Original Paper

Renin-Angiotensin System Polymorphisms and Risk of Hypertension: Influence of Environmental Factors

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Renin-angiotensin system (RAS) polymorphisms have been studied as candidate risk factors for hypertension with inconsistent results, possibly due to heterogeneity among various environmental factors. We analyzed the association between RAS candidate gene polymorphisms and risk of hypertension among 2722 women and also explored whether these associations varied according to menopausal status, body mass index, and dietary factors. In a main-effects analysis of all 2722 women adjusted for age and race, homozygosity for the AT_1R A1166C polymorphism was associated with hypertension (odds ratio, 1.35; 95% confidence interval [CI], 1.03–1.78). We also found that a novel nonsense polymorphism in the aminopeptidase-A gene was associated with hypertension among postmenopausal women (hazard ratio, 1.54; 95% CI, 1.01–2.37), women with inadequate calcium intake (hazard ratio, 2.47; 95% CI, 1.29–4.72) and, marginally, women with inadequate vitamin D intake. In addition, angiotensin-converting enzyme and AT_1R A1166C polymorphisms were associated or marginally associated with incident hypertension among postmenopausal women and those with inadequate calcium and vitamin D intakes. These data suggest that demographic and dietary factors may influence the associations between RAS polymorphisms and hypertension and could explain heterogeneity in prior studies. J Clin Hypertens (Greenwich). *2008;10:459–466.* ©*2008 Le Jacq*

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Because the activity of the renin-angiotensin system (RAS) is intimately related to systemic blood pressure, dozens of association studies have examined the relation between blood pressure or hypertension and polymorphisms in RAS-related genes.[1] Findings have been conflicting, and no consensus has been reached regarding genetic causes of essential hypertension.[2]

Most hypertension is multifactorial. Apart from rare examples of monogenic hypertensive syndromes, single common genetic variants are unlikely to cause substantial elevations in blood pressure.[3] Rather, it is more likely that common genetic polymorphisms interact with demographic or dietary factors to produce a phenotype. For example, menopause and higher body mass index (BMI) may activate the RAS.[4–6] Dietary factors such as sodium intake,[7] but also intakes of calcium,[8] vitamin D,[9,10] potassium,[11–13] and magnesium,[14] could influence blood pressure and interact with the RAS. Therefore, heterogeneity among the populations that were investigated may partly explain contradictory conclusions about whether RAS polymorphisms are associated with hypertension.[1,7]

In this study, we examined the association between polymorphisms in genes related to the RAS

and the development of hypertension among 2722 women. We also investigated whether these associations varied by demographic and dietary factors.

METHODS

The Nurses' Health Study

The Nurses' Health Study (NHS) cohort was assembled in 1976 when 121,700 female nurses aged 30 to 55 years returned a mailed questionnaire. This prospective cohort has been described in detail elsewhere.[15] Subsequent questionnaires have been mailed every 2 years to update information on health-related behaviors and medical events. Follow-up during the past 30 years has been >90%.

Study Population

The study population for this analysis is part of a subcohort of the NHS that was designed to investigate the association between analgesics and a decline in renal function.[16] To derive this subcohort from the larger NHS cohort, the population was initially limited to the 32,826 participants who provided a blood sample in 1989. Women with a history of cancer (except nonmelanoma skin cancer) or with preexisting cardiovascular disease (myocardial infarction, angina, stroke, transient ischemic attack) were excluded from the blood sample collection in 1989. The characteristics of the women who provided blood samples were similar to those of the total cohort in terms of prevalent hypertension, age, weight, diabetes mellitus, and hyperlipidemia, but women who provided blood samples were less likely to be active smokers. The population was then further limited to 3123 women who answered a supplementary questionnaire about analgesic use and who provided a second blood sample in 2000 and finally to 2722 women who had serum creatinine measured on both blood samples. The institutional review board at Brigham and Women's Hospital reviewed and approved this study.

Genotyping

Polymorphisms were selected because of their relation to the RAS and proposed association with renal function decline or hypertension. They included the angiotensin-converting enzyme insertion/deletion (ACE I/D, no rs number), angiotensinogen M235T (AGT M235T, rs699), angiotensinogen A-20C (AGT A-20C, rs5050), angiotensin II type 1 receptor A1166C (AT_1R A1166C, rs5186), aldosterone synthase T-344C (AS T-344C, rs1799998), and kallikrein-1 R53H (KLK1 R53H, rs5515) polymorphisms. In addition, we analyzed a novel polymorphism that encodes a putative stop codon in aminopeptidase-A (AP-A stop, unpublished data, no rs number assigned); AP-A is the enzyme responsible for metabolizing angiotensin II (an octapeptide) into angiotensin III (a septapeptide) by cleaving the N-terminal amino acid.[17,18]

DNA was extracted from the white blood cells of blood samples and amplified. Genotyping of all polymorphisms was performed at the Harvard-Partners Genotyping Facility using Taqman (Applied Biosystems, Foster City, CA) or Sequenom (Sequenom, San Diego, CA). Failure to genotype occurred to varying degrees for each polymorphisms, but none more than 9.2%, which was the failure rate for ACE I/D.

Ascertainment of Hypertension

The initial and follow-up biennial questionnaires asked participants of the NHS to report whether a clinician had made a new diagnosis of hypertension during the preceding 2 years. Self-reported hypertension was shown to be highly reliable in the NHS.[19,20] In a subset of participants who reported hypertension in 1984, medical record review confirmed a documented systolic and diastolic blood pressure >140 and 90 mm Hg, respectively, in 100%.[19] Self-reported hypertension in the NHS is also highly predictive of subsequent cardiovascular events.[21]

Assessment of Other Factors

Menopausal status was ascertained with each biennial questionnaire. Postmenopausal status was assigned when a participant first reported a natural menopause or bilateral oophorectomy. If a woman reported a hysterectomy without bilateral oophorectomy, menopausal status was assigned at the age when natural menopause occurred in 90% of the cohort (56 years for nonsmokers and 54 for smokers). BMI was computed as weight in kilograms divided by the square of height in meters. Self-reported weight has been validated by direct measurement in a subset of locally residing participants, with excellent correlation (r=0.97). Race was self-reported.

Dietary intakes of calcium, vitamin D, potassium, and magnesium were ascertained from food frequency questionnaires (FFQs), which were sent to participants on every other questionnaire cycle (every 4 years rather than every 2 years, with the exception of an additional FFQ in 1986) starting in 1980. Thus, for cycles in which an FFQ was not sent to participants, the dietary intakes were carried forward from the prior FFQ. The correlation

coefficients for intakes of calcium (r=0.62), vitamin D (r=0.62 to 0.79 for the major sources of vitamin D), potassium (r=0.61), and magnesium (r=0.76) were good comparing the FFQ to the averaged intake computed from four 1-week dietary records. We specifically did not attempt to analyze interactions with FFQ sodium intake because of the large and variable contribution of salt added to processed food, in cooking, and at the table; historically, the correlation between self-reported sodium intake and 24-hour urine measurements ranged from 0.30 to 0.45.[22,23]

Statistical Analysis

For all polymorphisms, the genotype that *a priori* was hypothesized to confer the lowest risk of hypertension was used as the reference group. For example, if for an allele p/q the q allele was hypothesized to be associated with hypertension, then the p allele was used as the reference group. Genotype-hypertension associations were then analyzed in 3 ways: dose-response model (3 categories including pp as the reference group, pq, and qq); recessive model (pp and pq combined as the reference group); and dominant model (pp as the reference group, with pq and qq combined as the comparison group). For polymorphisms in which qq was not present, only pq vs pp models were analyzed.

Violations of Hardy-Weinberg equilibrium were analyzed using a chi-square test. The relations between genotypes and baseline age and BMI (in 1976) were tested using analysis of variance and log-transformation of age and BMI. The relation between genotype and race was tested using the chi-square test.

To examine the main effects (ie, unstratified analyses) of genotype on risk of hypertension, we used age- and race-adjusted logistic regression and all cases of hypertension that accrued in this population through 2002. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

In contrast, for stratified analyses, we excluded individuals in whom hypertension developed before the time we first ascertained information about the stratifying variable (BMI and menopausal status in 1976; diet in 1980). Women were considered to have incident hypertension if they reported an initial diagnosis of hypertension on subsequent questionnaires.

We used Cox proportional hazards regression adjusted for age and race to analyze all stratified models. This enabled us to update information on menopausal status and BMI with every questionnaire cycle (every 2 years) and update information on dietary factors with each FFQ (every 4 years); at questionnaire cycles in which FFQ information was not gathered, dietary information was carried forward from the prior FFQ. For each participant, person-time was counted from the date of return of the 1976 or 1980 questionnaire until the date of return of the 2002 questionnaire and allocated according to exposure status. Person-time was truncated when an event occurred. Women were censored either at the date of death or if they did not return a subsequent questionnaire, at the date the subsequent questionnaire was mailed.

Interactions between genotype and demographic or dietary factors were analyzed using stratified analyses: menopausal status (pre vs post); BMI (<25 kg/m^2 vs ≥25 kg/m^2); calcium intake (using the dietary reference intake, <1000 mg/d vs ≥1000 mg/d); vitamin D (using the dietary reference intake, <400 IU/d vs ≥400 IU/d); potassium intake (< or ≥ the cohort median); magnesium intake (< or ≥ the cohort median). Because intakes of calcium, vitamin D, potassium, and magnesium are correlated, these stratified models were adjusted for intake of the other 3 dietary variables in addition to age and race. Interaction P values were computed by creating appropriate interaction terms and employing the –2 log likelihood ratio test.

Hazard ratios from Cox models were reported as relative risks (RRs) and 95% CIs. All P values are 2-tailed. Statistical tests were performed using SAS statistical software, version 9 (SAS Institute Inc, Cary, NC).

RESULTS

Cohort Characteristics

The mean age of the women in the subcohort in 1976 was 44.7 years (median 45 years; interquartile range, 39–51 years) and the mean BMI was 23.5 kg/m^2 (median 22.7 kg/m^2; interquartile range, 20.9–25.1 kg/m^2). There were no significant differences among baseline age or BMI across any genotype (data not shown). Some genotypes differed among race; specifically, black race was more commonly associated with the AGT M235T polymorphism (P=.03) and less commonly associated with the AGT C-20A (P=.05) and AT_1R A1166C (P=.02) polymorphisms.

The cohort included 2722 participants, although the sample size analyzed for each genotype depended upon the number successfully genotyped; genotype frequencies for each polymorphism are shown in Table I. There were no homozygotes for the AP-A stop codon, and 2.5% were heterozygotes.

Table I. Genotype Frequencies Among 2722 Women From the Nurses' Health Study

Genotype	Genotype Frequency (%)		
AP-A stop	wt/wt	wt/stop	stop/stop
	2585 (97.5)	67 (2.5)	0 (0.0)
ACE I/D	I/I	I/D	D/D
	537 (21.5)	1196 (47.8)	767 (30.7)
AGT M235T	M/M	M/T	T/T
	924 (34.9)	1251 (47.3)	472 (17.8)
AGT A-20C	A/A	A/C	C/C
	1843 (69.8)	733 (27.7)	66 (2.5)
AT_1R A1166C	A/A	A/C	C/C
	1346 (50.8)	1064 (40.2)	240 (9.0)
AS T-344C	T/T	T/C	C/C
	785 (30.4)	1237 (47.9)	562 (21.7)
KLK1 R53H	R/R	R/H	H/H
	2463 (93.1)	182 (6.9)	1 (0.0)

Abbreviations: AP-A, aminopeptidase A; ACE, angiotensin converting enzyme; AGT, angiotensinogen; AT_1R, angiotensin II receptor type 1; AS, aldosterone synthase; KLK-1, kallikrein-1; Totals do not equal 2722 because of variable numbers with missing genotypes.

Similarly, there was a single homozygote for the KLK1 R53H polymorphism, and this individual was not included in further analyses for this polymorphism. Hardy-Weinberg equilibrium was not violated for any polymorphism (all *P* values ≥.07).

There were 1537 total cases of hypertension through 2002 that were included in the analyses of genotype main effects. After excluding 256 participants with prevalent hypertension in 1976, the analysis of menopause- and BMI-stratified models included 1281 cases. After excluding 413 women with prevalent hypertension in 1980, there were 1124 cases of incident hypertension analyzed in nutrient stratified models.

Genotype Main Effects

The age- and race-adjusted associations between polymorphisms and risk of hypertension are shown in Table II. The AP-A stop codon was associated with a nonsignificant 38% higher risk of hypertension among the entire study sample (OR, 1.38; 95% CI, 0.83–2.30). The ACE I/D polymorphism also showed a nonsignificant association with an increased risk of hypertension in recessive models (D/D vs combination of I/I and I/D: OR, 1.13; 95% CI, 0.95–1.34). The AT_1R A1166C polymorphism was associated with a higher risk of hypertension in both dose-response (CC vs AA: OR, 1.39; 95% CI, 1.05–1.84) and recessive models (OR, 1.35; 95% CI, 1.03–1.78) but was not significant in dominant models (OR, 1.12; 95% CI, 0.96–1.31). All other polymorphisms were nonsignificant.

Menopause and BMI

We tested whether the associations between these RAS polymorphisms and risk of hypertension varied by menopausal status and BMI (Table III). Among premenopausal women, RAS polymorphisms were not associated with hypertension. However, among postmenopausal women, AP-A stop heterozygotes (RR, 1.54; 95% CI, 1.01–2.37) and ACE D/D homozygotes (RR, 1.17; 95% CI, 1.00–1.36) were at elevated risk for developing hypertension. The association comparing homozygotes for the AT_1R A1166C polymorphism to nonhomozygotes and risk of hypertension among postmenopausal women was marginally significant (RR, 1.24; 95% CI, 0.98–1.56). Tests for interactions, however, were not statistically significant (*P*>.10).

Among BMI-stratified models, the only significant association observed was for AT_1R A1166C homozygotes with a BMI ≥25 kg/m^2, who had a 42% higher risk (RR, 1.42; 95% CI, 1.00–2.01). Test results for interaction were not significant (*P*>.10).

Dietary Factors: Calcium, Vitamin D, Potassium, and Magnesium

We also explored whether associations between RAS polymorphisms and the risk of hypertension varied by dietary factors (Table IV and Table V). Among women whose calcium intake was ≥1000 mg/d, no polymorphisms were associated with risk of incident hypertension. In contrast, among women with lower calcium intake, AP-A stop heterozygotes (RR, 2.47; 95% CI, 1.29–4.72), ACE D/D homozygotes (RR, 1.28; 95% CI, 1.03–1.57), and AT_1R A1166C homozygotes (RR, 1.37; 95% CI, 1.00–1.88) were at elevated risk for developing hypertension. Test results for interaction were not significant (*P*>0.10).

There were no associations between polymorphisms and risk of hypertension among women whose vitamin D intake was ≥400 IU/d. Among women whose vitamin D intake was <400 IU/d, ACE D/D homozygotes (compared with nonhomozygotes) had a 30% increased risk of incident hypertension (RR, 1.30; 95%, 1.07–1.58). There was a trend toward a higher risk of hypertension among women whose vitamin D intake was <400 IU/d who were AP-A stop heterozygotes (RR, 1.63; 95% CI, 0.97–2.76), those who were homozygous for the AT_1R A1166C polymorphism (RR, 1.28; 95% CI, 0.95–1.73), and those heterozygotes for

Table II. Association Between Gene Polymorphisms and Risk of Hypertension, Adjusted for Age and Race

	Dose-Response Model	Dominant Model	Recessive Model
AP-A stop[a]	1.38 (0.83–2.30)	N/A	N/A
ACE I/D[b]	I/D: 0.93 (0.78–1.12) D/D: 1.08 (0.88–1.33)	0.99 (0.83–1.17)	1.13 (0.95–1.34)
AGT M235T[c]	M/T: 1.03 (0.87–1.22) T/T: 1.13 (0.91–1.42)	1.06 (0.90–1.24)	1.12 (0.91–1.37)
AGT A-20C[d]	A/C: 1.10 (0.93–1.31) C/C: 0.73 (0.44–1.20)	1.06 (0.90–1.26)	0.71 (0.43–1.17)
AT_1R A1166C[e]	A/C: 1.07 (0.91–1.26) C/C: 1.39 (1.05–1.84)	1.12 (0.96–1.31)	1.35 (1.03–1.78)
AS T-344C[f]	T/C: 0.98 (0.83–1.17) C/C: 0.83 (0.67–1.03)	0.93 (0.79–1.10)	0.84 (0.70–1.01)
KLK1 R53H[g]	1.13 (0.83–1.54)	N/A	N/A

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AP-A, aminopeptidase A; AS, aldosterone synthase; AT_1R, angiotensin II type 1 receptor; KLK-1, kallikrein-1; [a]Reference = wt; [b]reference = I/I (dose-response and dominant models) or I/I + I/D (recessive model); [c]reference = M/M (dose-response and dominant models) or M/M + M/T (recessive model); [d]reference = A/A (dose-response and dominant models) or A/A + A/C (recessive model); [e]reference = A/A (dose-response and dominant models) or A/A + A/C (recessive model); [f]reference = T/T (dose-response and dominant models) or T/T + T/C (recessive model); [g]reference = R/R.

Table III. Age- and Race-Adjusted Associations Between Gene Polymorphisms and Risk of Hypertension Stratified by Menopausal Status and BMI

	Menopausal Status		BMI	
	Premenopausal	Postmenopausal	<25 kg/m²	≥25 kg/m²
AP-A stop	0.81 (0.27–2.37)	1.54 (1.01–2.37)	1.37 (0.67–2.80)	1.19 (0.68–2.09)
ACE I/D (recessive)	0.86 (0.63–1.22)	1.17 (1.00–1.36)	1.20 (0.97–1.49)	1.08 (0.86–1.35)
AGT M235T (recessive)	1.09 (0.73–1.63)	1.03 (0.86–1.24)	1.01 (0.78–1.30)	1.07 (0.83–1.39)
AGT A-20C (recessive)	1.00 (0.35–2.91)	0.66 (0.40–1.07)	1.03 (0.59–1.80)	0.44 (0.19–1.06)
AT_1R A1166C (recessive)	1.14 (0.72–1.81)	1.24 (0.98–1.56)	1.19 (0.86–1.64)	1.42 (1.00–2.01)
AS T-344C (recessive)	0.79 (0.54–1.15)	0.97 (0.82–1.15)	0.92 (0.72–1.17)	0.97 (0.76–1.23)
KLK1 R53H	1.14 (0.62–2.10)	1.08 (0.83–1.41)	1.41 (0.99–2.01)	1.07 (0.71–1.60)

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AP-A, aminopeptidase A; AS, aldosterone synthase; AT_1R, angiotensin II type 1 receptor; BMI, body mass index; KLK1, kallikrein-1; stop; polymorphism encoding a stop codon.

the KLK-1 R53H polymorphism (RR, 1.36; 95% CI, 0.99–1.89). The only significant association we observed among women whose vitamin D intake was ≥400 IU/d was for AGT A-20C homozygotes (RR, 0.36; 95% CI, 0.14–0.94). Test results for interaction between vitamin D intake and genotype were not significant ($P>0.10$).

Women who were homozygous for the AT_1R A1166C polymorphism (compared with nonhomozygotes) had an increased risk of hypertension (RR, 1.53; 95% CI, 1.13–2.10) if their potassium intake was below the median, and women who were ACE D/D homozygous had an increased risk (RR, 1.26; 95% CI, 1.03–1.55) if their magnesium intake was above the median. In addition, homozygotes for the AGT A-20C polymorphism had a lower risk of incident hypertension compared with nonhomozygotes if their intake of magnesium (RR, 0.43; 95% CI, 0.19–0.96) was below the median. Women homozygous for the AS T-344C polymorphism also had a lower risk compared with nonhomozygotes if their magnesium intake was below the median (RR, 0.71; 95% CI, 0.54–0.94).

DISCUSSION

A novel polymorphism encoding a putative stop codon in AP-A was associated with hypertension in postmenopausal women, women with lower calcium intakes and, marginally, with hypertension in women with lower vitamin D intakes. Women who were homozygous for the ACE D/D polymorphism and the AT_1R A1166C polymorphism had a significant or a nearly significant increased risk of hypertension if they were postmenopausal or had lower calcium and vitamin D intakes.

Many studies have examined the association between RAS gene polymorphisms and blood pressure or the development of hypertension. Results have varied. Three of these, the ACE I/D, AGT M235T, and AT_1R A1166C polymorphisms, have received the most attention. Regarding ACE I/D, reports can be found supporting a direct association with the D genotype,[24,25] no association,[26–28] or an inverse association.[29] A meta-analysis of 23 studies showed no association between the ACE I/D polymorphism and risk of hypertension.[30] The AGT M235T polymorphism likewise has been

Table IV. Multivariable-Adjusted Associations Between Gene Polymorphisms and Risk of Hypertension Stratified by Calcium and Vitamin D Intake

	Calcium Intake		Vitamin D Intake	
	<1000 mg/d	≥1000 mg/d	<400 IU/d	≥400 IU/d
AP-A stop	2.47 (1.29–4.72)	1.03 (0.53–1.99)	1.63 (0.97–2.76)	1.35 (0.61–3.01)
ACE I/D (recessive)	1.28 (1.03–1.57)	1.10 (0.88–1.37)	1.30 (1.07–1.58)	1.15 (0.89–1.48)
AGT M235T (recessive)	0.99 (0.76–1.27)	1.05 (0.81–1.37)	1.15 (0.91–1.44)	0.84 (0.62–1.15)
AGT A-20C (recessive)	0.71 (0.37–1.36)	0.68 (0.34–1.33)	0.79 (0.47–1.34)	0.36 (0.14–0.94)
AT_1R A1166C (recessive)	1.37 (1.00–1.88)	1.02 (0.74–1.42)	1.28 (0.95–1.73)	1.18 (0.82–1.70)
AS T-344C (recessive)	0.83 (0.65–1.05)	0.91 (0.71–1.16)	0.80 (0.64–1.00)	0.97 (0.75–1.28)
KLK1 R53H	1.17 (0.82–1.67)	1.06 (0.71–1.60)	1.36 (0.99–1.89)	0.87 (0.55–1.39)

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AP-A, aminopeptidase A; AS, aldosterone synthase; AT_1R, angiotensin II type 1 receptor; KLK1, kallikrein-1; stop; polymorphism encoding a stop codon.

Table V. Multivariable-Adjusted Associations Between Gene Polymorphisms and Risk of Hypertension Stratified by Potassium and Magnesium Intake

	Potassium Intake		Magnesium Intake	
	<Median	≥Median	<Median	≥Median
AP-A stop	1.54 (0.87–2.73)	1.36 (0.64–2.87)	1.66 (0.83–3.32)	1.50 (0.83–2.68)
ACE I/D (recessive)	1.18 (0.95–1.45)	1.22 (0.97–1.55)	1.18 (0.93–1.50)	1.26 (1.03–1.55)
AGT M235T (recessive)	1.18 (0.92–1.50)	0.96 (0.72–1.28)	1.14 (0.86–1.50)	0.97 (0.76–1.24)
AGT A-20C (recessive)	0.49 (0.24–1.02)	0.82 (0.42–1.61)	0.43 (0.19–0.96)	0.75 (0.41–1.37)
AT_1R A1166C (recessive)	1.54 (1.13–2.10)	1.05 (0.74–1.50)	1.34 (0.92–1.94)	1.26 (0.93–1.71)
AS T-344C (recessive)	0.85 (0.67–1.07)	0.92 (0.71–1.20)	0.71 (0.54–0.94)	0.90 (0.72–1.12)
KLK1 R53H	1.12 (0.80–1.59)	1.04 (0.68–1.58)	1.06 (0.71–1.59)	1.02 (0.71–1.47)

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AP-A, aminopeptidase A; AS, aldosterone synthase; AT_1R, angiotensin II type 1 receptor; KLK1, kallikrein-1; stop; polymorphism encoding a stop codon. Dietary stratified models were adjusted for age, race, and the other 3 dietary variables. All *P* values for interaction were >.05.

demonstrated to have a direct association,[31,32] no association,[27,29,33] or an inverse association with hypertension.[26] Three meta-analyses have examined the AGT M235T polymorphism, combining results from 25 to 32 studies. Each described an increased risk of hypertension with TT compared with MM, with RRs ranging from 1.20 to 1.31.[26,34,35] However, publication bias was noted in the one meta-analysis that inspected for this type of bias.[34] Finally, evidence can be produced to show that the AT_1R A1166C polymorphism is directly,[25,36] inversely,[37] or not associated with hypertension or blood pressure.[27,29,38] By comparison, fewer studies have examined potential demographic and dietary modifiers of these associations.

We observed associations or marginal associations between the AP-A stop, ACE I/D, and AT_1R A1166C polymorphisms and risk of incident hypertension among postmenopausal but not premenopausal women. However, interaction *P* values were not significant, so we cannot assert that risk indeed varies by menopausal status. Nevertheless, the prevalence of hypertension in women is greatly increased after menopause, and many investigators suggest that the loss of normal menstrual cycles leads to enhanced RAS activity.[4,5,39] This view is supported by the demonstration of an increased prevalence of salt sensitivity in postmenopausal women,[40,41] as well as a striking nonlinear increase in abnormal adrenal responsiveness to infused angiotensin II among age groups typical for the onset of menopause.[42] Thus, if menopause is associated with enhanced RAS activity, then menopause could potentially amplify the effect of genetic polymorphisms that also may enhance RAS activity. An alternate explanation is that menopause, with loss of estrogen, may unmask prior genetic predisposition.

We also observed associations or marginal associations between the AP-A stop, ACE I/D, and AT_1R A1166C polymorphisms and risk of incident hypertension among women with lower intakes of calcium and vitamin D. However, we cannot assert that these risks indeed varied by intake, as our tests for interaction were null. Yet lower intakes of calcium and vitamin D might influence the RAS.[8] While the blood pressure–lowering effect of higher calcium intake is controversial (as evidenced by meta-analyses of numerous randomized trials that demonstrate a minimal, if any, effect), higher calcium intake may indeed lower blood pressure in

certain populations.[43,44] In a study that segregated hypertensive individuals into low-renin and higher-renin categories, calcium supplementation had a blood pressure–lowering effect in low-renin (ie, salt-sensitive) hypertension but not higher-renin hypertension.[45] In another study, a high-salt diet was associated with blood pressure changes only on a low-calcium but not a high-calcium diet.[46] From these and other studies, Resnick[8,47] has concluded that a higher calcium intake has protective effects in salt-sensitive individuals. Vitamin D suppresses the RAS directly by suppressing renin expression in the juxtaglomerular apparatus[10]; in addition, vitamin D is important for the absorption of dietary calcium.[48] Therefore, it is possible that the effect of polymorphisms that may enhance RAS activity may be amplified by lower intakes of calcium and vitamin D.

There are limitations of this study. First, because we examined 7 polymorphisms and performed stratified analyses among 2 demographic and 4 dietary variables, multiple statistical tests were conducted. As the number of tests increases, so does the likelihood that a significant result would occur by random chance. On the other hand, we found an increased risk of hypertension among the same 3 polymorphisms with both postmenopausal status and with lower calcium and vitamin D intakes. While indeed these may be chance findings, the consistency of these results argues against them being random. Second, we had insufficient power to detect effect modification. For example, only 67 women had the AP-A stop polymorphism; even though stratifying by calcium yielded impressive findings, the confidence intervals were wide. Third, we did not examine effect modification by sodium intake because, unlike the other dietary variables, historic validation studies demonstrated weak correlations between self-reported sodium intake and measured 24-hour urine sodium concentration. Fourth, we did not directly measure our participants' blood pressure, yet self-reported hypertension has been shown previously to be reliable in this cohort of health professionals.

The association between RAS polymorphisms and hypertension may depend on other critical factors that have not typically been measured in prior studies. Our study suggests but does not prove that factors such as menopause and intake of calcium and vitamin D might influence these relations. We propose that such factors could partly explain the conflicting results of prior studies. Future genetic studies of hypertension should consider these other potentially important modifiers.

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