphygmomanometry in each field center of the NHLBI-FHS study, which included Forsyth County, NC; Minneapolis, Minn; Framingham, Mass; and Salt Lake City, Utah. Both SBP and DBP were measured 3 times, and the mean of the second and the third measurements was used for analysis. Hypertension is defined as SBP ≥140 mm Hg or DBP ≥90 mm Hg, or currently under hypertension medication. All subjects were fasting for 12 hours before the blood draws. The measurement of leptin was performed on EDTA plasma using Human Leptin RIA Kit (LINCO Research). Stepwise multiple regression models were used to adjust for the effects of age and field center on the mean and the variance of SBP and DBP within sex, similar to our previous studies, which is hereafter referred to as regular adjustment in the current study. However, we further adjusted for the effects of BMI in addition to the factors mentioned above, which is hereafter referred to as BMI adjustment. The association analyses were based on the residual SBP and DBP, the distributions of which were standardized to a limit normal. In the later phenotype association studies between plasma leptin level and blood pressure/hypertension, regular and BMI adjustments were also applied to leptin levels. In the association studies between LEP single nucleotide polymorphisms (SNPs) and plasma leptin, only regular adjustments were applied to leptin levels. There are 105 subjects with measured SBP and DBP already taking antihypertensive medication (53 males and 52 females), and their blood pressures are recoded as missing in the quantitative association analysis to avoid the confounding effects of antihypertensive medications on blood pressure. Menopausal status was ascertained based on self-report in a reproductive questionnaire for women.

### Genotyping and Quality Control

SNP selection, genomic DNA preparation, SNP typing methods, and bioinformatic searching for transcription factor binding sites (TFBSs) were described in detail in the previous study. The data contain 695 subjects from 82 white families in the NHLBI-FHS study. The families contribute strong support to linkage evidence for BMI on chromosome 7q13, and the multipoint logarithm of odds score for this data set is 17.09 at 136.95 cM (Genetic Map Index web site; Center for Medical Genetics). All SNPs were selected according to Assays-on-Demand SNP Genotyping Products, previous publications, or the human SNP database in the Celera Discovery System. TFBSs were described in detail in the previous study. In bioinformatic searching for transcription factor binding sites (TFBSs) were described in detail in the previous study. Genotyping and Quality Control

### Table 1. SNP Summary

<table>
<thead>
<tr>
<th>SNP No.</th>
<th>Marker</th>
<th>Gene</th>
<th>SNP</th>
<th>Frequency</th>
<th>NCBI Location</th>
<th>SNP Type</th>
<th>rs No.</th>
<th>Haplotype</th>
<th>Block</th>
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was extracted from whole blood, and the SNPs were genotyped using the ABI TaqMan Technology. The pedigree relationships were verified previously using the linkage STR marker panel by graphical representation of relationship errors. Additional quality control measures such as checks for Mendelian errors and Hardy–Weinberg equilibrium were performed. Double recombinants were detected using MERLIN and were set as missing genotypes.

**Family-Based Association Analysis**

To test the association between LEP SNPs with SBP, DBP, and plasma leptin level, Quantitative Transmission Disequilibrium Test (QTDT) version 2.425 was used to test single SNP association, as well as haplotype association based on the 6 SNPs with the most significant marginal P values. We input estimated haplotypes from MERLIN23 for the haplotype analyses. TRANSMIT26,27 version 2.5.4 was used to perform the Transmit Disequilibrium Tests (TDT) to test the association between LEP SNPs and hypertension. Asymptotic P values were reported in the results.

Additional powerful haplotype analyses were performed to test the association between LEP SNPs and hypertension using SPTDT (Sequential Peeling Transmission Disequilibrium Test). We performed SPTDT based on 2 sets of SNPs. The first set contains SNPs in blocks located at the 5’ TFBS and promoter region (SNPs 6 to 18 in blocks 2 to 3; Table 1), and the second set contains SNPs in blocks located at the intron/exon region, 3’UTR, and further downstream (SNPs 19 to 29 in blocks 4 to 7; Table 1). Haplotypes with frequencies <0.005 were excluded from the analysis, and 10,000 permutations were used to determine empirical P values.

**Phenotype Association**

Pearson correlations were estimated by SAS to explore the relationship between plasma leptin levels and blood pressures using the residuals of blood pressures and plasma leptin levels after regular adjustment. We further estimated the correlation between leptin levels and blood pressures, accounting for the effects of BMI, in SAS.

To test the association between leptin levels and hypertension, we used a logistic function in generalized estimating equations to adjust for the nonindependence of subjects within families. We first analyzed the residuals of leptin levels after regular adjustment as the independent variable and hypertension as the dependent variable in the model. We also investigated leptin levels additionally adjusted for BMI.

Because of potential interactions between endogenous hormones and LEP plasma leptin in the regulation of blood pressure, all of the above genotype and phenotype association studies were stratified by gender and self-reported menopausal status. Phenotypes of the subjects who do not belong to the categories of interest were coded as missing.

**Results**

**SNP Location and Characteristics**

Twenty-nine SNPs within and around LEP were analyzed in the current study. Their location and characteristics are presented in Table 1, ordered by their NCBI bp location. The range of minor allele frequencies is from 0.092 to 0.495. The SNPs are in 7 linkage disequilibrium blocks identified by Haplovie, using confidence-bound estimates for D’. A block is defined when ≥95% of SNP pairs meet the criteria of 1-sided upper 95% confidence bound of D’>0.98 and a lower bound of >0.7.

**Phenotype Distribution**

The characteristics of the sample are shown in Table 2. The level of plasma leptin is significantly higher in female subjects (P<0.05), and SBP and DBP are significantly higher in male subjects (P<0.05). Postmenopausal women have significantly higher SBP, DBP, plasma leptin levels, and hypertension prevalence compared with premenopausal women (P<0.05).

**Single SNP Association Studies Between LEP and Blood Pressure/Hypertension**

QTDT analyses evidence significant associations between SBP and a 5’ TFBS SNP (P=0.0028), 3 SNPs within the promoter region (P<0.01), and 2 intronic SNPs (P<0.002), all in women only (Table 3). QTDT analysis also showed significant association between these SNPs and DBP, also in women. After further adjusting SBP and DBP for BMI, the associations remained significant (Figure 1). To account for multiple testing, we calculated the false discovery rates, and the corresponding q values are significant for SBP (q<0.05) and borderline significant for DBP (q=0.15). No significant association was detected in men (P>0.05). Stratifying the data by menopausal status revealed that the significant associations are only in postmenopausal women. In the postmenopausal group, significance level of LEP SNPs is increased 2- to 80-fold regarding the associations of SBP, and the significance level is also slightly increased regarding the associations of DBP (Figure 2). The QTDT P values and false discovery rate q values for SBP in all women and postmenopausal women are shown in Table 3.

TDTs also identified significant associations between the above 6 SNPs with hypertension in women (Figure 1). Further analysis stratified by menopausal status supported the association of these SNPs in postmenopausal women, with improved significance levels (Figure 2).

**LEP Haplotype Associations With Blood Pressure/Hypertension**

The 6 LEP SNPs that consistently show association in women using marginal SNP association tests (rs13245201, rs13245202, rs13245203, rs13245204, rs13245205, and rs13245206) were extracted from whole blood, and the SNPs were genotyped using the ABI TaqMan Technology. The pedigree relationships were verified previously using the linkage STR marker panel by graphical representation of relationship errors. Additional quality control measures such as checks for Mendelian errors and Hardy–Weinberg equilibrium were performed. Double recombinants were detected using MERLIN and were set as missing genotypes.

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**Results**

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<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males</th>
<th>Females</th>
<th>Premenopausal Women</th>
<th>Postmenopausal Women</th>
</tr>
</thead>
<tbody>
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<td>No.</td>
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<td>235</td>
<td>100</td>
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<td>SBP, mm Hg</td>
<td>115.85±12.97</td>
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<td>DBP, mm Hg</td>
<td>70.57±9.81</td>
<td>66.70±9.56</td>
<td>64.73±8.89</td>
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<td>BMI, kg/m²</td>
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<td>Plasma leptin, ng/mL</td>
<td>8.44±7.69</td>
<td>24.58±18.98</td>
<td>21.86±18.21</td>
<td>27.19±20.24</td>
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<tr>
<td>Percentage with hypertension</td>
<td>25.27%</td>
<td>23.79%</td>
<td>6.45%</td>
<td>49.23%</td>
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</tbody>
</table>

Mean±SD of age, SBP, DBP, BMI, and plasma leptin are based on subjects with SBP or DBP measurements not using antihypertensive medications.

Hypertension is defined as SBP ≥140 mm Hg or DBP ≥90 mm Hg or currently taking antihypertensive medication.

### Table 2. Phenotype Distribution by Gender and Menopausal Status (Mean±SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males</th>
<th>Females</th>
<th>Premenopausal Women</th>
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<td>49.23%</td>
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rs7799039, rs6467166, rs12536535, rs10244329, and rs11763517) can be analyzed as a haplotype, which is also associated with SBP and DBP. The *P* values in the haplotype analysis for DBP are 0.0054 with regular adjustment, and 0.0076 with BMI adjustment in women, whereas the *P* values in the haplotype analysis for SBP are 0.0032 with regular adjustment, and 0.0036 with BMI adjustment in women. Among postmenopausal women, haplotype analysis also yielded higher significance of association with DBP (*P* = 0.0018 with both regular and BMI adjustment), as well as higher significance of association with SBP (*P* = 0.0007 with regular adjustment and *P* = 0.0002 with BMI adjustment).

SPTDT results indicate that the SNPs located at *LEP* 5' TFBS and promoter region (SNPs 6 to 18; ie, rs6947095 to rs13228777; Table 1) are jointly significantly associated with hypertension in women, and the global adjusted empirical *P* value is 0.0065. The SNPs located at blocks in *LEP* introns, exons, 3'UTR region, and further downstream (SNPs 19 to 28; ie, rs2167270 to rs10279576; Table 1) are also jointly significantly associated with hypertension in women, with the global empirical adjusted *P* value at 0.0020. The haplotype association is restricted to women, and no association is seen in men. Among postmenopausal women, SPTDT provides empirical *P* values as 0.0064 and 0.0043 regarding the above TFBS and promoter region (SNPs 6 to 18; Table 1), as well as *LEP* intron, exon, and 3'UTR regions (SNPs 19 to 28; Table 1), respectively.

**Phenotype Association Studies**

Plasma leptin levels are correlated with SBP and DBP in women (Table 4). However, after SBP and DBP are additionally adjusted by BMI, the correlation appreciably diminishes, suggesting that the relationship between plasma leptin levels and blood pressure is mediated by BMI. Generalized estimating equation logistic models reveal a significant association between plasma leptin levels and hypertension in women (*P* < 0.001; *β* = 0.4830; 95% CI, 0.2498 to 0.7162). However, no significant association remains after further BMI adjustment (*P* = 0.1540; *β* = 0.1293; 95% CI, 0.0485 to 0.3072; Table 4). No significant association was detected in men.

The association between plasma leptin levels and SBP, DBP, or hypertension is also localized in postmenopausal women. After accounting for BMI, the association either diminishes or is weakened appreciably (Table 4).

**Single SNP Association Studies Between LEP and Plasma Leptin Levels**

QTD results suggest that there are no appreciable significant associations between the *LEP* SNPs and plasma leptin in men or women, regardless of menopausal status (Figure 3). The only marginally significant signals observed are at rs2060715 in premenopausal women (Table 4) and rs28954369 in premenopausal women (Table 4).

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Table 3. QTDT Single SNP Analysis Results of SBP

<table>
<thead>
<tr>
<th>SNP No.</th>
<th>SNP Name</th>
<th>Female Regular Adjustment <em>P</em> Value</th>
<th>Female Regular Adjustment FDR <em>q</em></th>
<th>Postmenopausal Regular Adjustment <em>P</em> Value</th>
<th>Postmenopausal Regular Adjustment FDR <em>q</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>rs13245201</td>
<td>0.0028</td>
<td>0.2030</td>
<td>0.0003</td>
<td>0.0029</td>
</tr>
<tr>
<td>14</td>
<td>rs7799039</td>
<td>0.0005</td>
<td>0.0131</td>
<td>0.0002</td>
<td>0.0029</td>
</tr>
<tr>
<td>15</td>
<td>rs6467166</td>
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<td>0.0131</td>
<td>0.0002</td>
<td>0.0029</td>
</tr>
<tr>
<td>16</td>
<td>rs12536535</td>
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<td>0.0131</td>
<td>0.0002</td>
<td>0.0029</td>
</tr>
<tr>
<td>23</td>
<td>rs10244329</td>
<td>0.0006</td>
<td>0.0131</td>
<td>0.0002</td>
<td>0.0029</td>
</tr>
<tr>
<td>24</td>
<td>rs11763517</td>
<td>0.0017</td>
<td>0.0164</td>
<td>0.0008</td>
<td>0.0033</td>
</tr>
</tbody>
</table>

---

Figure 1. Single SNP association between *LEP* and blood pressure/hypertension in female subjects. sbp-bmi-adj indicates SBP BMI adjustment; dbp-bmi-adj, DBP BMI adjustment; sbp-regular-adj, SBP regular adjustment; dbp-regular-adj, DBP regular adjustment; hyperten, hypertension. The correspondence between SNP numbers and rs numbers is shown in Table 1.

Figure 2. Single SNP association between *LEP* and blood pressure/hypertension in postmenopausal women. sbp-bmi-adj indicates SBP BMI adjustment; dbp-bmi-adj, DBP BMI adjustment; sbp-regular-adj, SBP regular adjustment; dbp-regular-adj, DBP regular adjustment; hyperten, hypertension. The correspondence between SNP numbers and rs numbers is shown in Table 1.
SBP


dence between SNP numbers and rs numbers is shown in Table 1.

pre-meno, results from premenopausal women. The correspon-

results from men; meno, results from postmenopausal women;

plasma leptin. Female indicates results from women; male,

blood pressure with

leading to diminished associations of blood pressure.

Thus, effects toward blood pressure regulation from genes

underlying BMI would disappear after adjusting for BMI,

rule out that the absence of association between plasma leptin

values of Association Between Leptin Level and SBP, DBP, and Hypertension

Table 4. Pearson Correlation Coefficients and P Values of Association Between Leptin Level and SBP, DBP, and Hypertension

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Female Regular Adjustment</th>
<th>Female BMI Adjustment</th>
<th>Postmenopausal Regular Adjustment</th>
<th>Postmenopausal BMI Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.2829</td>
<td>0.0324</td>
<td>0.3326</td>
<td>0.1579</td>
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<tr>
<td>P</td>
<td>0.0001</td>
<td>0.6235</td>
<td>0.0004</td>
<td>0.0996</td>
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<tr>
<td>DBP</td>
<td>0.2575</td>
<td>0.1103</td>
<td>0.3498</td>
<td>0.2116</td>
</tr>
<tr>
<td>P</td>
<td>0.0001</td>
<td>0.0939</td>
<td>0.0002</td>
<td>0.0265</td>
</tr>
<tr>
<td>Hypertension</td>
<td>P&lt;0.001</td>
<td>P=0.1540</td>
<td>P=0.0045</td>
<td>P=0.3593</td>
</tr>
</tbody>
</table>

Discussion

The current study shows evidence of associations between

SNPs in LEP with blood pressure and hypertension in postmenopausal women, and it also provides evidence of

significant associations between plasma leptin levels with

blood pressure and hypertension in women regardless of

menopausal status. The associations were identified across a

variety of approaches and persisted after false discovery rate

correction for multiple comparisons. Moreover, the meno-
pausal dependence of the associations is unique compared

with previous studies.15–19

One interesting finding is that the association between LEP

genotype and blood pressure is robust to the adjustment of

blood pressure for BMI, but the association between plasma

leptin levels and blood pressure diminishes or is weakened

appreciably after adjustment for BMI. It is well known that

plasma leptin levels are closely related to BMI (r=0.74 in

female subjects in the current data), so adjusting blood

pressure for BMI could account for the association with

plasma leptin levels. Because LEP SNP variants are not

associated with BMI in women,20 it stands to reason that

adjusting for BMI does not ameliorate the association of

blood pressure with LEP SNPs in women. Finally, we cannot

rule out that the presence of association between plasma leptin

levels and blood pressure after BMI adjustment may be

caused by pleiotropic effects of genes other than LEP

underlying BMI and blood pressure (Figure 4). Genes influ-

encing BMI could also affect blood pressure through BMI.

Thus, effects toward blood pressure regulation from genes

underlying BMI would disappear after adjusting for BMI,

leading to diminished associations of blood pressure.

Figure 3. Single SNP association results between LEP and

plasma leptin. Female indicates results from women; male,

results from men; meno, results from postmenopausal women;

pre-meno, results from premenopausal women. The correspon-
dence between SNP numbers and rs numbers is shown in Table 1.

Figure 4. The relationship among LEP, leptin, blood pressures,

and BMI in women. G indicates genes other than LEP.
promoter region influence transcription factor binding and promoter activity in a sex-specific manner in the SNS, as well as how intrinsic variation influences protein level and activity in the SNS. Many studies report higher leptin levels in women than in men. The sexual dimorphism of leptin levels is thought attributable to the endogenous hormones. Testosterone functions as a regulator of leptin, and Elbers et al demonstrated that suppression of testosterone in men leads to an increase of leptin to levels similar to those of women. Furthermore, other leptin regulators such as proopiomelanocortin contain estrogen- and testosterone-responsive elements. However, additional studies are needed to clarify the mechanisms of how endogenous hormones influence leptin levels in the SNS.

In the current study, we extend the previous reports of LEP variant influence on blood pressure by demonstrating that the effect is dependent on both sex and menopausal status. It is possible that previous studies (eg, Gaukrodger et al) might have failed to find association in the overall data because of the group specificity of the association. Previous studies demonstrating male-specific association of LEP variants with BMI, as well as the dependence on menopausal status, suggest a possible interaction between leptin and endogenous hormones.

Perspectives
In the current study, we identified significant or suggestive associations between LEP SNPs and leptin levels with blood pressure and hypertension in women, specifically among postmenopausal women. The results suggest a role for LEP variants in blood pressure regulation that appear to be subject to hormonal milieu and provide insight into the mechanisms involved in the gender differences of hypertension.

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Disclosures
None.

References


