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Association of renin-angiotensin and endothelial nitric oxide synthase gene polymorphisms with blood pressure progression and incident hypertension: prospective cohort study

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Abstract

Objective—The renin-angiotensin system and endothelial function have both been associated with hypertension. The aim of the present study was to assess the relationship of six previously characterized gene variants in the renin-angiotensin system and the *NOS3* gene with blood pressure progression and incident hypertension.

Methods—We analyzed data from 18436 Caucasian women who participated in a prospective cohort study and were free of hypertension at baseline. Six previously characterized single nucleotide polymorphisms (*NOS3* rs1800779, *NOS3* rs3918226, *NOS3* rs1799983, *ACE* rs1799752, *AGT* rs699, and *AGTR1* rs5186) were genotyped. Blood pressure progression at 48 months and incident hypertension during the entire follow-up according to the different genotypes and inferred haplotypes were assessed by logistic regression and Cox proportional-hazards models, respectively.

Results—At 48 months, 47.4% of the women had blood pressure progression. The odds ratios (95% confidence intervals (CI)) for blood pressure progression associated with *NOS3* rs1800779, *NOS3* rs3918226, *NOS3* rs1799983, *ACE* rs1799752, *AGT* rs699, and *AGTR1* rs5186 were 1.00 (0.96–1.05), 1.00 (0.92–1.09), 0.99 (0.94–1.04), 0.96 (0.92–1.01), 1.04 (0.99–1.08), and 1.03 (0.98–1.08). During 9.8 years of follow-up, 29.6% of women developed incident hypertension. The hazard ratios (95% CI) for the six polymorphisms were 1.01 (0.97–1.06), 1.06 (0.99–1.14), 1.05 (1.01–1.09), 0.99 (0.95–1.02), 1.01 (0.97–1.05) and 0.99 (0.95–1.04). *NOS3*-haplotypes were not significantly associated with blood pressure progression (p=0.91) or incident hypertension (p=0.10).

Conclusion—Blood pressure progression and incident hypertension are not consistently associated with six well-characterized genetic polymorphisms of the renin-angiotensin system and the *NOS3* gene in a large cohort of Caucasian women.

Keywords

Blood pressure; Hypertension; Gene polymorphism; Nitric oxide synthase; Angiotensin-converting enzyme; Renin-angiotensin System

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Introduction

In a Western population, the cumulative lifetime risk of developing hypertension approaches 90% (1). Although obesity and other environmental factors (2) substantially contribute to the high incidence of hypertension, twin studies suggest that in human beings a significant part of the inter-individual variability of blood pressure is heritable (3). However, genetic studies of multifactorial disorders such as hypertension have proven difficult due to the multiplicity of genes underlying complex phenotypes and the modest effect of individual polymorphisms or genes.

Endothelial function is closely associated with blood pressure and hypertension (4,5), and several studies found endothelial dysfunction in normotensive siblings of hypertensive individuals (6,7). Endothelial nitric oxide synthase (eNOS), an enzyme that generates nitric oxide, is a major determinant of endothelial function (8), suggesting that polymorphisms in the eNOS gene (*NOS3*) may influence the likelihood of blood pressure progression or incident hypertension in an individual person.

Similarly, the renin-angiotensin system plays a central role in blood pressure regulation (9). Angiotensin II is a powerful vasoconstrictor and stimulates the reabsorption of sodium in the kidney. Chronic production of angiotensin II may result in remodeling and restructuring in various cardiovascular organs (9). Several genetic variants in the renin-angiotensin system have been associated with functional differences in the corresponding genes, contributing up to 50% of the phenotypic variation (10–13). Genetic polymorphisms of the renin-angiotensin system may therefore be implicated in the pathogenesis of arterial hypertension.

In this context, we assessed the relationship of six previously characterized gene variants in the *NOS3* gene and the renin-angiotensin system with blood pressure progression and incident hypertension in a large cohort of prospectively followed Caucasian women.

Methods

Participants

All study subjects were participants of the Women's Health Study, a completed randomized trial evaluating the risks and benefits of low dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer. Details of the study design have been described previously (14–16).

Briefly, information on baseline variables was collected using mailed questionnaires. Follow-up questionnaires asking participants about study outcomes and other information were sent every six months during the first year and every 12 months thereafter. Follow-up information from randomization through the end of the trial, March 31, 2004 was used for this analysis. For the present study, we included 18436 Caucasian women who were free of hypertension and did not receive antihypertensive drugs at baseline, and who had all six genotypes of interest determined. Median follow-up for this sample population was 9.8 years (interquartile range 6.6–10.5 years).

Study variables

Blood pressure at randomization was self-reported by the female health professionals, a group where self-report of blood pressure has proven highly accurate (17–19). Participating women categorized their blood pressure levels into nine categories of systolic blood pressure and seven categories of diastolic blood pressure. For the purpose of this study, women were classified into three predefined blood pressure categories: below 120 mmHg for systolic and 75 mmHg for diastolic blood pressure; 120 to 129 mmHg for systolic or 75 to 84 mmHg for diastolic

blood pressure; and 130 to 139 mmHg for systolic or 85 to 89 mmHg for diastolic blood pressure (20). Women with discordant systolic and diastolic blood pressure categories were classified into the higher category.

Covariates of interest were ascertained at study entry and included age, smoking, history of hypercholesterolemia (self-reported cholesterol of at least 240 mg/dl (6.22 mmol/l)), body mass index (weight in kilograms divided by the square of height in meters), history of diabetes, exercise, alcohol consumption, highest education level achieved, hormone replacement use and menopausal status at baseline.

Outcome assessment

First, we assessed blood pressure progression at 48 months. For this analysis, we created categories of self-reported blood pressure at 48 months of follow-up identical to those at baseline. Blood pressure progression was defined by progressing at least one blood pressure category compared to baseline, or by a new diagnosis of hypertension during the first 48 months. Blood pressure information at 48 months was missing in 1874 women, and 182 women with complete blood pressure information had a cardiovascular event or died during the first 48 months of follow-up. After excluding these women, 16380 participants remained in the analysis for blood pressure progression.

Second, we assessed cases of incident hypertension during the entire follow-up period of 9.8 years. Incident cases of hypertension were defined by meeting at least one of the following criteria: self-report of a new physician diagnosis of hypertension assessed at years 1, 3 and yearly thereafter; self-report of antihypertensive treatment assessed at years 1, 3 and 4; or self-reported systolic blood pressure of at least 140 mmHg or diastolic blood pressure of at least 90 mmHg.

Women reporting a new physician diagnosis of hypertension also provided month and year of diagnosis. For a diagnosis defined by another criterion or a missing date for a physician diagnosis, a date between the current and the previous questionnaire was randomly assigned. Women who developed cardiovascular disease for which the management may affect blood pressure levels, were censored at the date of diagnosis and not considered at risk for incident hypertension thereafter. All 18436 women were included in the incident hypertension analyses.

Genotype determination

We analyzed six previously described polymorphisms: rs1799752 (I/D) within the *ACE* gene, rs699 (235 T>C) within the angiotensinogen (*AGT*) gene, rs5186 (1166 A>C) within the angiotensin II type 1 receptor (*AGTR1*) gene, and three genetic polymorphisms within the *NOS3* gene: rs1800779, rs3918226 and rs1799983 (894 G>T). These polymorphisms were part of a larger panel of candidate gene polymorphisms, selected from biochemical pathways implicated in the development and progression of cardiovascular disease (21). Genotyping was performed in the context of a multimarker assay using an immobilized probe approach, as previously described (Roche Molecular Systems, Alameda, CA) (21). In brief, each DNA sample was amplified by polymerase chain reaction (PCR) with biotinylated primers. Each PCR product pool was then hybridized to a panel of sequence-specific oligonucleotide probes immobilized in a linear array. The colorimetric detection method was based on the use of streptavidin–horseradish peroxidase conjugate with hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine as substrates. Genotype assignment was performed using proprietary Roche molecular systems image processing software. To confirm genotype assignment, scoring was carried out by two independent observers. Discordant results (<1% of all scoring) were resolved by a joint reading, and where necessary, a repeat genotyping.

Statistical analysis

We calculated allele frequencies and performed a Hardy–Weinberg equilibrium test using the Fisher probability test statistics. Baseline characteristics according to the different genotype groups were compared using chi square tests for categorical variables and ANOVA for continuous variables.

Next, we performed logistic regression analysis to assess the association between blood pressure progression at 48 months and each of the six genetic variants. Separate models were constructed for each genotype, always assuming an additive model. To test the validity of the additive model assumption we compared the additive models to models with two indicator variables for allele heterogeneity and minor allele homogeneity using likelihood ratio chi square tests. The assumption was not rejected for any model. The common wild type was used as the reference group. In a first step, age-adjusted models are presented. Thereafter, we fitted multivariable models adjusting for age, smoking, baseline blood pressure category, history of diabetes, body mass index, history of hypercholesterolemia, exercise, alcohol consumption, highest education level, hormone replacement therapy, baseline menopausal status and randomized treatment assignments (aspirin, vitamin E and beta carotene).

We calculated age-adjusted incidence rates for every genetic variant using internal standardization. Subsequently, we fitted Cox proportional-hazards models to compare the risk of incident hypertension during the entire follow-up period across the six genotype groups. The additive model assumption was again found to be valid for all models. For multivariable adjustment, we used the same variables as described above.

To further assess the effect of the six genetic variants, we stratified the overall study population in three groups according to baseline blood pressure category. Subsequently, we repeated all regression analyses described above within each blood pressure stratum. Differences according to baseline blood pressure category were also assessed by including baseline blood pressure by genotype interaction terms into the non-stratified models. The significance of the interaction was assessed by comparing the likelihood ratio with and without the interaction terms in the model.

For the three *NOS3* genotypes, haplotype estimation and inference was determined using PHASE v. 2.1.1 (22–24). Subsequently, the same multivariable regression models as described above were constructed using inferred haplotypes as the predictor of interest. We pre-specified that haplotypes with an estimated frequency <0.01 would not be analyzed individually, and only women with an inferred haplotype probability of >0.80 were retained for these analyses. Statistical significance was based on the difference of the likelihood ratio with and without all haplotype indicator variables in the fully adjusted models. The most common haplotype was used as reference category for all analyses.

Categorical variables were entered in the regression models using binary indicator variables. The proportional hazards assumption was examined for all models by including a genotype by logarithm of time interaction into the model (25). The p value of this interaction was of nominal statistical significance for two genotype models (p=0.02 for *ACE* rs1799752, and p=0.02 for *NOS3* rs1799983). Given the large power of this study to detect small deviations from proportional hazards, we did not consider these p values as evidence for a significant violation of this assumption. All analyses were carried out using SAS version 9 (SAS Institute Inc, Cary, NC). A two-tailed p value <0.05 was considered to indicate statistical significance. P values are not adjusted for multiple testing.

Results

Baseline characteristics of the 18436 women are shown in Table 1. Across all six genotypes considered in this study, there was only one of these baseline variables with significant differences across at least one polymorphism: Women with the *NOS3* rs1800779 GG genotype had a significantly lower body mass index compared to women with the AA or the AG genotype (24.9 kg/m^2 versus 25.2 kg/m^2 and 25.1 kg/m^2, p=0.02).

Allele and genotype frequencies are shown in Table 2. Allele frequencies were above five percent for all polymorphisms under study. The genotype distribution of the *ACE* rs1799752 variant was not in Hardy-Weinberg equilibrium (p<0.001). We repeated genotyping of this polymorphism in a representative subgroup of our cohort and did not find genotyping errors that might be responsible for this finding (data not shown).

At 48 months of follow-up, 7756 out of 16380 women (47.4%) had blood pressure progression. The risk of blood pressure progression across the six genotypes after adjustment for age and other potential confounders is shown in Table 3. None of the genotypes under study was significantly associated with blood pressure progression at 48 months. All confidence intervals were narrow and overlapped 1.0.

In total, 5451 out of 18436 women (29.6%) progressed to hypertension during 9.8 years of follow-up. Age adjusted incidence rates across genotypes ranged from 34.2 events per 1000 person-years to 38.0 events per 1000 person-years, with the exception of women with two copies of the rs3918226 minor allele. In this small group (n=127), the event rate per 1000 person-years was 45.6. The hazard ratios relating each genetic variant to incident hypertension were not significantly different from 1.0 in five of the six genotypes. Only the association between the *NOS3* rs1799983 genotype and incident hypertension reached statistical formal significance (hazard ratio (95% confidence interval) 1.05 (1.01–1.09), p=0.02).

Haplotype analyses for blood pressure progression and incident hypertension were based on 16375 and 18429 women with an estimated haplotype probability >0.80, respectively. Among these women, haplotype frequencies were 0.54, 0.09, 0.14, 0.16 and 0.08 for the A-C-G, A-C-T, G-C-G, G-C-T and the G-T-T haplotypes. The A-T-G, A-T-T and G-T-G haplotypes were rare (<0.01) and not analyzed individually. Multivariable regression models did not reveal a significant association between *NOS3*-haplotypes and the risk of blood pressure progression or incident hypertension during follow-up. Adding indicator variables for the five haplotypes with a frequency >0.01 simultaneously to the multivariable models did not improve model fit for blood pressure progression (p=0.91 by likelihood ratio test) or incident hypertension (p=0.10 by likelihood ratio test), indicating that *NOS3* haplotypes are not associated with either outcome.

To assess whether baseline blood pressure modifies the effect of the genotypes on blood pressure progression and incident hypertension, we stratified our cohort into three groups according to blood pressure at randomization. The relative risks were very consistent and did not depend on baseline blood pressure for both outcome variables analyzed (Table 4). Accordingly, all confidence intervals widely overlapped and none of the blood pressure category by genotype interaction terms was statistically significant.

Discussion

In this large prospective study, we found no evidence of a consistent association between six previously characterized genetic polymorphisms in the *ACE, AGT, AGTR1* or *NOS3* genes and the risk of blood pressure progression or incident hypertension. None of the polymorphisms was associated with blood pressure progression and the only observed association was between

the *NOS3* rs1799983 genotype and the risk of incident hypertension (p=0.02). However, given the large number of statistical tests performed in this study and the absence of an association in the *NOS3*-haplotype analysis, the most likely explanation would be a chance finding. In this context, it is important to note that the present study had enough power to detect small to moderate associations. For incident hypertension, assuming a univariate-additive model, a power of 80% and an alpha level of 0.05, the study had the ability to detect a relative risk of >1.10 if the minor allele frequency is 0.50, and of >1.30 if the minor allele frequency is 0.01.

After the implication of the renin-angiotensin system in the pathogenesis of arterial hypertension (9), multiple association studies have explored the association between genetic variants within this system and the risk of arterial hypertension (26–32). These predominantly small studies have found inconsistent results. A recent meta-analysis suggested a small but significant association between the *AGT* rs699 polymorphism, plasma levels of angiotensinogen and the risk of hypertension (odds ratio (95% confidence interval) for hypertension 1.08 (1.01–1.15) in heterozygotes and 1.19 (1.10–1.30) in minor allele homozygotes) (26). Despite the availability of a similar number of cases, we were unable to confirm these findings. As shown recently for apolipoprotein E genotypes and polymorphisms of the *NOS3* gene, selective publication of small studies with significant findings may introduce substantial bias in meta-analyses of genetic association studies (33,34).

The Framingham Heart Study found significant associations between the *ACE* I/D genotype, blood pressure levels and hypertension among men, but not among women. The present study confirms the lack of an association in women. The potential relationship between the *ACE* I/D genotype and blood pressure or hypertension in men deserves further study. Finally, we were unable to confirm a significant association between the *AGTR1* rs5186 polymorphism and arterial hypertension (30).

Studies on the relationship between *NOS3* gene polymorphisms and arterial hypertension have also reported inconsistent findings (34–40). We were unable to confirm a significant association between three well-characterized *NOS3* gene polymorphisms and blood pressure progression or incident hypertension in this large cohort of Caucasian women. Several *NOS3* polymorphisms and haplotypes have been associated with blood pressure and hypertension, but no consistent patterns emerged (34,35). As documented recently by Pereira et al, the literature on *NOS3* polymorphisms and hypertension has been subject to important heterogeneity and publication bias (34). Therefore, this large, prospective study adds to the literature by showing that three *NOS3* polymorphisms and *NOS3* haplotypes are not consistently associated with the risk of blood pressure progression and incident hypertension. However, we cannot exclude that a more comprehensive haplotype tagging may have detected an association between blood pressure progression or incident hypertension and those *NOS3* haplotypes that were not covered by the present analysis.

Finally, in recent years, genome-wide association studies of complex diseases using 300,000 to 500,000 polymorphisms per individual have become available. The only large genome wide association study for hypertension published so far failed to identify polymorphisms that are significantly associated with hypertension after adjustment for multiple testing (41), highlighting the need for more large scale, carefully designed studies in this important area. In this context, participants of the WGHS are currently undergoing genotyping for more than 360,000 polymorphisms, and more detailed results concerning the association between genetic polymorphisms and hypertension are expected in future analyses (42).

Strengths and limitations

Strengths of the present study are the large sample size, the prospective design and the long-term follow-up with a large number of cases. Potential limitations of our study also require

discussion. First, this study included only Caucasian female health professionals, and our findings may not be generalizable to other populations. Second, we used self-reported blood pressure and hypertension status. The prognostic value of self-reported blood pressure in cohort studies involving US health professionals is similar compared to directly measured blood pressure values in participants of other cohort studies (17). Furthermore, the validity of this approach has been examined in the comparable Nurses' Health Study, where 99% of the women who reported high blood pressure levels had their diagnosis confirmed based on medical record review (18). However, it has to be emphasized that the potential contribution of a single genetic polymorphism to blood pressure progression or the development of hypertension is likely to be small. Therefore, because of the limited precision of self-reported blood pressure assessment in categories at baseline, we cannot exclude that the genetic variants evaluated in the current study do have small effects on blood pressure levels, and more precise measurement techniques may be necessary to unravel them (43).

Conclusion

In this large prospective cohort of Caucasian women, six well-characterized genetic polymorphisms of the renin-angiotensin system and the *NOS3* gene were not consistently associated with blood pressure progression or incident hypertension.

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Table 1

Baseline characteristics

Characteristic	N=18436
Age, years	54 ± 7
Body mass index, kg/m^2	25.1 ± 4.4
History of diabetes (%)	246 (1.3)
History of hypercholesterolemia (%)	4595 (24.9)
Smoking (%)	
Current	2179 (11.8)
Former	6823 (37.0)
Never	9423 (51.1)
Exercise, times/week (%)	
Rarely/never	6472 (35.1)
<1	3651 (19.8)
1–3	6107 (33.1)
>3	2199 (11.9)
Alcohol consumption (%)	
Rarely/never	7678 (41.7)
1–3 drinks per month	2473 (13.4)
1–6 drinks per week	6356 (34.5)
≥1 drink per day	1924 (10.4)
Highest education level (%)	
Less than a bachelor's degree	9804 (54.1)
Bachelor's degree	4470 (24.7)
Master's degree or doctorate	3848 (21.2)
Hormone replacement therapy (%)	
Current	7829 (42.5)
Past	1447 (7.9)
Never	9128 (49.6)
Baseline blood pressure category (%)	
<120/75 mmHg	8188 (44.4)
120–129/75–84 mmHg	7217 (39.2)
130–139/85–89 mmHg	3031 (16.4)

Data are mean ± standard deviation or counts (percentages). Number of women across categories may not sum to the number in total study because of missing data.

Table 2

Allele and genotype frequencies

N=18436	Allele frequencies	Genotype frequencies	p value for HWE
***NOS3* rs1800779**	A = 0.63 G = 0.37	AA = 0.39 AG = 0.47 GG = 0.14	0.19
***NOS3* rs3918226**	C = 0.92 T = 0.08	CC = 0.85 CT = 0.14 TT = 0.01	0.13
***NOS3* 894 G>T (rs1799983)**	G = 0.67 T = 0.33	GG = 0.45 GT = 0.44 TT = 0.11	0.59
***ACE* I/D (rs1799752)**	I = 0.48 D = 0.52	II = 0.24 ID = 0.46 DD = 0.29	<0.001
***AGT* 235 T>C (rs699)**	T = 0.59 C = 0.41	TT = 0.35 TC = 0.48 CC = 0.17	0.36
***AGTR1* 1166 A>C (rs5186)**	A = 0.70 C = 0.30	AA = 0.48 AC = 0.42 CC = 0.09	0.78

Data are proportions

HWE Hardy-Weinberg equilibrium

NOS3 Endothelial Nitric Oxide Synthase

ACE Angiotensin Converting Enzyme

I/D Insertion/Deletion

AGT Angiotensinogen

AGTR1 Angiotensin II type 1 receptor

Table 3

Risk of blood pressure progression and incident hypertension across different genotypes

Polymorphism	BP progression* Odds ratio (95% CI)†	p value	Incident hypertension* Hazard ratio (95% CI)†	p value
***NOS3* rs1800779**				
Age-adjusted	0.99 (0.95–1.04)	0.76	1.00 (0.96–1.04)	0.99
Multivariable adjusted‡	1.00 (0.96–1.05)	0.89	1.01 (0.97–1.06)	0.52
***NOS3* rs3918226**				
Age-adjusted	0.98 (0.91–1.07)	0.70	1.05 (0.98–1.12)	0.17
Multivariable adjusted‡	1.00 (0.92–1.09)	0.97	1.06 (0.99–1.14)	0.09
***NOS3* 894 G>T (rs1799983)**				
Age-adjusted	0.99 (0.95–1.04)	0.73	1.04 (1.00–1.08)	0.05
Multivariable adjusted‡	0.99 (0.94–1.04)	0.77	1.05 (1.01–1.09)	0.02
***ACE* I/D (rs1799752)**				
Age-adjusted	0.97 (0.93–1.01)	0.13	0.99 (0.96–1.03)	0.72
Multivariable adjusted‡	0.96 (0.92–1.01)	0.08	0.99 (0.95–1.02)	0.45
***AGT* 235 T>C (rs699)**				
Age-adjusted	1.03 (0.98–1.08)	0.21	1.02 (0.98–1.06)	0.34
Multivariable adjusted‡	1.04 (0.99–1.08)	0.14	1.01 (0.97–1.05)	0.77
***AGTR1* 1166 A>C (rs5186)**				
Age-adjusted	1.03 (0.98–1.08)	0.26	0.99 (0.95–1.03)	0.61
Multivariable adjusted‡	1.03 (0.98–1.08)	0.21	0.99 (0.95–1.04)	0.77

The multivariable (age-adjusted) blood pressure progression models are based on 7468 (7756) events in 15761 (16380) women; the multivariable (age-adjusted) incident hypertension models are based on 5230 (5451) incident events in 17724 (18436) women.

BP Blood pressure

CI Confidence interval

NOS3 Endothelial Nitric Oxide Synthase

ACE Angiotensin Converting Enzyme

I/D Insertion/Deletion

AGT Angiotensinogen

AGTR1 Angiotensin II type 1 receptor

[*] Blood pressure progression was defined as progression of at least 1 blood pressure category or progressing to hypertension during the first 48 months of follow-up. Incident hypertension was defined as developing hypertension during the entire follow-up period.

[†] Assuming an additive model. The common wild type was used as reference group, except for the *ACE* I/D polymorphism, where the II genotype was used as reference group.

[≠] Adjusted for age, smoking, baseline blood pressure category, history of hypercholesterolemia, history of diabetes, body mass index, exercise, alcohol consumption, highest education level, hormone replacement use and menopausal status at baseline and randomized treatment assignments.

Table 4

Risk of blood pressure progression and incident hypertension stratified by baseline blood pressure category

Outcome/polymorphism	Baseline blood pressure category, mmHg <120/75	120–129/75–84	130–139/85–89	p value†
		Odds Ratio (95% confidence interval)‡		
Blood pressure progression*				
NOS3 rs1800779	1.01 (0.94–1.08)	0.98 (0.91–1.06)	1.05 (0.94–1.18)	0.58
NOS3 rs3918226	0.96 (0.84–1.08)	1.07 (0.93–1.23)	0.99 (0.80–1.21)	0.47
NOS3 rs1799983	0.99 (0.92–1.06)	0.99 (0.92–1.07)	1.03 (0.92–1.16)	0.78
ACE rs1799752	0.94 (0.88–1.01)	1.00 (0.93–1.08)	0.91 (0.82–1.02)	0.27
AGT rs699	1.08 (1.01–1.16)	0.97 (0.90–1.05)	1.04 (0.93–1.17)	0.11
AGTR1 rs5186	1.07 (0.99–1.15)	1.01 (0.93–1.10)	0.98 (0.87–1.11)	0.52
		Hazard Ratio (95% confidence interval)‡		
Incident hypertension*				
NOS3 rs1800779	1.02 (0.94–1.11)	0.99 (0.93–1.06)	1.03 (0.96–1.10)	0.52
NOS3 rs3918226	1.15 (1.00–1.34)	1.06 (0.95–1.18)	0.98 (0.87–1.11)	0.43
NOS3 rs1799983	1.09 (1.00–1.19)	1.03 (0.97–1.10)	1.04 (0.97–1.11)	0.67
ACE rs1799752	1.01 (0.93–1.10)	1.02 (0.97–1.08)	0.94 (0.88–1.00)	0.06
AGT rs699	1.02 (0.93–1.10)	1.03 (0.97–1.09)	0.97 (0.91–1.04)	0.50
AGTR1 rs5186	1.01 (0.92–1.10)	1.00 (0.94–1.06)	0.99 (0.92–1.06)	0.90

NOS3 Endothelial Nitric Oxide Synthase

ACE Angiotensin Converting Enzyme

I/D Insertion/Deletion

AGT Angiotensinogen

AGTR1 Angiotensin II type 1 receptor

* Blood pressure progression was defined as progression of at least 1 blood pressure category or progressing to hypertension during the first 48 months of follow-up. Incident hypertension was defined as developing hypertension during the entire follow-up period.

† p value for heterogeneity of the genotype effect on blood pressure progression and incident hypertension across different baseline blood pressure categories

‡ Women with the common wild type constitute the reference group. All relative risk estimates are adjusted for age, smoking, history of hypercholesterolemia, diabetes, body mass index, exercise, alcohol consumption, highest education level, hormone replacement use and menopausal status at baseline and randomized treatment assignments.