Sex and Body Mass Index Specific Regulation of Blood Pressure by CYP19A1 Gene Variants

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Abstract—Sexual dimorphism in blood pressure (BP) regulation has been observed both in humans and experimental animals, and estrogens have been shown to contribute to this epidemiological observation. A key enzyme in determining estrogen levels is aromatase cytochrome P450. The aim of this study was to evaluate the role of the gene encoding aromatase, CYP19A1, as an independent risk factor for hypertension and its relationship with systolic and diastolic BP measures. We genotyped 2 polymorphisms within the CYP19A1 gene, IVS4 rs11575899 and 3′UTR rs10046, in 3448 individuals. In quantitative analysis, we observed significant associations between the 2 polymorphisms and BP values in women, being these associations dependent on BMI and independent of menopause status. The case–control analysis revealed that the most prominent associations were found for nonobese women in diastolic hypertension (DHT): the IVS4_22 and 3′UTR_11 are risk genotypes (OR=1.61, P=0.027 and OR=1.59, P=0.012, respectively), whereas IVS4_11 and 3′UTR_22 genotypes have a protective effect against DHT (OR=0.63, P=0.009, and OR=0.61, P=0.020, respectively). Haplotype analysis confirmed the above associations: among nonobese women the haplotype 21 is overrepresented in hypertensive women (OR=1.33, P=0.004, for DHT and OR=1.25, P=0.026, for systolic hypertension, SHT) and, conversely, the haplotype 12 protects against hypertension (OR=0.78, P=0.015 for DHT and OR=0.82, P=0.04 for SHT). Our study has shown that the CYP19A1 gene may be involved in the genetic regulation of BP in women. This effect is dependent on BMI and independent of menopause status, suggesting that this action is mainly driven by aromatase activity in fat tissue. (Hypertension. 2007;50:884-890.)

Key Words: essential hypertension ■ body mass index ■ genetics ■ estrogens ■ gender

Essential hypertension (EH) is the most common cardiovascular disease, with a prevalence of nearly 27% worldwide.1 EH is a major risk factor for stroke, heart disease, and end-stage renal disease. High arterial blood pressure is a complex and multifactorial disorder that results from the interaction of multiple genetic and environmental influences.2 Genetic factors account for 30% to 50% of interindividual variability in blood pressure (BP),3–5 and there is evidence suggesting that each of the polygenes contributing to hypertension has only a modest effect.

Sexual dimorphism in the regulation of BP has been demonstrated in several population studies6,7 and in experimental animal models.7 Age-adjusted BP is consistently higher in men than women, but these differences are attenuated when women enter menopause.8 These findings suggest the presence of distinct mechanisms of BP regulation in males and females, emphasizing the importance of the sex hormonal levels in determining BP. Thus, genes involved in testosterone-estradiol shunt are strong candidates to explain, at least in part, the genetic influence on hypertension. The aromatase cytochrome P450 is the enzyme responsible for catalyzing the final step of conversion of androgens into estrogens.9 The importance of this enzyme in BP regulation has been highlighted in animal studies. Aromatase inhibitors have antihypertensive effects in rats with genetic and experimental hypertension.10,11 Thus, genetic variations in CYP19A1, the gene encoding aromatase, might contribute to alterations in aromatase expression and enzyme activity,
which are related to the estrogens/androgens balance. We and other researchers have recently shown that common polymorphisms of the aromatase gene (rs11575899 and rs10046) are associated with estradiol and androgen serum levels in premenopausal and postmenopausal women.13–16 Furthermore, it has been reported that the effect of these genetic variants of CYP19A1 gene are dependent of body fat accumulation in women.16,17 In men and postmenopausal women, when ovarian estrogen synthesis has concluded, adipose tissue becomes the main source of estrogen and, consequently, circulating estrogen levels are correlated with body mass index (BMI).18,19

There are only a few studies analyzing the role of different CYP19A1 markers in hypertension, and their results are inconsistent. Two independent groups reported no significant association between BP and variation in CYP19A1 gene in 2 population-based studies comprising 270 women and 729 individuals, respectively.16,20 Recently, a study including 1780 unrelated participants from the Framingham Heart Study’s offspring cohort21 found suggestive evidence of gender-specific contributions of CYP19A1 gene to BP variation.

Given the role of CYP19A1 alleles in androgen/estrogen levels, we aimed at investigating the role of CYP19A1 gene as an independent risk factor for hypertension and its relationship with systolic (SBP) and diastolic (DBP) blood pressure measures. With this purpose, we genotyped 2 intragenic polymorphisms, IVS4 rs11575899 and 3′ UTR rs10046, in 3448 individuals. This article presents a comprehensive set of results for the association between variations in CYP19A1 gene and BP in the Spanish population.

Methods

Subjects

The quantitative studies of hypertension were performed in the Segovia and VIVA cohorts (n=2474), which are unrelated Spanish men (n=1140) and women (n=1334) recruited by a simple random sampling approach from a cross-sectional population-based epidemiological survey to describe the prevalence of cardiovascular risk factors in general population.22,23 Clinical characteristics of study subjects are summarized in Table 1.

The case-control study of hypertension included 1682 unrelated patients with hypertension and 1766 unrelated individuals with normal values of BP. Among the patients with hypertension, 974 were recruited at outpatient clinics from a multicenter study, and 708 patients, as the 1766 control individuals, were from Segovia and VIVA cohorts. The referral centers involved in this research are Hospital Universitario de Valme (Sevilla), Hospital Universitario de la Princesa (Madrid), Hospital Clínico San Carlos (Madrid), University Hospital Ramón y Cajal (Madrid), and Hospital Clínico San Carlos (Madrid). Informed written consent was obtained from all study participants. The study protocol was designed in accordance with institutional guidelines for human research and was approved by the Ethics Committees of all referral centers.

To perform phenotype-genotype correlations, patients were classified in 2 groups: (1) systolic hypertension (SHT): systolic BP values ≥140 mm Hg, (2) diastolic hypertension (DHT): diastolic BP values ≥90 mm Hg. Systolic and diastolic BPs were measured 3 times in the seated position after 10 minutes of rest to the nearest digit by use of a random-zero sphygmomanometer.

Genotyping

We obtained 5 mL of peripheral blood from all patients and controls to isolate germline DNA from leukocytes. DNA extraction was performed in a MagNa Pure LC Instrument (Roche Diagnostics) according to the manufacturer’s instructions. To perform polymerase chain reactions (PCRs), we prepared aliquots of DNA at a concentration of 5 ng/μL. The rest of the stock was cryopreserved at −20°C.

We selected 2 common bi-allelic polymorphisms of the CYP19A1 gene (MIM 107910) with a high heterozygosity value (Hz > 47.1%): a 3′ untranslated region (3′ UTR) C>T change (rs10046) and the deletion/insertion polymorphism located in the intron 4, IVS4 [-TCT][rs11575899]. PCRs were run on a thermal cycler machine (MJ Research Inc) using a final volume of 20 μL. The genotypes were carried out by using the pyrosequencing technology protocols.24 The selected primers for pyrosequencing analysis are shown in Table S1 (available online at http://hyper.ahajournals.org). The pyrosequencing machine was programmed in accordance with the manufacturer’s recommendation (Biotage).

Statistical Analysis

To analyze deviation from Hardy-Weinberg equilibrium, we used tests adapted from Sasieni25 at the online resource available at the Institute for Human Genetics, Munich, Germany (http://ihg.gsdf.org). For genotypic association analysis of qualitative traits, we used logistic regression analysis. To assess homogeneity of odds ratio (OR) between the different population subgroups analyzed, we introduced an interaction term in the logistic regression. Common odds ratio was estimated by logistic regression or Mantel-Haenszel stratified analysis. In both cases, we have employed SPSS software (Ver. 13.0.0., LEAD Technologies, Inc).

For genotypic association analysis of quantitative traits, we performed an analysis of variance using the GLM procedure included in the SPSS software. Normality of the dependent variables was assessed by Kolmogorov-Smirnov test. The Levene test of equality of error variances was also performed for each analysis. In addition, only individuals untreated for hypertension and, in the case of women, not taken hormonal replacement therapy were included in the quantitative analysis (n=2058).

The estimation of linkage disequilibrium (LD) between both polymorphisms and the qualitative haplotypic analyses were performed using THESIAS software, based on the SEM algorithm (GeneCanvas [http://ecgene.net/genecanvas/news.php]26). This software allows the estimation of haplotypic frequencies and effects by comparison to a reference haplotype (haplotype 11 in our study). We have also performed a regression-based haplotype association test using Whap software (Purcell laboratory [http://pngu.mgh.harvard.

### Table 1. Clinical Characteristics of Subjects From Population-Based Cohort Included in the Quantitative Analysis

<table>
<thead>
<tr>
<th>Cohort (sample size)</th>
<th>Women (1053)</th>
<th>Men (1005)</th>
<th>All (2058)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.2±9.7</td>
<td>49.8±10.2</td>
<td>49.5±9.9</td>
</tr>
<tr>
<td>Physically active (%)</td>
<td>490 (46.5)</td>
<td>443 (44.1)</td>
<td>933 (45.3)</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>535 (50.8)</td>
<td>838 (83.4)</td>
<td>1373 (66.7)</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>201 (19.1)</td>
<td>432 (43.0)</td>
<td>633 (30.8)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.2±4.5</td>
<td>27.3±4.4</td>
<td>27.2±4.0</td>
</tr>
<tr>
<td>Waist, mm</td>
<td>840.3±100.4</td>
<td>940.3±91.0</td>
<td>890.2±110.4</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.6±1.1</td>
<td>5.4±1.5</td>
<td>5.2±1.3</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>122±18</td>
<td>125±17</td>
<td>123±18</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>76±10</td>
<td>79±11</td>
<td>79±11</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.0±0.6</td>
<td>1.5±1.2</td>
<td>1.3±0.9</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.5±1.0</td>
<td>5.7±1.1</td>
<td>5.6±1.1</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.5±0.4</td>
<td>1.3±0.4</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.5±0.9</td>
<td>3.8±0.97</td>
<td>3.6±1.0</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure. The results are mean±SEM.
edu/~purcell/) for the quantitative haplotype analysis and to confirm results obtained by the SEM approach. This software estimates haplotype effects by comparison with the remaining haplotypes.

All the studies were adjusted for age, BMI, smoking (defined as present or past history of smoking of at least five cigarettes per day for a minimum of 5 years), alcohol consumption (defined as a daily intake of more than 10 g), and physical activity. Sex adjustment was also used in the combined analyses. Interaction effects of cohort, age, sex, BMI, and menopause were explored. Women with >1 year from their last menstrual period were considered to have entered menopause.

The sample size is adequate to detect a SNP or haplotype with a modest effect (power is 0.886 for OR = 1.3 or higher). The statistical significance threshold was established in 0.05. We have not applied correction for multiple comparisons because we consider it very conservative.

Results

Genotype Analysis

Genotypes were determined in all 3448 individuals. The observed allelic frequencies in Spanish general population were 0.61 for allele 1 (alleles Ins [TCT]) in IVS4 and 0.51 for allele 1 (alleles C) in 3’UTR. All genotype frequencies observed during this study are in accordance with the Hardy-Weinberg equilibrium law.

We carried out quantitative genotype association analysis (QTL) of the 2 selected markers of CYP19A1 gene with SBP and DBP levels in the population-based sample (n = 2058, 1005 men and 1053 women). In combined general population analysis, no significant association was observed, but we detected statistically significant interaction with sex and BMI for BP phenotypes, whereas no interaction effect of cohort, age, or menopause status was detected (P > 0.11).

Only in women, we found significant differences in DBP values associated with the genotype distribution of the 2 markers (IVS4 2df, P = 0.008 and 3’UTR df2, P = 0.032; Table 2). Homozygous presence of IVS4 allele 2 was associated with higher diastolic levels (P = 0.003, mean ± error: IVS4_22, 78.04 ± 0.73 and IVS4_11 + IVS4_12, 75.68 ± 0.39). Homozygotes for 3’UTR allele 1 also have increased DBP levels (P = 0.008, mean ± error: 3’UTR_11, 77.29 ± 0.58 and 3’UTR_12 + 3’UTR_22, 75.63 ± 0.41). In addition, we found that the effect of these genotypes was modified by BMI in women (P ≤ 0.025), so we stratified the analysis by the presence/absence of obesity. In this study, we found that the association was especially apparent in women with BMI < 30, for IVS4_22 and 3’UTR_11 genotypes (P = 0.026, mean ± error: 76.18 ± 0.86 versus 74.17 ± 0.41, and P = 0.005, 75.96 ± 0.66 versus 73.93 ± 0.43; respectively), and there was not significance in obese women (BMI ≥ 30).

To confirm these results, we performed a case-control study of hypertension including 1682 cases and 1766 controls. As in QTL analysis, we observed significant differences in the genotypes distribution of polymorphisms between hypertensive patients and controls only in women, being this association dependent on BMI values (Table S2). The greater differences were found in nonobese women with DHT: the IVS4_22 and 3’UTR_11 genotypes were overrepresented in controls (OR = 1.61, P = 0.02 and OR = 1.59, P = 0.012, respectively), and conversely, the IVS4_11 and 3’UTR_22 genotypes were underrepresented in cases compared with controls (OR = 0.63, P = 0.009 and OR = 0.61, P = 0.020, respectively). In SHT, just the 3’UTR_11 genotype was associated in nonobese women (OR = 1.52, P = 0.025). In obese women, the 3’UTR_11 genotype was associated with DHT although the effect was opposite to those observed in nonobese women: it has a risk effect in nonobese women and a protective effect in obese women (OR = 1.59, P = 0.012 and OR = 0.59, P = 0.017, respectively) (Figure 1).

Given the high correlation between diastolic and systolic hypertension, these associations remain significant when we consider women with both pressures elevated (data not shown). We have not found evidence of association of CYP19A1 genotypes with hypertension in men.

Haplotype Analysis

Haplotype information can be of great interest for investigating the role of a candidate gene in the etiology of complex diseases. The two examined polymorphisms are at the same block of Linkage Disequilibrium (D’ = −0.94, P < 0.0001). The haplotype frequencies in general population were: 49.1% for haplotype 12 (IVS4 allele 1 and 3’UTR allele 2), 38.4% for haplotype 21 (IVS4 allele 2 and 3’UTR allele 1) and 12.5% for haplotype 11 (allele 1 for both markers). To minimize loss of power, a fourth haplotype (haplotype 22) with a frequency of only 1% was excluded from the analysis.

The quantitative haplotype analysis of BP levels was performed in the general population-based sample (Table 3). According to the quantitative genotype analysis, significant association between diastolic and systolic pressure measure-

<table>
<thead>
<tr>
<th>Marker</th>
<th>Genotype</th>
<th>n</th>
<th>SBP</th>
<th>DBP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP19A1 IVS4</td>
<td>11</td>
<td>388</td>
<td>124.45 ± 0.91</td>
<td>78.84 ± 0.58</td>
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<td></td>
<td>12</td>
<td>465</td>
<td>124.93 ± 0.87</td>
<td>79.21 ± 0.56</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>152</td>
<td>124.13 ± 1.33</td>
<td>78.52 ± 0.86</td>
</tr>
<tr>
<td>CYP19A1 UTR</td>
<td>11</td>
<td>239</td>
<td>124.45 ± 1.10</td>
<td>78.54 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>506</td>
<td>124.61 ± 0.83</td>
<td>79.23 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>260</td>
<td>124.54 ± 1.07</td>
<td>78.67 ± 0.69</td>
</tr>
</tbody>
</table>

Analysis adjusted for age, BMI, smoking, alcohol, and physical activity. SBP indicates systolic blood pressure; DBP, diastolic blood pressure.*P = 0.044; †P = 0.003; ‡P = 0.055; §P = 0.008. The SBP and DBP values are mean ± SEM.
ments and haplotype distribution, after adjustment for covariates (age, BMI, smoking, alcohol, and physical activity) was observed only in women, whereas no significant association was found in men. In women, the test of a global haplotypic association with DBP levels was significant (P<0.034, 2 d.f.) and only a trend for association with SBP levels was detected (P=0.074, 2 d.f.). The haplotype 21 was associated with a significant increase of diastolic and systolic values (P=0.009 and P=0.015, respectively) and, in contrast, haplotype 12 was associated with a reduction of both values (P=0.007 and P=0.039, respectively).

In the case-control haplotype analysis, once again, the highest global haplotypic effect was found for DHT in women with BMI <30 (χ²=8.71 2d.f., P=0.012) (Table S3). The haplotype 21 is overrepresented in nonobese women with DHT and SHT compared with nonobese women with normal SBP and DBP values (OR=1.33, P=0.004; OR=1.25, P=0.026, respectively). Conversely, the haplotype 12 has a protective effect against DHT and SHT in nonobese women (OR=0.78, P=0.015; OR=0.82, P=0.04, respectively). In obese women we observed the opposite effect than in nonobese women, the haplotype 21 confers a protective effect against hypertension (SHT: OR=0.75, P=0.026; DHT: OR=0.77, P=0.04), and the haplotype 12 confers risk for hypertension, but it did not reach the statistical significance level (Figure 2).

Thus, hypertension risk associated with these haplotypes is dependent on sex and BMI status.

### Discussion

CYP19A1 is a key gene in determining androgen and estrogen levels. This important function is being investigated at the genetic level in multiple complex diseases suspicious to be influenced by estrogens. However, relatively little is known regarding the relationship of CYP19A1 variants on BP. We conducted a quantitative analysis in general population and a case-control analysis of hypertension using 2 variants in the CYP19A1 gene. In the quantitative analysis, we observed a significant interaction with sex and BMI for BP levels, but we did not detect interaction with age or menopause status. In

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Adjusted Mean</th>
<th>CI</th>
<th>Adjusted Mean</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12</td>
<td>0.491</td>
<td>122.21</td>
<td>118.20–126.23</td>
<td>82.14</td>
<td>79.66–84.63</td>
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<td>21</td>
<td>0.384</td>
<td>122.45</td>
<td>118.49–126.41</td>
<td>82.32</td>
<td>79.88–84.78</td>
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<tr>
<td>11</td>
<td>0.125</td>
<td>124.68</td>
<td>120.74–128.62</td>
<td>82.59</td>
<td>80.16–85.02</td>
</tr>
</tbody>
</table>

**Global test (2 df) P=0.287**

**Global test (2 df) P=0.239**

**Men**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Adjusted Mean</th>
<th>CI</th>
<th>Adjusted Mean</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.503</td>
<td>120.69</td>
<td>116.24–125.14</td>
<td>79.75</td>
<td>76.71–82.80</td>
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<tr>
<td>21</td>
<td>0.374</td>
<td>120.71</td>
<td>116.34–125.06</td>
<td>79.89</td>
<td>76.91–82.87</td>
</tr>
<tr>
<td>11</td>
<td>0.123</td>
<td>120.68</td>
<td>116.41–124.98</td>
<td>79.64</td>
<td>76.70–82.59</td>
</tr>
</tbody>
</table>

**Global test (2 df) P=0.871**

**Global test (2 df) P=0.822**

**Women**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Adjusted Mean</th>
<th>CI</th>
<th>Adjusted Mean</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.481</td>
<td>112.03*</td>
<td>107.57–116.49</td>
<td>74.87†</td>
<td>72.36–77.39</td>
</tr>
<tr>
<td>21</td>
<td>0.393</td>
<td>112.41†</td>
<td>108.09–116.70</td>
<td>75.08§</td>
<td>72.64–77.52</td>
</tr>
<tr>
<td>11</td>
<td>0.126</td>
<td>113.80</td>
<td>109.57–118.02</td>
<td>76.02</td>
<td>73.64–78.40</td>
</tr>
</tbody>
</table>

**Global test (2 df) P=0.074**

**Global test (2 df) P=0.034**

Adjusted for sex age, body mass index (BMI), alcohol consumption, smoking, and physical activity. *P=0.039; †P=0.015; §P=0.007; ††P=0.009.

Figure 1. Odds ratio (95% confidence interval) for the association between the IVS_22 and UTR_11 genotypes vs the IVS_11+IVS_12 and UTR_12+UTR_22 genotypes of the CYP19A1 gene and hypertension in women stratified by the presence or absence of obesity. A, DHT; B, SHT.
this population, CYP19A1 variants are not associated with BMI (data not shown). Interestingly, we observed statistically significant associations between the 2 markers in CYP19A1 gene and BP in women. This association was especially apparent in DBP and genotype effects were BMI-dependent. In the case-control study, we stratified the population by both sex and BMI status. Genotype analysis revealed that the most prominent associations were found for nonobese women (BMI <30) with diastolic hypertension: the IVS4_22 and 3'UTR_11 are risk genotypes, and conversely, the IVS4_11 and 3'UTR_22 genotypes confer a protective effect against DHT. Haplotype analysis confirmed the above associations, the haplotype 21 is a risk haplotype and the haplotype 12 is a protective haplotype in nonobese women for both DHT and SHT. Nevertheless, the haplotype 21 was associated with both SHT and DHT in obese women (BMI ≥30), but with opposite effect.

We found no evidence of CYP19A1 gene variants being associated with risk of hypertension in men. Our data are in accordance with those previously reported in the Framingham Heart Study by Peter et al, which found suggestive evidence of gender-specific contributions of polymorphisms in the CYP19A1 gene to DBP variation in women. However, the weak association observed in women could be due to the fact that the authors did not analyze the BMI-specific contribution of CYP19A1 variants or a smaller sample size.

The incidence of cardiovascular disease among women is low before menopause, but increases afterward. This phenomenon is believed to result in part from the reduction of estrogen levels caused by the loss of the gonadal function, and indicates that estrogen may play an important role in the prevention of cardiovascular disease in women. However, aromatase deficiency or inhibition causes lower BP in animal models. Aromatase-deficient (ArKO) female mice, which are deficient in estrogens because of the deletion of the aromatase gene, have lower DBP than wild-type female mice. The mechanisms by which estrogens affect BP are thus complex and multifaceted, involving direct effects on vascular, renal, and heart cells, as well as indirect effects mediated by humoral and hormonal factors. In the last 2 decades, several studies have revealed original signaling mechanisms by which estrogen receptors also trigger rapid actions of steroid hormones that are determined outside the nuclear compartment. These rapid signaling actions are independent of the synthesis of mRNA or protein and, therefore, are known as nongenomic actions as opposed to the classical genomic mechanisms.

Nongenomic signaling of estrogens plays a prominent role in nonreproductive tissues, and is particularly important at the vascular endothelium. At this level, estrogens activate rapid vasodilatation, exert antiinflammatory effects, stimulate endothelial growth and migration, and protect the vessels from atherosclerotic degeneration by elevating nitric oxide and prostaglandin levels. In addition, estrogens inhibit the vasoconstrictor pathway mediated by the sympathetic nervous system and renin-angiotensin system.

We have shown that the CYP19A1 gene may be involved in the genetic regulation of BP in women and that this gene may have different roles depending on BMI. In a previous study, we reported that the effect of these two CYP19A1 variants in osteoporosis was also modified by BMI. The independence of the effect of the CYP19A1 variants on BP from menopause status suggests that it is mainly related to aromatase activity in fat tissue.

A plausible explanation for the fact that the same genetic variants have opposite effect in nonobese women and in obese women could be that the fat tissue acts as a modulating factor. Estrogen biosynthesis in adipose tissue is driven by an adipose-specific promoter (distal promoter I.4) and regulated by hormones, class I cytokines, and growth factors. Among these, there are several molecules that have been shown to play an important role in determining obesity. Aromatase expression in adipose tissue has been shown to be regulated by leptin, a hormone produced in this tissue that regulates body weight which is increased in obese individuals. Interestingly, leptin levels are gender-dependent, being higher in women than in men. Adiponectin, a protein synthesized in the adipose tissue which is deficient in obese individuals, has been reported to inhibit aromatase transcription. Other factor that stimulates aromatase expression in adipose stromal cells is tumor necrosis factor (TNF) alpha. In addition to the above cited factors, the peroxisome proliferator-activated receptor-γ (PPARγ) nuclear receptor, a key protein in adipogenesis, may be another important modulator of estrogen production, because ligands for PPARγ inhibit aromatase expression via the specific fat tissue promoter I.4 of the CYP19A1 gene. Thus, the differential levels of these molecules in obese and nonobese women could underly the observed opposite effects driven by aromatase gene variants. Gene-gene interaction analysis and functional studies are necessary to shed more light on the effect of CYP19A1 variants in adipose tissue site-specific
regulation. The mechanisms that regulate arterial pressure comprise one of the most complex physiological systems and it must be expected that a full understanding of the genetic basis of hypertension will not be quickly reached.

Although the polymorphisms we studied do not induce changes in the amino acid sequence of aromatase, we cannot rule out that these variants could have functional consequences. It is also possible that these markers can be in linkage disequilibrium with another causal polymorphism situated within the adipose-specific promoter region. Further studies analyzing a larger number of SNPs distributed throughout the promoter region and functional studies to determine their relationship on gene expression are needed to clarify the role of aromatase in BP regulation. In addition, we cannot discard the possibility that a type I error occurs in our results, although it probably is not given the large size and the homogeneity of the studied population.

Our results have shown that CYP19A1 gene may be involved in the genetic regulation of blood pressure in women. Interestingly, this association is dependent on BMI suggesting that it could be due to a differential aromatase activity in fat tissue.

Perspectives
This article presents a comprehensive set of results in the largest series used to date, providing evidence of BMI and sex-specific contributions of CYP19A1 gene to BP variation. Our results underscore the importance of CYP19A1 gene in hypertension in women and represent a step toward identification of genes and pathophysiological pathways of relevance for essential hypertension. However, the direct or indirect effects of these markers in BP levels remain to be determined. A confirmation of our findings in independent populations and functional studies are necessary to shed more light on the role of CYP19A1 gene in BP regulation and the incidence of hypertension.

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Disclosures
None.

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