Clinical and Genetic Correlates of Aldosterone-to-Renin Ratio and Relations to Blood Pressure in a Community Sample

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Abstract—Aldosterone:renin ratio (ARR) is used to screen for hyperaldosteronism. Data regarding correlates of ambulatory ARR in the community and its relation to hypertension incidence are limited. We defined clinical correlates of ARR, determined its heritability, tested for association and linkage, and related ARR to blood pressure (BP) progression in nonhypertensive individuals among 3326 individuals from the Framingham Heart Study (53% women; mean age: 59 years). Ambulatory morning ARR (serum aldosterone and plasma renin concentrations) were related to clinical covariates, genetic variation across the *REN* locus, a 10-cM linkage map, and among nonhypertensive participants (n=1773) to progression of ≥1 Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure BP category (optimal: <120/80 mm Hg, normal: 120 to 129/80 to 84 mm Hg, high normal: 130 to 139/85 to 89 mm Hg, hypertension: ≥140/90 mm Hg), or incident hypertension (systolic BP: ≥140 mm Hg, diastolic BP: ≥90 mm Hg, or use of antihypertensive treatment). ARR was positively associated with age, female sex, untreated hypertension, total/high-density lipoprotein cholesterol ratio, hormone replacement therapy, and β-blocker use, but negatively associated with angiotensin-converting enzyme inhibitor and diuretic use. ARR was heritable (h^2=0.40), had modest linkage to chromosome 11p (logarithm of the odds: 1.89), but was not associated with 17 common variants in *REN* (n=1729). On follow-up (mean: 3 years), 607 nonhypertensive individuals (34.2%) developed BP progression, and 283 (16.0%) developed hypertension. Higher baseline logARR was associated with increased risk of BP progression (odds ratio per SD increment: 1.23; 95% CI: 1.11 to 1.37) and hypertension incidence (odds ratio per SD increment: 1.16; 95% CI: 1.00 to 1.33). ARR is a heritable trait influenced by clinical and genetic factors. There is a continuous gradient of increasing risk of BP progression across ARR levels in nonhypertensive individuals. (***Hypertension*. 2007;49:846-856.)**

Key Words: aldosterone ■ renin ■ hypertension, essential ■ blood pressure ■ genetics ■ population ■ risk factors

An elevated ratio of serum aldosterone to plasma renin level can point toward the diagnosis of primary hyperaldosteronism (PH), described first by Conn in 1955.[1] Recent studies using the aldosterone:renin ratio (ARR) as a screening measure have estimated the frequency of PH to be as much as 5% to 15%.[2–7]

Data regarding the clinical and genetic correlates of ARR in the general population are limited, particularly so in nonhypertensive individuals. In referral samples of hypertensive individuals, ARR (or its components aldosterone and renin) has been found to vary with age,[4,8–11] sex,[9] body mass index and obesity,[12–14] blood pressure (BP) and hypertension,[15] diuretic[16–18] and other antihypertensive use,[19] menopausal status, hormone replacement therapy,[20–23] total/high-density lipoprotein cholesterol,[24,25] urinary sodium excretion,[8] posture,[18,26] time of day,[26] and race.[9,27–29]

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Furthermore, the prognostic significance of ARR in community-based nonhypertensive individuals is unknown. We demonstrated recently that increasing serum aldosterone predicts a future increase in BP and incident hypertension in nonhypertensive participants in the Framingham Heart Study.[30] Subsequent to that investigation, we measured plasma renin concentrations in our cohort to evaluate the independent and conjoint contributions of both hormones to longitudinal BP outcomes using the ARR. Given the interest in ARR as a screening test and the emergence of novel antihypertensive agents targeting both renin and aldosterone,[31,32] a study of the clinical and genetic correlates of ARR in the community and the influence of ARR on BP progression and hypertension incidence among nonhypertensive subjects could have diagnostic, therapeutic, and prognostic importance. Accordingly, we evaluated the clinical and genetic correlates of ARR and examined the relationship of ARR in nonhypertensive individuals to BP progression and incident hypertension in a large, community-based sample.

Methods

Study Participants

The design and selection criteria of the Framingham Offspring Study have been reported previously.[33] Approximately every 4 years, offspring study participants undergo detailed medical history, assessment of medication use, physical examination, and assessment of vascular risk factors. The present investigation included 3532 participants who attended the sixth examination cycle (1995–1998). The institutional review board at Boston Medical Center approved the study, and all of the participants gave written informed consent.

Participants were excluded if they were missing values for plasma renin (n=74), serum aldosterone (n=97), or any of the covariates (n=35). After exclusions, 3326 attendees were eligible for the study of clinical correlates. Heritability analyses were conducted in 2271 participants in 998 families. The genetic linkage analysis included 1225 genotyped participants in 328 families (1597 sibling pairs and 4 parent–offspring pairs). The association analysis included 1729 unrelated cohort participants with covariate and genotype data. Analyses of the relations of baseline ARR to BP progression and incident hypertension were restricted to the 1773 nonhypertensive participants at the sixth examination who attended the seventh examination.

Measurements of Plasma Renin, Serum Aldosterone, and BP

Fasting whole blood samples were drawn by venipuncture after ≈10 minutes of rest in a supine position in the morning, typically between 7:30 and 9:00 AM. Participants were instructed to take all routine medications. Blood samples were centrifuged and the serum/plasma fraction stored at −70 to −80°C until it was thawed for analysis. Plasma renin concentration (milliunits per liter) was measured using an immunochemiluminometric assay (Nichols Advantage Direct Renin assay). The assay was sensitive with intraassay coefficients of variation ranging from 2% for high concentrations to 10% for low concentrations. Serum aldosterone (nanograms per liter) was measured using a radioimmunoassay (Quest Diagnostics), as described previously.[34] Spot urinary sodium concentration was measured using an automated ion-electrode method and indexed to urinary creatinine (expressed as millimoles of sodium per gram of urinary creatinine), as described previously.[34]

Participants rested in a chair for ≥5 minutes before BP measurement in the Framingham clinic. A physician measured systolic and diastolic BP twice in the left arm of seated participants with a mercury column sphygmomanometer, a cuff of appropriate size, and a standardized written quality control protocol.[35] The average of 2 readings constituted the examination BP.

Clinical Covariates

We considered the following potential covariates of ARR for analyses: age, sex, total:high-density lipoprotein cholesterol ratio, diabetes, menopausal status (premenopausal, postmenopausal with hormone replacement therapy, or postmenopausal without hormone replacement therapy), hypertension (systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg at the sixth examination), and use of the following classes of antihypertensive agents: diuretics, angiotensin-converting enzyme (ACE) inhibitors, β-blockers, calcium channel blockers, α-1 adrenergic receptor antagonists, and other antihypertensive agents. Methods of risk factor ascertainment in the Framingham Heart Study have been reported elsewhere.[36] Because the urinary sodium:creatinine ratio was only available in a subset of 2850 participants, we performed secondary analyses adjusting for the urinary sodium:creatinine ratio.

Selection and Genotyping of Tag Single Nucleotide Polymorphisms and Microsatellites

We genotyped 32 polymorphic single nucleotide polymorphisms (SNPs) in a reference sample of 93 individuals of European ancestry,[37] spanning a genomic distance of 30 kb surrounding the *REN* locus. These data revealed 2 blocks of strong linkage disequilibrium (LD) defined using the "spine of LD" method implemented in Haploview 2.03 (Figure S1, available online at http://hyper.ahajournals.org).[38] We selected 14 tag SNPs for genotyping in the Framingham Heart Study samples that captured haplotypes within blocks at frequency ≥0.05 at an $r^2 \geq 0.8$,[39] as well as an additional 3 SNPs to better define the region between the 2 blocks of strong LD. Genotyping of microsatellite markers at an average 10 cM spacing (Weber set 8A) for the linkage analyses in the related sample was conducted through the Mammalian Genotyping Center at Marshfield Clinic as described previously.[40]

Statistical Analysis

Renin and aldosterone levels were natural-log transformed because of their positively skewed distributions. We evaluated the sex-specific distributions of logARR in several participant subgroups: nonhypertensive; untreated hypertensive; and hypertensive on diuretics, ACE inhibitors, or β-blockers, separately. We used stepwise multivariable linear regression to evaluate the clinical correlates of logARR in the entire sample of 3326 participants.[41] We then used generalized estimating equations to account for correlations among related individuals[42]; a $P<0.05$ was used for retention in the final multivariable model. We examined the correlates of log-aldosterone and log-renin separately to clarify whether any observed relation of logARR was driven by the relations of covariates to the numerator (aldosterone), the denominator (renin), or both. All of the analyses were carried out in SAS.[43]

Heritability and Genetic Analyses

For genetic analyses, we normalized the residuals from regression models (age-, sex-, and multivariable-adjusted) in the entire sample. Using variance components methods implemented in sequential oligogenic linkage analysis routines (SOLAR),[44] we determined the heritability of logARR residuals from age-, sex-, and multivariable-adjusted models. We carried out multipoint linkage analysis using Genehunter 2.0 for the logARR residuals from both models.[45] Linkage results are reported in logarithm of the odds (LOD) scores. The LOD score is the log base 10 of the likelihood ratio under the hypotheses of linkage and nonlinkage. We tested the association of logARR residuals with each SNP (n=1729 unrelated subjects) individually using a 2-degree of freedom general test. Within the 2 blocks of strong LD, we tested haplotypes with frequency ≥0.05 for association with logARR residuals from age-, sex-, and multivariable-adjusted models using the score-based haplo.stat program (available at http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm).[46] We considered global haplotype tests as the primary statistical test of significance.

TABLE 1. Characteristics of Framingham Heart Study Sample and Nonhypertensive Subgroup at Exam 6

	Entire Sample		Nonhypertensive* Sample	
Clinical Characteristic	Men (n=1574)	Women (n=1752)	Men (n=760)	Women (n=1013)
Age, y	59 (10)	59 (10)	55 (9)	56 (9)
Body mass index, kg/m^2	28.6 (4.4)	27.4 (5.7)	28.1 (4.4)	26.3 (5)
% change in weight	0.5 (5.8)	0.7 (6.6)	0.6 (5.6)	1.0 (6.5)
Systolic BP, mm Hg	130 (17)	127 (20)	121 (10)	117 (12)
Diastolic BP, mm Hg	77 (9)	74 (9)	75 (7)	71 (8)
Hypertension at exam 6, %†	45	39		
On any treatment	32	26		
On diuretics	7	10		
On ACE inhibitors	14	9		
On β-blockers	12	9		
On calcium channel blockers	12	8		
On α-1 AR blockers	3	1		
Total cholesterol:HDL ratio	4.9 (2.0)	4.0 (1.4)	4.8 (1.4)	3.8 (1.4)
Diabetes, %‡	14	9	6	3
Current smoker, %	14	16	18	17
Atrial fibrillation, %	1	0	3	1
Prevalent CVD, %	15	7	2	0
Premenopausal, %		20		27
Postmenopausal on HRT, %		25		25
Postmenopausal no HRT, %		56		48
Serum creatinine, mg/dL	1.3 (0.2)	1.1 (0.2)	1.2 (0.2)	1.1 (0.1)
Urine Na/Cr ratio mmol/g§	103 (90)	123 (100)	99 (90)	117 (100)
Serum aldosterone ng/L	90 [70 to 130]	110 [70 to 150]	90 [70 to 130]	100 [70 to 130]
Plasma renin, mU/L	14 [8 to 25]	11 [6 to 19]	14.0 [9.0 to 22.5]	11.0 [7.0 to 17.0]
ARR, ng/L per mU/L	6.7 [3.8 to 11.4]	10 [5.5 to 16.7]	6.0 [4 to 10]	10 [6 to 10]
ARR $\geq$26 ng/L per mU/L, %‖	7	13	3	9

Values are reported as mean (SD) for continuous traits and % for dichotomous traits. Because serum aldosterone, plasma renin and ARR are skewed, median values [25th percentile to 75th percentile] are shown. CVD indicates cardiovascular disease; Na, sodium; Cr, creatinine; AR, adrenergic receptor; HDL, high-density lipoprotein.

*Nonhypertensive participants at baseline exam 6 were defined by systolic BP $<$140 mm Hg, diastolic BP $<$90mm Hg, and absence of antihypertensive treatment.

†Shown are the percentages of participants with treated or untreated hypertension.

‡Diabetes determined by use of hypoglycemic agents or fasting glucose $\geq$126 mg/dL.

§Note that urine Na/Cr ratio was available for the subset of 1337 men and 1513 women.

‖Shown is the percentage exceeding one among many thresholds proposed in screening hypertensive subjects for primary hyperaldosteronism.[49]

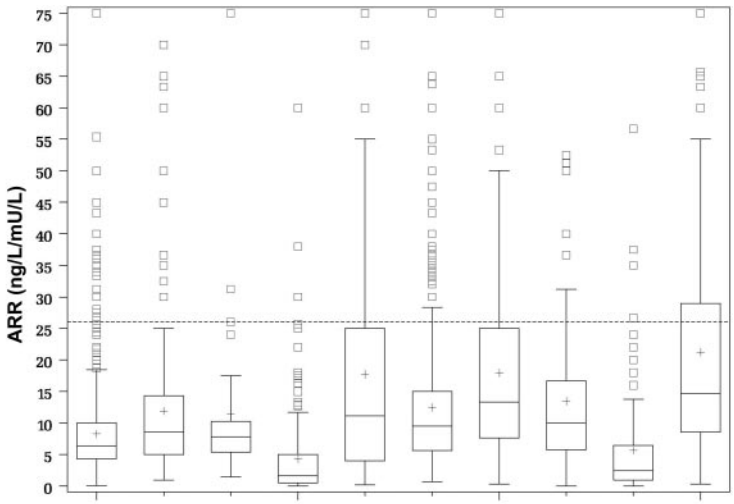
ARR Relation to BP Progression and Incident Hypertension

We used multivariable logistic regression to relate ARR (modeled as a continuous, log-transformed variable and as sex-specific quartiles) in nonhypertensive participants at examination 6 to the risk of progression of $\geq$1 BP category (optimal: $<$120/80 mm Hg, normal: 120 to 129/80 to 84 mm Hg, high normal: 130 to 139/85 to 89 mm Hg, hypertension: $\geq$140/90 mm Hg)[35] and incident hypertension (systolic BP: $\geq$140 mm Hg, diastolic BP: $\geq$90 mm Hg, or use of antihypertensive treatment) at examination 7 after a mean follow-up of 3 years.[47] We examined a model adjusting for age and sex and a multivariable model adjusting for age, sex, baseline BP stage, systolic BP, diastolic BP, smoking status, diabetes, serum creatinine, body mass index, and weight change percentage on follow-up, as described previously.[48] We performed secondary analyses incorporating urine sodium:creatinine ratio as a covariate. We tested for effect modification by age, sex, body mass index, and urine sodium:creatinine ratio by incorporating appropriate interaction terms in multivariable models with logARR. To understand the relative contributions of aldosterone versus renin to any potential association of ARR with BP progression and incident hypertension, we assessed rates of BP progression and incident hypertension by tertiles of aldosterone cross-classified by tertiles of renin; tertiles were used instead of quartiles to avoid small numbers in individual cells. Furthermore, we examined models that included continuous hormone levels of aldosterone and renin each alone and together as separate linear predictors and compared the results with models containing aldosterone and renin together as a fixed ratio (ARR).

Results

Characteristics of the study Cohort

The characteristics of the entire study sample and of the nonhypertensive individuals are shown in Table 1. The cohort is middle-aged to elderly and of European descent.



	Men					Women				
N	864	215	100	168	116	1096	229	181	111	88
% >26 ng/L/mU/L	3.1%	7.9%	9.0%	4.8%	26%	8.9%	23.1%	12.7%	7.2%	34%
95%ile	20	35	41	26	60	35	50	40	35	63
90%ile	15	22	25	15	50	25	35	30	16	50
75%ile	10	15	12	5.1	28	15	25	15	8.2	33
50%ile	6.4	8.8	6.3	1.7	14	9.5	13	9	2.7	20
25%ile	4.3	5.0	1.7	0.6	6.7	5.6	7.7	4.1	1.1	12.4
10%ile	2.6	3.3	0.4	0.3	3.3	4.0	4.6	1.1	0.3	7.5
5%ile	2.2	2.2	0.2	0.2	2.0	3.0	3.5	0.6	0.2	5.0

Figure 1. Distribution of ARR. Box and whisker plots of ARR (nanograms per liter per milliunit per liter) separately for men and women with no hypertension, untreated hypertension, hypertension on diuretics, hypertension on an ACE inhibitor (ACEI), and hypertension on a β-blocker (BB). A line is drawn at the 26 ng/L/mU/L value suggested previously to indicate an increased probability of primary hyperaldosteronism.[49] Because of the right skew of the untransformed ARR measure, values >75 ng/L/mU/L have been set to 75 ng/L/mU/L for display purposes. Shown below the plots are the percentage in each group with an ARR >26 ng/L/mU/L and the ARR values in nanograms per liter per milliunit per liter corresponding with the 5th, 25th, 50th, 75th, and 95th percentiles for each group.

Distribution of ARR

Figure 1 displays the sex-specific distributions of ARR for the following subgroups: nonhypertensive, untreated hypertensive, hypertensive on a diuretic, hypertensive on ACE inhibitors, and hypertensive subjects on β-blockers. The ARR was higher in women than in men and in untreated hypertensive subjects compared with nonhypertensive subjects. The ARR was lower in men and women on ACE inhibitors and higher in men and women on β-blockers compared with untreated hypertensive subjects. Overall, ARR values exceeded 26 ng/L per mU/L (only 1 screening threshold among many proposed in the literature[49]) in 7.9% and 23.1% of untreated hypertensive men and women and in 24.6% and 31.1% of men and women on β-blockers. This threshold was exceeded by 3.1% of nonhypertensive men and 8.8% of nonhypertensive women (Figure 1).

Clinical Correlates of LogARR

Log-renin was positively associated with the use of diuretics and ACE inhibitors and inversely related to age, female sex, hypertensive status, and β-blocker and hormone replacement therapy use (Table 2). We confirmed our previously reported[34] finding that serum aldosterone was positively associated with female sex and diuretic use and demonstrated an additional positive association with calcium channel blocker use and a negative association with ACE inhibitor use (Table 2). In stepwise regression modeling, logARR was positively related to age, female sex, hypertensive status, total cholesterol:high-density lipoprotein cholesterol ratio, and β-blocker and hormone replacement therapy use (Table 2). We observed that logARR was inversely related to diuretic and ACE inhibitor use. Several covariates were not significantly related to log-renin or log-aldosterone individually but were significant correlates of logARR (Table 2).

The multivariable-adjusted logARR model explained 28% of the interindividual variation in ARR. The inclusion of urinary sodium:creatinine ratio as a covariate minimally altered the regression coefficients for other covariates (data not shown).

Heritability and Linkage

The heritability for age and sex-adjusted logARR was 0.36 (SE: 0.06; $P<10^{-4}$) and for multivariable-adjusted logARR was 0.40 (SE: 0.06; $P<10^{-4}$). The heritability of multivariable-adjusted log-renin was 0.22 (SE: 0.06; $P<10^{-4}$). The heritability of multivariable-adjusted log-aldosterone as modeled here was 0.11 (SE: 0.05; $P=0.01$), quite consistent with our previous report on aldosterone that used slightly different covariates in the model.[34] Exclusion of all of the individuals on antihypertensive therapy reduced the sample size substantially (740 sibpairs), so models including antihypertensive users with adjustment for treatment were considered the primary analysis.

The results of genome-wide linkage analysis of multivariable-adjusted logARR are shown in Figure 2. We observed modest evidence of linkage to chromosome 11p with a maximum multipoint LOD score of 1.89 at 2 cM and to chromosome 5p with a maximum multipoint LOD score of 1.60 at 30.8 cM for residuals from multivariable-adjusted models. In an initial analysis, we did not adjust for ACE inhibitor and β-blocker use when generating logARR residuals. In linkage analyses of those residuals, we observed suggestive evidence of linkage to a locus on chromosome 7p21-22 (24.6 cM) with a maximum multipoint

TABLE 2. Multivariable Clinical Correlates of LogARR, Log-Aldosterone and Log-Renin in the Entire Framingham Heart Study Sample

	LogARR (Model $R^2=0.28$)					
Covariate	β (SE)	Fold Change in ARR	95% CI	*P*	Log-Aldosterone β (SE)	Log-Renin β (SE)
Age, per 10 y	+0.11 (0.02)	1.11	1.07 to 1.15	<0.0001	−0.03 (0.01)	−0.13 (0.02)*
Sex, women (vs men)	+0.37 (0.05)	1.45	1.31 to 1.60	<0.0001	+0.13 (0.03)†	−0.23 (0.05)*
Hypertensive‡	+0.18 (0.04)	1.19	1.09 to 1.30	<0.0001	+0.01 (0.03)	−0.17 (0.04)*
Diuretic use	−0.22 (0.08)	0.80	0.68 to 0.94	0.005	+0.48 (0.04)*	+0.70 (0.08)*
ACE inhibitor use	−1.58 (0.08)	0.21	0.17 to 0.24	<0.0001	−0.23 (0.03)*	+1.35 (0.08)*
β-Blocker use	+0.47 (0.07)	1.60	1.40 to 1.83	<0.0001	−0.06 (0.04)	−0.53 (0.07)*
Calcium channel blocker use	+0.06 (0.07)	1.06	0.92 to 1.21	0.42	+0.14 (0.04)*	+0.09 (0.07)
α-1 AR blocker use	−0.13 (0.15)	0.87	0.65 to 1.17	0.37	+0.04 (0.08)	+0.16 (0.14)
Total cholesterol:HDL ratio	+0.03 (0.01)	1.03	1.02 to 1.05	0.0001	+0.02 (0.01)	−0.01 (0.01)
Postmenopausal on HRT§	+0.29 (0.06)	1.33	1.19 to 1.49	<0.0001	+0.06 (0.04)	−0.23 (0.06)*
Postmenopausal no HRT§	−0.06 (0.06)	0.94	0.84 to 1.06	0.32	−0.07 (0.04)	−0.02 (0.06)

β indicates linear regression coefficient; AR, adrenergic receptor; HRT, hormone replacement therapy; HDL, high-density lipoprotein. Covariates tested in stepwise multivariable regression included (data not shown): age, sex, diastolic BP, systolic BP, hypertension, ACE inhibitor use, β-blocker use, calcium channel blocker use, and α-1 adrenergic receptor antagonist use, total:high-density lipoprotein cholesterol, postmenopausal on hormone replacement therapy, and postmenopausal not on hormone replacement therapy. Shown are the β-coefficients, fold change in ARR, 95% CI, and *P* for all covariates significant at $P<0.05$ in GenMod linear regression modeling, accounting for sibling correlations. For comparison, the β-coefficients (SE) for the covariates in models predicting log-aldosterone and log-renin are shown. Note that coefficients for covariates predicting log-aldosterone differ from those previously published[30] because of differences in regression modeling and samples. A β-coefficient for age of 0.11 indicates that for a 10-year increase in age, the ARR increases 1.11-fold (e^{β}) or by 11%.

*$P<0.0001$.

†$P<0.01$.

‡The hypertensive group is compared with the nonhypertensive group.

§The postmenopausal state, on or not on hormone replacement therapy, is compared with the premenopausal state.

LOD score of 2.94 and 2.78 for age-, sex-, and multivariable-adjusted ARR, respectively. However, adjusting for ACE inhibitor and β-blocker use in the multivariable-adjusted models (our principal analytical strategy) resulted in a LOD score at this locus of 1.14. To explore the attenuation of the linkage signal with more complete drug class modeling, we conducted linkage analysis on chromosome 7p in participants without ACE inhibitor use (LOD: 0.43), without β-blocker use (LOD: 2.02), and in individuals using neither (LOD: 0.53). These results raise the possibility of confounding of linkage results in the larger sample by ACE inhibitor use when not adjusted for.

Single SNP and Haplotype Association Results

We genotyped 32 SNPs falling in 2 blocks of strong LD in the Centre d'Etude du Polymorphisme Humain (CEPH) reference samples to construct a high-resolution LD map of the *REN* locus with an average inter-SNP distance of 880 bp (Figure S1). In the 1729 unrelated participants, we genotyped 17 SNPs that capture the majority of common variation across the *REN* locus.

We tested 17 SNPs across the *REN* locus and observed no single SNP to have a nominal $P<0.01$ for association with logARR (Table S1). We tested all of the haplotypes with frequency $\geq 5\%$ in the 2 blocks of strong LD across the *REN* locus and found no global $P<0.01$.

Relations of ARR to BP Progression and Incident Hypertension

On follow-up (mean: 3.0 years), 607 nonhypertensive individuals (34.2%) experienced BP progression, and 283 (16.0%) developed hypertension. The rates of BP progression and hypertension incidence rose across ARR quartiles in graded fashion (Table 3). In multivariable analyses, an SD increment in logARR was associated with a 23% increased risk of BP progression ($P<0.0001$) and a 16% increased risk of hypertension ($P=0.05$). The top ARR quartile was associated with an 89% increased risk of BP progression ($P<0.0001$) and a 53% increased risk of hypertension ($P=0.045$) compared with the lowest ARR quartile. Models incorporating urine sodium:creatinine ratio yielded essentially similar results (data not shown). We did not observe any effect modification by age, sex, body mass index, or urine sodium:creatinine ratio on risk of BP progression or incident hypertension.

Rates of BP progression and incident hypertension rose across increasing tertiles of aldosterone (for any tertile of renin) and decreased across increasing tertiles of renin (for any tertile of aldosterone; Figure 3). To better understand the contributions of aldosterone and renin to the prognostic ability of ARR, we compared age-, sex-, and multivariable-adjusted models predicting BP progression and incident hypertension that incorporated aldosterone and renin, separately and together as linear predictors, as well as in a fixed ratio (ARR; Table 4). β-Coefficients for log-aldosterone and log-renin incorporated in separate multivariable models were opposite in direction (positive for log-aldosterone and negative for log-renin) and statistically significant with the exception of log-renin for the outcome of incident hypertension ($P=0.004$ and 0.02 for log-aldosterone for BP progression and incident hypertension; correspondingly, $P=0.04$ and 0.77 for log-renin; Table 4). Models incorporating both log-aldosterone and log-renin together as linear predictors had gen-

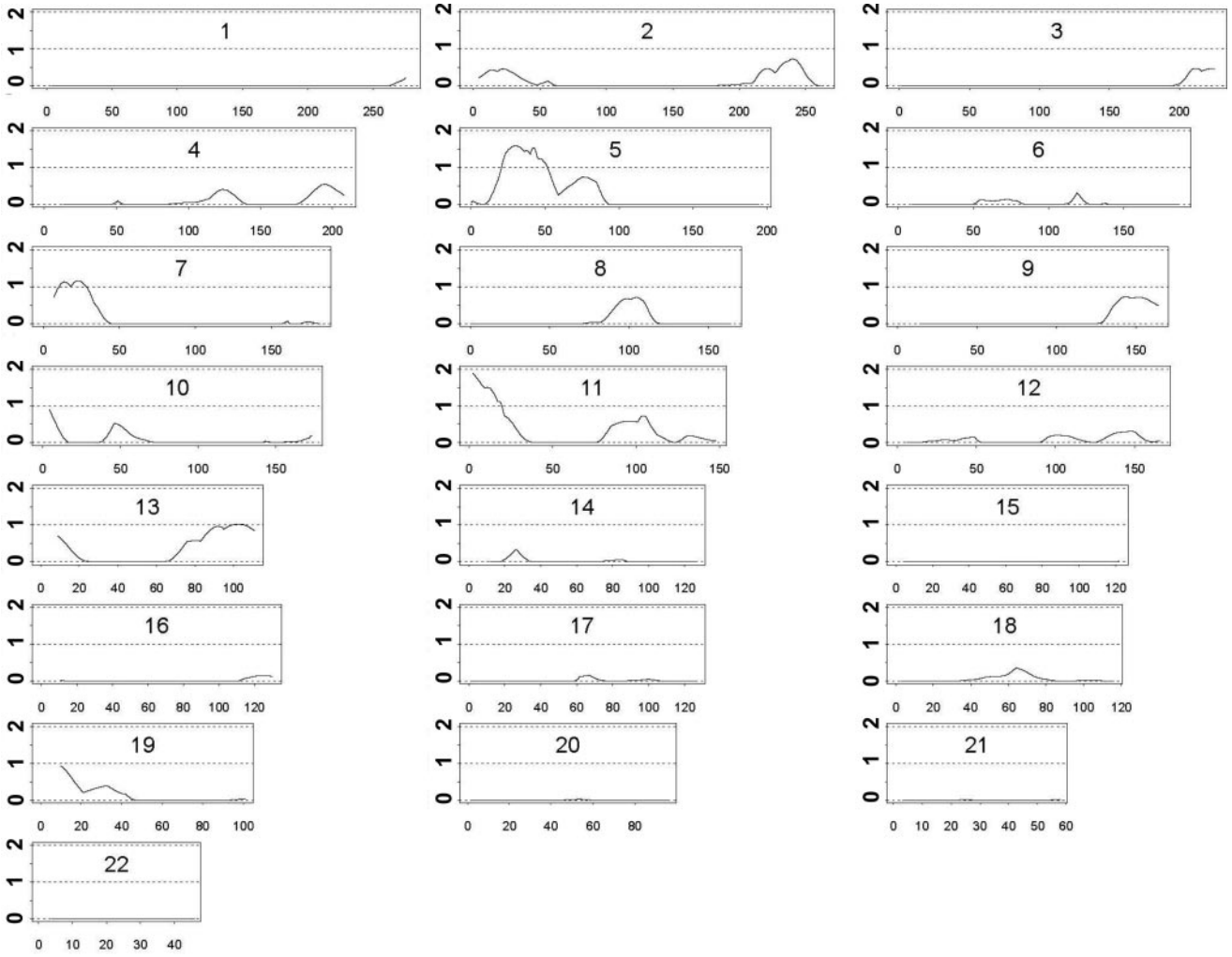


Figure 2. Genome-wide linkage to logARR. Multipoint linkage analysis of normalized residuals of multivariable-adjusted logARR for 22 autosomes in 1225 Framingham Heart Study participants in 328 families. The x-axis represents genetic distance from the p-terminus in centimorgan (cM) and the y-axis represents LOD score.

erally higher c-statistics compared with models incorporating either hormone alone. In these models, log-aldosterone and log-renin had β-coefficients more extreme in magnitude and, again, opposite in direction (for BP progression, $P=0.0002$ and 0.002 for log-aldosterone and log-renin, respectively; for hypertension incidence, $P=0.01$ and 0.19 for log-aldosterone and log-renin, respectively; Table 4). Models with ARR did not differ substantially from models containing both log-aldosterone and log-renin as separate linear predictors (c-statistics of 0.68 to 0.69 and 0.82 for BP progression and hypertension incidence, respectively, for both sets of models).

Discussion

We observed significant association of ARR with several clinical factors in our ambulatory community-based sample. ARR demonstrated significant heritability, and we identified 2 chromosomal regions with modest evidence of linkage to ARR. We did not find significant association of ARR with 17 common variants across the renin gene (*REN*) locus. In nonhypertensive participants, we observed that a graded increase in baseline ARR was associated with significantly increased risk of BP progression and incident hypertension in a continuous fashion and that renin and aldosterone are jointly more predictive of BP outcomes than either hormone alone.

To our knowledge, the current report is the largest study of the correlates of ARR in a community-based setting. Olivieri et al[4] have reported the distribution of ARR values in a community-based sample of hypertensive subjects withdrawn from antihypertensive agents, but that study did not examine the entire BP distribution in the community or explore several potential covariate relations to ARR. The significant associations of ARR with age, sex, use of ACE inhibitors, β-adrenergic receptor blockers, diuretics, and hormone replacement therapy observed in our sample are consistent with previous reports.[9,16–22,50–52] Our finding of the association of higher total:high-density lipoprotein cholesterol ratio with increased ARR differs from the results of a previous report that investigated people on a high-salt diet.[25]

We examined the distribution of ARR in nonhypertensive participants and in untreated and treated hypertensive participants. We observed that ARR level in individuals on ACE inhibitors was significantly lower and on β-blockers was significantly higher compared with untreated hypertensive individuals. This is in agreement with previous findings in

TABLE 3. Rates and Odds Ratios for 3-Year Progression by ≥1 BP Category and Incident Hypertension for Increasing LogARR Among Individuals Without Hypertension

			Age- and Sex-Adjusted Model		Multivariable Model	
ARR Category	Events/at Risk	Incidence Rate, %*	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*
Progression by ≥1 BP category at 3 years						
Per SD increase logARR			1.29 (1.17 to 1.43)	<0.0001	1.23 (1.11 to 1.37)	<0.0001
Q1	115/441	27 (19 to 35)	1.00 (referent)		1.00 (referent)	
Q2	151/444	32 (24 to 42)	1.42 (1.06 to 1.90)	0.02	1.44 (1.06 to 1.96)	0.02
Q3	151/456	36 (29 to 44)	1.33 (1.00 to 1.78)	0.05	1.26 (0.93 to 1.71)	0.14
Q4	190/432	42 (33 to 53)	2.09 (1.57 to 2.79)	<0.0001	1.89 (1.39 to 2.56)	<0.0001
Per quartile increase			1.24 (1.13 to 1.36)	<0.0001	1.19 (1.08 to 1.31)	0.0003
Incidence of hypertension at 3 years						
Per SD increase logARR			1.34 (1.18 to 1.53)	<0.0001	1.16 (1.00 to 1.33)	0.05
Q1	50/441	11 (5 to 21)	1.00 (referent)		1.00 (referent)	
Q2	63/444	14 (7 to 28)	1.20 (0.80 to 1.80)	0.37	1.14 (0.73 to 1.76)	0.57
Q3	66/456	17 (9 to 29)	1.18 (0.79 to 1.76)	0.41	0.96 (0.62 to 1.49)	0.87
Q4	104/432	22 (11 to 37)	2.19 (1.50 to 3.18)	<0.0001	1.53 (1.01 to 2.32)	0.045
Per quartile increase			1.29 (1.14 to 1.45)	<0.0001	1.13 (0.99 to 1.29)	0.07

Q1 to Q4 indicate quartiles of increasing sex-specific ARR (ng/L/μU/mL) defined as follows: men Q1 <4.35, Q2 4.35 to 6.40, Q3 6.40 to 10.0, and Q4 >10.0; women Q1 <5.6, Q2 5.6 to 9.6, Q3 9.6 to 15.0, and Q4 >15.0. The multivariable model was adjusted for age, baseline BP stage, systolic BP, diastolic BP, smoking status, diabetes, creatinine, body mass index, and percentage of weight change on follow-up. The first ARR quartile, Q1, was used as the referent category. Three dummy variables of ARR corresponding to Q2, Q3, and Q4 were included in the multivariable models of quartiles, and in the trend analysis across quartiles, the ARR quartile variable (coded 0, 1, 2, and 3) was included. 1 SD of logARR = 0.7.

*Age- and sex-adjusted.

which hypertensive subjects were randomly assigned to ACE inhibitor and β-blocker therapy.[19] We noted significant heterogeneity in the proportion of hypertensive participants exceeding 1 among many thresholds proposed for PH screening (ARR >26 ng/L per mU/L), according to their sex and treatment status.[49] Given the differences in ARR by hypertension and treatment status, as well as by multiple covariates, the use of a single threshold in diagnostic testing of population-based samples may not be appropriate.

We demonstrated the heritability of multivariable-adjusted ARR to be 0.40, higher than for aldosterone[34] or renin alone. We are unaware of other studies that have sought to quantify the heritability of ARR in unselected individuals in the community, although familial aggregation of hyperaldosteronism has been demonstrated (eg, familial hyperaldosteronism type II).[53] Evidence of substantial heritability of plasma renin activity has been reported in some studies,[54,55] but other investigators have reported more modest evidence that heritable factors influence renin.[56,57]

We observed modest evidence that a locus on chromosome 11p is linked to ARR (multivariable-adjusted ARR LOD score 1.89) and 5p (LOD 1.60). The *REN* and *CYP11B2* (aldosterone synthase gene) loci, residing on different chromosomes, had no significant linkage signals. A review of known genes under the 2 linkage peaks with LOD>1.5 revealed few obvious candidate genes on the basis of involvement in steroid metabolism or renal physiology: natriuretic peptide receptor C/guanylate cyclase (*NPR3*, chromosome 5) and 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (*HMGCS1*, chromosome 5).[58] However, the possibility of a false-positive result cannot be excluded. Replication in other samples would be essential before further examination of candidate genes under any linkage peak would be warranted. We note that, in linkage analysis without adjustment for ACE inhibitor or β-blocker use, we observed suggestive evidence of linkage to a region on chromosome 7p that has been linked previously to ARR in families with familial hyperaldosteronism II or nonglucocorticoid remediable hyperaldosteronism.[59,60] However, the statistical support for linkage to this locus was markedly attenuated when we adjusted for use of specific antihypertensive therapy. The marked attenuation in the linkage signal observed with exclusion of ACE inhibitor users supports familial aggregation of medication use as a potential cause of the linkage signal that we observed in analyses that did not account for medication use in the regression models. We are unaware of any previous linkage study of ARR in an unselected community-based sample that included a wide spectrum of hypertensive and nonhypertensive subjects.

We observed no clear association of 17 common genetic variants across the *REN* locus with ARR, although small effects could have been missed. Our study of common variants cannot assess the possibility that rare variants at this locus contribute to ARR. Moreover, the lack of linkage to the *REN* locus does not exclude a possible influence of rare or common variants on ARR given the limited statistical power of linkage for complex traits.[61]

Among nonhypertensive participants, we observed a strong and graded influence of baseline ARR on the risk of BP progression and incident hypertension on 3-year follow-up.

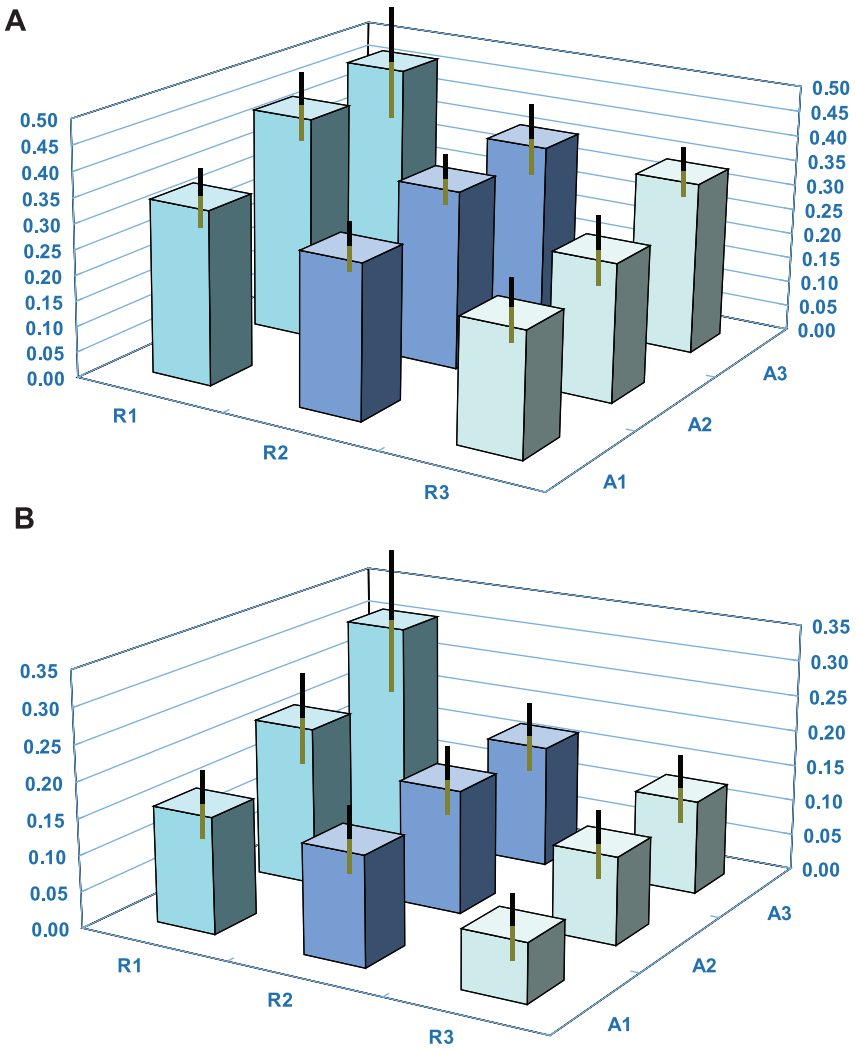


Figure 3. Rates of BP progression and incident hypertension among nonhypertensive participants. Age- and sex-adjusted incidence rates of BP progression (A) and incident hypertension (B) at a mean of 3 years are shown across tertiles of aldosterone (A1 to 3=first to third tertiles) and renin (R1 to 3=first to third tertiles). Graded, continuous increases in the risk of BP progression and incident hypertension are found across increasing aldosterone and decreasing renin tertiles. Olive and black error bars represent lower and upper 95% confidence bounds.

These findings extend our earlier report linking serum aldosterone assessed alone to the risk of BP progression and hypertension.[30] We observed that aldosterone and renin independently and jointly predict future BP progression and hypertension and that this effect is not driven by 1 hormone alone. The temporal relationship suggests that the renin–angiotensin system contributes to the development of hypertension in nonhypertensive individuals, but our study cannot exclude the possibility that ARR is simply a marker (as opposed to a risk factor) of incipient hypertension. Examination of the role of the renin–angiotensin system in BP change over time could yield insights into BP physiology and suggest novel targets for preventive strategies. We are not aware of any published reports linking ARR to hypertension incidence in the community.

The large, community-based sample, the adjustment for multiple clinical covariates, the comprehensive evaluation of heritability, linkage and association with variation at the *REN* locus, and the longitudinal follow-up to assess prognostic significance of ARR strengthen our investigation. Nonetheless, several limitations must be acknowledged. We examined ambulatory ARR measurements in people on a random sodium diet who were not withdrawn from antihypertensive medications. The constraints of a large, longitudinal observational cohort precluded a longer duration in the supine position, standardization of sodium intake, or medication withdrawal. However, not controlling these factors would be expected to bias our findings toward the null rather than toward the false inference of association. Our study reflects a community-based sample and is not directly comparable to the examination of hypertensive subjects in a research setting to diagnose hyperaldosteronism. The middle-age-to-elderly composition and predominant European ancestry of our cohort may limit the generalizability of our results to younger individuals or those of different ancestry; racial differences in serum aldosterone and renin values have been reported.[9] We tested for association with SNPs at the *REN* locus but not at the *CYP11B2* (aldosterone synthase) locus; a comprehensive set of SNP genotypes at *CYP11B2* was not available. The finding that ARR predicts BP progression and incident hypertension does not address the use of population-based screening of nonhypertensive subjects, which would require studies designed to address this specific question. Lastly, our community-based study cannot address the role of ARR for identifying individuals with PH, because we did not carry out further diagnostic testing beyond the assessment of ARR.

TABLE 4. Comparison of Models With Aldosterone and Renin Separately and Jointly Predicting BP Progression and Incident Hypertension in Nonhypertensive Participants at 3 Years

Model	Term	β (SE)	OR (95%CI)	*P*	C Statistic
BP Progression					
Aldo alone					
Age, sex	logaldo	0.27 (0.10)	1.32 (1.08 to 1.60)	0.006	0.58
Multivariable	logaldo	0.30 (0.11)	1.35 (1.10 to 1.67)	0.004	0.68
Renin alone					
Age, sex	logrenin	−0.23 (0.07)	0.79 (0.69 to 0.91)	0.001	0.58
Multivariable	logrenin	−0.16 (0.07)	0.86 (0.74 to 0.99)	0.04	0.68
Aldo, renin both					
Age, sex	logaldo	0.44 (0.11)	1.56 (1.26 to 1.93)	<0.0001	0.60
Age, sex	logrenin	−0.33 (0.08)	0.72 (0.62 to 0.84)	<0.0001	
Multivariable	logaldo	0.43 (0.12)	1.54 (1.23 to 1.93)	0.0002	0.69
Multivariable	logrenin	−0.25 (0.08)	0.78 (0.66 to 0.91)	0.002	
ARR					
Age, sex	logARR	0.36 (0.07)	1.43 (1.24 to 1.65)	<0.0001	0.60
Multivariable	logARR	0.30 (0.08)	1.35 (1.16 to 1.57)	0.0001	0.68
Incident hypertension					
Aldo alone					
Age, sex	logaldo	0.32 (0.13)	1.37 (1.06 to 1.77)	0.02	0.65
Multivariable	logaldo	0.34 (0.15)	1.40 (1.05 to 1.88)	0.02	0.82
Renin alone					
Age, sex	logrenin	−0.23 (0.09)	0.80 (0.67 to 0.95)	0.01	0.65
Multivariable	logrenin	−0.03 (0.10)	0.97 (0.80 to 1.18)	0.77	0.82
Aldo, renin both					
Age, sex	logaldo	0.52 (0.14)	1.69 (1.27 to 2.23)	0.0003	0.66
Age, sex	logrenin	−0.38 (0.10)	0.69 (0.56 to 0.84)	0.0002	
Multivariable	logaldo	0.42 (0.16)	1.52 (1.11 to 2.08)	0.01	0.82
Multivariable	logrenin	−0.14 (0.11)	0.87 (0.70 to 1.08)	0.19	
ARR					
Age, sex	logARR	0.41 (0.09)	1.51 (1.25 to 1.82)	<0.0001	0.66
Multivariable	logARR	0.20 (0.10)	1.23 (1.00 to 1.50)	0.05	0.82

The multivariable model was adjusted for age, baseline BP stage, systolic BP, diastolic BP, smoking status, diabetes, creatinine, body mass index, and percentage of weight change on follow-up. OR indicates odds ratio; logaldo, log-aldosterone.

Perspectives

In our large, community-based sample, ARR varied with some clinical characteristics, suggesting that these features be taken into consideration when interpreting ARR values in ambulatory settings. Genetic factors influence ARR as evidenced by significant heritability; ongoing and planned genome-wide genotyping in the Framingham Heart Study may identify variants that influence ARR. We found that ARR has a higher heritability than either aldosterone or renin alone and that aldosterone and renin are independent predictors of BP progression and incident hypertension. The accruing evidence that interindividual variation in ARR predicts BP progression, hypertension, and myocardial infarction[62,63] raises the possibility that identifying genetic and nongenetic contributors to ARR could refine our understanding of the biology of the renin–angiotensin system, identify novel therapeutic targets, and improve risk prediction.

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Disclosures

None.

References

1. Conn JW. Presidential address. I. Painting background. II. Primary aldosteronism, a new clinical syndrome. *J Lab Clin Med*. 1955;45:3–17.
2. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC. High incidence of primary aldosteronism in 199 patients referred with hypertension. *Clin Exp Pharmacol Physiol*. 1994;21:315–318.

3. Lim PO, Rodgers P, Cardale K, Watson AD, MacDonald TM. Potentially high prevalence of primary aldosteronism in a primary-care population. *Lancet*. 1999;353:340.
4. Olivieri O, Ciacciarelli A, Signorelli D, Pizzolo F, Guarini P, Pavan C, Corgnati A, Falcone S, Corrocher R, Micchi A, Cressoni C, Blengio G. Aldosterone to renin ratio in a primary care setting: the Bussolengo study. *J Clin Endocrinol Metab*. 2004;89:4221–4226.
5. Lim PO, MacDonald TM. Primary aldosteronism, diagnosed by the aldosterone to renin ratio, is a common cause of hypertension. *Clin Endocrinol (Oxf)*. 2003;59:427–430.
6. Kaplan NM. The current epidemic of primary aldosteronism: causes and consequences. *J Hypertens*. 2004;22:863–869.
7. Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L, Gomez-Sanchez CE, Veglio F, Young WF Jr. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab*. 2004;89:1045–1050.
8. Weidmann P, Beretta-Piccoli C, Ziegler WH, Keusch G, Gluck Z, Reubi FC. Age versus urinary sodium for judging renin, aldosterone, and catecholamine levels: studies in normal subjects and patients with essential hypertension. *Kidney Int*. 1978;14:619–628.
9. James GD, Sealey JE, Muller F, Alderman M, Madhavan S, Laragh JH. Renin relationship to sex, race and age in a normotensive population. *J Hypertens*. 1986;4(suppl):S387–S389.
10. Tsunoda K, Abe K, Goto T, Yasujima M, Sato M, Omata K, Seino M, Yoshinaga K. Effect of age on the renin-angiotensin-aldosterone system in normal subjects: simultaneous measurement of active and inactive renin, renin substrate, and aldosterone in plasma. *J Clin Endocrinol Metab*. 1986;62:384–389.
11. Crane MG, Harris JJ. Effect of aging on renin activity and aldosterone excretion. *J Lab Clin Med*. 1976;87:947–959.
12. Haenni A, Reneland R, Lind L, Lithell H. Serum aldosterone changes during hyperinsulinemia are correlated to body mass index and insulin sensitivity in patients with essential hypertension. *J Hypertens*. 2001;19:107–112.
13. Umemura S, Nyui N, Tamura K, Hibi K, Yamaguchi S, Nakamaru M, Ishigami T, Yabana M, Kihara M, Inoue S, Ishii M. Plasma angiotensinogen concentrations in obese patients. *Am J Hypertens*. 1997;10:629–633.
14. Goodfriend TL, Egan BM, Kelley DE. Aldosterone in obesity. *Endocr Res*. 1998;24:789–796.
15. Weidmann P, Hirsch D, Beretta-Piccoli C, Reubi FC, Ziegler WH. Interrelations among blood pressure, blood volume, plasma renin activity and urinary catecholamines in benign essential hypertension. *Am J Med*. 1977;62:209–218.
16. Lammintausta R, Lammintausta K. The renin-aldosterone system in low-dose chlorothiazide treatment of hypertensive subjects. *Int J Clin Pharmacol Ther Toxicol*. 1980;18:329–331.
17. Lijnen P, Fagard R, Staessen J, Amery A. Effect of chronic diuretic treatment on the plasma renin-angiotensin-aldosterone system in essential hypertension. *Br J Clin Pharmacol*. 1981;12:387–392.
18. Montori VM, Schwartz GL, Chapman AB, Boerwinkle E, Turner ST. Validity of the aldosterone-renin ratio used to screen for primary aldosteronism. *Mayo Clin Proc*. 2001;76:877–882.
19. Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, Veglio F. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension*. 2002;40:897–902.
20. Schunkert H, Danser AH, Hense HW, Derkx FH, Kurzinger S, Riegger GA. Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women. *Circulation*. 1997;95:39–45.
21. Zacharieva S, Shigarminova R, Nachev E, Orbetzova M, Genov N, Kamenov Z, Atanassova I, Stoynev A, Doncheva N, Borissova AM, Zingilev D. Ambulatory blood pressure monitoring and active renin in menopausal women treated with amlodipine and hormone replacement therapy. *Gynecol Endocrinol*. 2004;19:26–32.
22. Zacharieva S, Kirilov G, Kalinov K, Shigarminova R, Nachev E, Orbetzova M, Atanassova I. Effect of different hormone replacement therapy regimens on circadian blood pressure profile and active renin in postmenopausal women. *Gynecol Endocrinol*. 2002;16:461–467.
23. Steingold KA, Matt DW, DeZiegler D, Sealey JE, Fratkin M, Reznikov S. Comparison of transdermal to oral estradiol administration on hormonal and hepatic parameters in women with premature ovarian failure. *J Clin Endocrinol Metab*. 1991;73:275–280.
24. Lind L, Lithell H, Wide L, Ljunghall S. Metabolic cardiovascular risk factors and the renin-aldosterone system in essential hypertension. *J Hum Hypertens*. 1992;6:27–29.
25. Goodfriend TL, Egan B, Stepniakowski K, Ball DL. Relationships among plasma aldosterone, high-density lipoprotein cholesterol, and insulin in humans. *Hypertension*. 1995;25:30–36.
26. Tiu SC, Choi CH, Shek CC, Ng YW, Chan FK, Ng CM, Kong AP. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. *J Clin Endocrinol Metab*. 2005;90:72–78.
27. Helmer OM. Renin activity in blood from patients with hypertension. *Can Med Assoc J*. 1964;90:221–225.
28. He J, Klag MJ, Appel LJ, Charleston J, Whelton PK. The renin-angiotensin system and blood pressure: differences between blacks and whites. *Am J Hypertens*. 1999;12:555–562.
29. Luft FC, Rankin LI, Bloch R, Weyman AE, Willis LR, Murray RH, Grim CE, Weinberger MH. Cardiovascular and humoral responses to extremes of sodium intake in normal black and white men. *Circulation*. 1979;60:697–706.
30. Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, Benjamin EJ, Levy D. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med*. 2004;351:33–41.
31. Himmelmann A, Bergbrant A, Svensson A, Hansson L, Aurell M. Remikiren (Ro 42-5892)–an orally active renin inhibitor in essential hypertension. Effects on blood pressure and the renin-angiotensin-aldosterone system. *Am J Hypertens*. 1996;9:517–522.
32. Gradman AH, Schmieder RE, Lins RL, Nussberger J, Chiang Y, Bedigian MP. Aliskiren, a novel orally effective renin inhibitor, provides dose-dependent antihypertensive efficacy and placebo-like tolerability in hypertensive patients. *Circulation*. 2005;111:1012–1018.
33. Kannel WB, Feinleib M, McNamara PM. An investigation of coronary heart disease in families: the Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281–290.
34. Kathiresan S, Larson MG, Benjamin EJ, Corey D, Murabito JM, Fox CS, Wilson PW, Rifai N, Meigs JB, Ricken G, Lifton RP, Levy D, Vasan RS. Clinical and genetic correlates of serum aldosterone in the community: the Framingham Heart Study. *Am J Hypertens*. 2005;18:657–665.
35. The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Intern Med*. 1997;157:2413–2446.
36. Cupples LA, D'Agostino R. Some risk factors related to the annual incidence of cardiovascular disease and death using pooled repeated biennial measurements: Framingham Study, 30-year follow-up. In: *The Framingham Heart Study: An Epidemiological Investigation of Cardiovascular Disease Section 34.* Kannel WB, Wolf PA, Garrison RJ, eds. Washington, DC: Government Printing Office; 1987.
37. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. *Science*. 2002;296:2225–2229.
38. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2004;21:263–265.
39. Stram DO, Haiman CA, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Pike MC. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered*. 2003;55:27–36.
40. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. *Hypertension*. 2000;36:477–483.
41. Kleinbaum DG, Kupper LL, Muller KE. *Applied Regression Analysis and Other Multivariable Methods.* 2nd ed. Boston, MA: PWS-Kent Publishing Company; 1988.
42. Liang KY, Zeger SL. Longitudinal data analysis using generalized estimating linear models. *Biometrika*. 1986;73:12–22.
43. SAS Institute. *SAS/STAT User's Guide, Version 8.1*. Cary, NC: SAS Institute; 2000.
44. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet*. 1998;62:1198–1211.
45. Pratt SC, Daly MJ, Kruglyak L. Exact multipoint quantitative-trait linkage analysis in pedigrees by variance components. *Am J Hum Genet*. 2000;66:1153–1157.
46. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet*. 2002;70:425–434.

47. Hosmer DW, Lemeshow S. *Applied Logistic Regression.* New York, NY: John Wiley and Sons; 1989.
48. Vasan RS, Larson MG, Leip EP, Kannel WB, Levy D. Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham Heart Study: a cohort study. *Lancet*. 2001;358: 1682–1686.
49. Perschel FH, Schemer R, Seiler L, Reincke M, Deinum J, Maser-Gluth C, Mechelhoff D, Tauber R, Diederich S. Rapid screening test for primary hyperaldosteronism: ratio of plasma aldosterone to renin concentration determined by fully automated chemiluminescence immunoassays. *Clin Chem*. 2004;50:1650–1655.
50. Kaplan NM, Kem DC, Holland OB, Kramer NJ, Higgins J, Gomez-Sanchez C. The intravenous furosemide test: a simple way to evaluate renin responsiveness. *Ann Intern Med*. 1976;84:639–645.
51. Weinberger MH, Dowdy AJ, Nokes GW, Luetscher JA. Plasma renin activity and aldosterone secretion in hypertensive patients during high and low sodium intake and administration of diuretic. *J Clin Endocrinol Metab*. 1968;28:359–371.
52. Lebel M, Grose JH, Belleau LJ. Dissociation between renin and aldosterone during chronic diuretic therapy. Effect of the addition of a beta blocker, timolol. *J Clin Pharmacol*. 1979;19:424–427.
53. Stowasser M, Gordon RD. Primary aldosteronism: learning from the study of familial varieties. *J Hypertens*. 2000;18:1165–1176.
54. Grim CE. Evolution of diagnostic criteria for primary aldosteronism: why is it more common in "drug-resistant" hypertension today? *Curr Hypertens Rep*. 2004;6:485–492.
55. Kotchen TA, Kotchen JM, Grim CE, George V, Kaldunski ML, Cowley AW, Hamet P, Chelius TH. Genetic determinants of hypertension: identification of candidate phenotypes. *Hypertension*. 2000;36:7–13.
56. Williams RR, Hasstedt SJ, Hunt SC, Wu LL, Hopkins PN, Berry TD, Stults BM, Barlow GK, Kuida H. Genetic traits related to hypertension and electrolyte metabolism. *Hypertension*. 1991;17:I69–I73.
57. Rossi GP, Narkiewicz K, Cesari M, Winnicki M, Bigda J, Chrostowska M, Szczech R, Pawlowski R, Pessina AC. Genetic determinants of plasma ACE and renin activity in young normotensive twins. *J Hypertens*. 1999;17:647–655.
58. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. The human genome browser at UCSC. *Genome Res*. 2002; 12:996–1006.
59. So A, Duffy DL, Gordon RD, Jeske YW, Lin-Su K, New MI, Stowasser M. Familial hyperaldosteronism type II is linked to the chromosome 7p22 region but also shows predicted heterogeneity. *J Hypertens*. 2005;23:1477–1484.
60. Lafferty AR, Torpy DJ, Stowasser M, Taymans SE, Lin JP, Huggard P, Gordon RD, Stratakis CA. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *J Med Genet*. 2000;37:831–835.
61. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science*. 1996;273:1516–1517.
62. Brunner HR, Laragh JH, Baer L, Newton MA, Goodwin FT, Krakoff LR, Bard RH, Buhler FR. Essential hypertension: renin and aldosterone, heart attack and stroke. *N Engl J Med*. 1972;286:441–449.
63. Alderman MH, Madhavan S, Ooi WL, Cohen H, Sealey JE, Laragh JH. Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension. *N Engl J Med*. 1991;324:1098–1104.