Genome-Wide Meta-Analyses of Plasma Renin Activity and Concentration Reveal Association With the Kininogen 1 and Prekallikrein Genes

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Background—The renin–angiotensin–aldosterone system (RAAS) is critical for regulation of blood pressure and fluid balance and influences cardiovascular remodeling. Dysregulation of the RAAS contributes to cardiovascular and renal morbidity. The genetic architecture of circulating RAAS components is incompletely understood.

Methods and Results—We meta-analyzed genome-wide association data for plasma renin activity (n=5275), plasma renin concentrations (n=8014), and circulating aldosterone (n=13289) from ≤4 population-based cohorts of European and European-American ancestry, and assessed replication of the top results in an independent sample (n=6487). Single-nucleotide polymorphisms (SNPs) in 2 independent loci displayed associations with plasma renin activity at genome-wide significance ($P<5\times10^{-8}$). A third locus was close to this threshold (rs4253311 in *kallikrein B* [KLKB1], $P=5.5\times10^{-8}$). Two of these loci replicated in an independent sample for both plasma renin and aldosterone concentrations (SNP rs5030062 in *kininogen 1* [KNG1]: $P=0.001$ for plasma renin, $P=0.024$ for plasma aldosterone concentration; and rs4253311 with $P<0.001$ for both plasma renin and aldosterone concentration). SNPs in the *NEBL* gene reached genome-wide significance for plasma renin concentration in the discovery sample (top SNP rs3915911; $P=8.81\times10^{-9}$), but did not replicate ($P=0.81$). No locus reached genome-wide significance for aldosterone. SNPs rs5030062 and rs4253311 were not related to blood pressure or renal traits; in a companion study, variants in the *kallikrein B locus* were associated with B-type natriuretic peptide concentrations in blacks.

Conclusions—We identified 2 genetic loci (*kininogen 1* and *kallikrein B*) influencing key components of the RAAS, consistent with the close interrelation between the kallikrein–kinin system and the RAAS. **(*Circ Cardiovasc Genet*. 2015;8:131-140. DOI: 10.1161/CIRCGENETICS.114.000613.)**

Key Words: aldosterone ■ genome-wide association study ■ renin-angiotensin system

The renin–angiotensin–aldosterone system (RAAS) is a central pathway in cardiovascular and renal physiology. Through a series of enzymatic reactions, the liver-derived protein angiotensinogen is transformed into angiotensin I (this conversion is catalyzed by renin) and subsequently into angiotensin II, which is the key effector of the RAAS, mediating multiple biological effects in the kidneys, the heart, and the vasculature through its local and systemic effects.[1–4] Angiotensin II is a potent vasoconstrictor and adversely affects cardiac and vascular remodeling.[1–3] On a parallel note, angiotensin II stimulates the production of aldosterone in the adrenal glands, which enhances sodium and water reabsorption in

Received March 14, 2014; accepted October 27, 2014.

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Guest Editor for this article was Päivi Pajukanta, MD, PhD.

The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.114.000613/-/DC1.

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the kidneys.[1,2] Thus, the RAAS is a major determinant of fluid and electrolyte hemostasis, blood pressure (BP) regulation and cardiovascular remodeling.[1,3] Consequently, RAAS activity influences the development and progression of cardiovascular disease,[1,2] and pharmacological inhibition of the RAAS has been shown to improve patient outcomes in a variety of clinical settings.[2] Moreover, recent studies suggested a cross talk between adipose tissue and the adrenal gland, and high aldosterone concentrations were reported to be associated with the metabolic syndrome and type 2 diabetes mellitus.[5]

Clinical Perspective on p 140

Despite its clinical significance, the genetic architecture of the RAAS, as evident in circulating levels of its components, is incompletely understood. Previous studies have established different circulating components of the RAAS as heritable traits.[6,7] To improve our understanding of the genetic determinants of the RAAS, we conducted a genome-wide association analysis of plasma renin (investigated by either its activity or concentration) and circulating aldosterone concentrations in ≤4 population-based cohorts with replication in a fifth independent cohort. Given the reported heritability of RAAS components,[6,7] we hypothesized that these circulating RAAS biomarkers will be associated with common genetic variation in the general population.

Methods

Please find more detailed information about sample description and biomarker measurements in the Data Supplement.

Study Samples

Framingham Heart Study (FHS) Samples

RAAS biomarkers were measured at examination cycle 6 (1995–1998) of the Framingham offspring cohort (Generation 2 [Gen 2])[8] and at the first examination cycle (2002–2005) of the Third generation cohort (Generation 3 [Gen 3]).[9] At each Heart Study visit, participants were comprehensively characterized with respect to cardiovascular risk factors and subclinical disease measures. All participants provided written informed consent, and the study protocol was approved by the institutional review board at the Boston University Medical Center.

Cooperative Health Research in the Region of Augsburg (KORA) Sample

KORA comprises several population-based cohort studies in the region of Augsburg, Southern Germany.[10] This analysis includes data from the follow-up examination KORA F4 (2006–2008) of the KORA S4 survey (1999/2000).[10] Participants with missing genotype or phenotype data were excluded from the present analyses, as were participants reporting intake of diuretics or participants with a renin or aldosterone concentration of >1000 ng/L. The final study sample comprised 1786 participants. The studies were approved by the ethics committee of the Bavarian Medical Association (Bayerische Landesärztekammer) and all study participants gave written informed consent.

Study of Health in Pomerania Sample

Study of Health in Pomerania (SHIP) is a population-based cohort study in West Pomerania, the north-east area of Germany.[11] The first follow-up examination (SHIP-1) was conducted from March 2003 to July 2006.[11] Participants were comprehensively phenotyped with respect to cardiometabolic traits and subclinical disease burden, as detailed elsewhere.[11] All participants gave written informed consent. The study protocol was approved by the Ethics Committee of the University of Greifswald.

Supplémentation en Vitamines et Minéraux Antioxydants (SUVIMAX) Study Sample

Participants of the SUVIMAX study were healthy volunteers free of hypertension, cardiovascular disease, or cancer at baseline.[12] The recruitment of the SUVIMAX cohort was performed in metropolitan France, and all individuals were of European descent. RAAS biomarkers and genetic information were available in a subsample of 1518 participants.

Prevention of REnal and Vascular ENd-stage Disease (PREVEND) Sample

PREVEND is a general population sample from the city of Groningen (The Netherlands). This longitudinal study was primarily designed to assess the association between urinary albumin excretion and cardiovascular and renal diseases.[13] The study design has been described in detail elsewhere.[14] Basic vascular risk factors were determined on 2 occasions in the examination center.[14] The sample for this analysis comprised 6487 participants.

RAAS Measurements

Measurements of Renin and Aldosterone

In FHS, KORA, and SUVIMAX, blood samples were taken in the early morning from seated participants after an overnight fast. In PREVEND, fasting blood samples were taken between 8 AM and 4 PM. In SHIP, blood was drawn throughout the day (between 8 AM and 8 PM) in nonfasting participants, while they were taking their regular medication. Aldosterone measurements were available in all cohorts (FHS Gen 2 and Gen 3, KORA, SHIP, SUVIMAX, and PREVEND; Figure 1); plasma renin concentrations (PRCs) were measured in the FHS Gen 2, KORA, SHIP, and PREVEND samples; plasma renin activity (PRA) was determined in the FHS Gen 3 and SUVIMAX samples (Figure 1). Details on the methods to determine RAAS biomarkers in the different samples are provided in the Data Supplement.

Genotyping

All cohorts were genotyped using genome-wide arrays from Affymetrix or Illumina. Details on the specific arrays used and the quality control measures applied to genetic data are detailed in the Data Supplement.

Statistical Analysis

Meta-Analysis of Genome-Wide Association Data and Replication

The basic study design is displayed in Figure 1. The biomarkers were natural logarithmically transformed before analyses. Within each cohort, the association between ≈2.5 million single-nucleotide polymorphisms (SNPs; exposure) and

biomarkers of the RAAS were assessed using linear regression models adjusted for age and sex, assuming an additive genetic model. Aldosterone levels, PRC, and PRA served as dependent variables (each biomarker considered separately). In the FHS sample, a linear mixed effects model was used to adjust for familial correlation.[15] For aldosterone levels, genome-wide association results from Gen 2 and Gen 3 cohorts were combined by using Obrien method[16] because aldosterone measurements were correlated between Gen 2 and Gen 3. The Genome-Wide Association Study (GWAS) data from ≤4 cohorts (FHS, KORA, SHIP, and SUVIMAX) were meta-analyzed with a sample size–weighted approach (combining sample size–weighted *Z* statistics from each cohort) using the METAL software.[17]

Corrections for genomic controls were performed within each cohort, and after the meta-analyses. To display the association results from the meta-analyses for each biomarker graphically, we plotted the *P* value of each SNP versus its chromosomal position (Manhattan plot). Furthermore, we also plotted the observed versus the expected *P*-value distribution under the null hypothesis of no association between biomarker and SNPs (quantile–quantile plots) for each biomarker. Genetic variants with a minor allele frequency (MAF) >0.05, imputation quality ratio >0.75, and a $P \leq 5 \times 10^{-8}$ for any of the 3 biomarkers were assessed for replication in the PREVEND sample. In PREVEND, only PRC and plasma aldosterone levels were available. Thus, the top loci associated with each RAAS biomarker in the meta-analyses of FHS, KORA, SHIP, and SUVIMAX were tested for association with PRCs and aldosterone levels in PREVEND using a linear regression model adjusting for age and sex (Figure 1). Furthermore, because PRC and aldosterone levels were also available in the discovery samples (Figure 1), we performed a look-up of the top renin activity SNPs with respect to renin and aldosterone concentrations in the individual discovery cohorts.

Finally, we performed fine mapping of the top regions associated with PRA (because these regions reached or were close to genome-wide significance) and of the candidate genes *REN* and *CYP11B2* (±500 kb around the gene) based on imputations to the 1000 genome data set. These 1000 genome imputations were available in FHS, SHIP, and KORA.

Look-Up of Top SNPs in Blacks

We performed a look-up of our top loci, associated with PRA or PRCs in Europeans, to assess their associations with PRA, renin concentration, and B-type natriuretic peptide levels in blacks in the Jackson Heart Study.[18]

Association of Top SNPs With BP Traits, Renal Traits, and Echocardiographic Left Ventricular Mass in Europeans

In secondary analyses, we tested the successfully replicated SNPs from our GWAS (rs5030062 and rs4253311) for associations with traits known to be influenced by the RAAS, that is, BP, left ventricular mass, and renal traits. For BP, we related our top SNPs to systolic and diastolic BP in the International Consortium for Blood Pressure Genome-Wide Association Studies.[19] We also assessed the association of these 2 SNPs with left ventricular mass in the EchoGen Consortium,[20] and with renal traits in the CKDGen Consortium[21,22] (Figure 1). In the latter consortium, we tested specifically the association of these SNPs with the estimated glomerular filtration rate, with the urinary albumin:creatinine ratio, and with chronic kidney disease (as binary trait).[21,22]

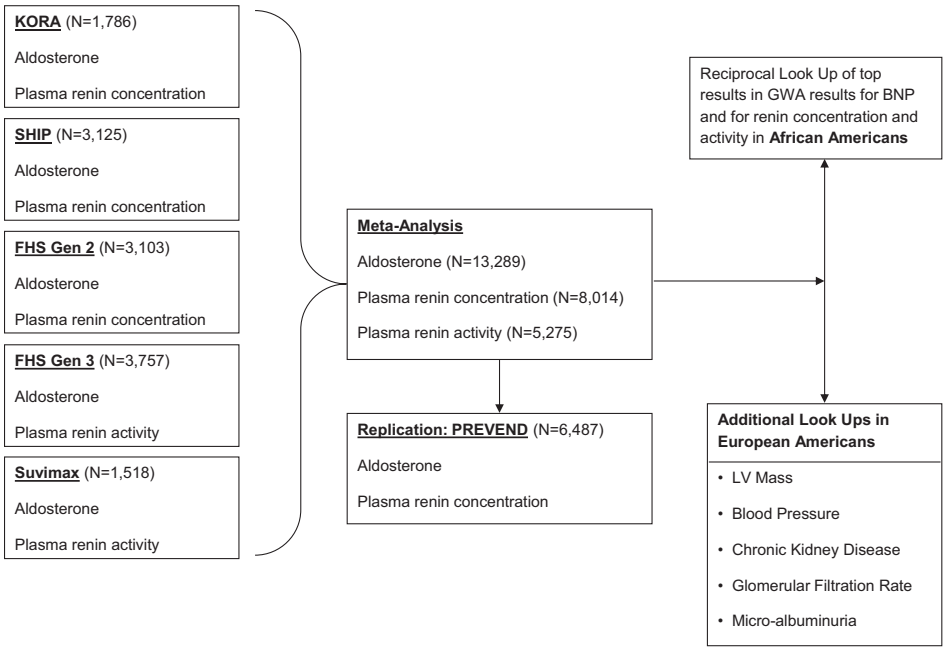

Figure 1. Basic study design. BNP indicates B-type natriuretic peptide; FHS, Framingham Heart Study; Gen, generation; GWA, Genome-Wide Association; KORA, Cooperative Health Research in the Region of Augsburg; LV Mass, left ventricular mass; PREVEND, Prevention of REnal and Vascular ENd-stage Disease; SHIP, Study of Health in Pomerania; and SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants.

Pathway Analyses

Pathway analyses provide a potential route to investigate the collective effects of multiple genetic variants on biological systems. For each genetic variant, we assigned an overall SNP score to indicate its association with RAAS-related traits. This SNP score was equivalent to the most significant *P* value among the 3 RAAS traits examined (PRC, PRA, and circulating aldosterone levels). The genetic variants with the score assigned were then mapped back to the human reference genome (NCBI Build 36, 2006) and we examined their locations relative to RefSeq genes (March 17, 2013), and gene scores were obtained. The gene score was defined as the most significant variant that was located within 110 kb upstream and 40 kb downstream of the gene's most extreme transcript boundaries. We selected these boundaries because they are expected to encompass the majority of *cis*-expression quantitative trait loci based on expression data.[23] Similar boundaries have been used in earlier studies.[24,25] Of the 23 696 genes evaluated, 548 reached a score $<1.0\times10^{-4}$. These genes were then imported into Ingenuity for pathway analyses (Ingenuity Systems, Redwood, CA). Fisher exact test was used to justify the enrichment of each of the canonical pathways.

Results

The discovery sample size comprised 13 289 participants for circulating aldosterone concentrations, 8014 participants for PRCs, and 5275 participants for PRA. Baseline clinical and biochemical characteristics of the study sample are shown in Table 1.

GWAS Meta-Analysis and Replication for Plasma Renin Activity, Plasma Renin Concentrations, and Circulating Aldosterone Levels

We identified 2 independent loci that were associated with PRA at a genome-wide significant level ($P<5\times10^{-8}$). A third locus was close to this threshold (tagged by rs4253311; $P=5.5\times10^{-8}$). The respective Manhattan plot and the quantile–quantile plot for PRA are shown in Figure 2A and 2B. The quantile–quantile plot for PRA in the FHS Gen 3 sample only is provided in Figure I in the Data Supplement. The top SNPs were rs12374220, an intronic variant in the *TENM3* gene, rs5030062 in intron 6 of the *kininogen 1 [KNG1]* gene, and rs4253311 in intron 11 of the *kallikrein B[KLKB1]* gene (Tables 2 and 3). The 2 latter SNPs could successfully be replicated in an independent sample (PREVEND), that is, they showed nominal statistically significant associations with PRCs and circulating aldosterone levels in PREVEND (Table 3; PRA was not available in PREVEND). However, except for an association of rs5030062 with aldosterone levels in KORA, SNPs rs12374220, rs5030062, and rs4253311 provided no evidence for association with renin concentrations (Table I in the Data Supplement) and aldosterone levels (Table II in the Data Supplement) in the individual discovery cohorts.

Regional plots of the *kallikrein B* locus and the *kininogen 1* locus are shown in **F**igure 3A and 3B, respectively. Analyzing these 2 regions using a data set imputed to the 1000 genome data[26] revealed essentially similar results. Regional plots based on the 1000 genome imputations are shown in Figure IIA and IIB in the Data Supplement.

SNP rs5030062 and rs4253311 explained 0.85% and 0.87% of PRA variance, respectively. PRA, stratified by rs5030062 genotype (Figure IIIA in the Data Supplement) and stratified by rs4253311 genotype (Figure IIIB in the Data Supplement), are displayed. Top loci associated with PRA stratified by sample, for example, in the FHS Gen 3 sample and in the SUVIMAX sample, are provided in Table III in the Data Supplement. The association results for all 3 SNPs were consistent in directionality in FHS and SUVIMAX but reached a higher level of statistical significance in FHS than in SUVIMAX, possibly because of the much larger sample size in FHS.

The Manhattan plot (Figure IVA in the Data Supplement) and the quantile–quantile plot (Figure IVB in the Data Supplement) for the genome-wide analysis for PRC are shown. SNPs in the *NEBL* gene reached genome-wide significance for PRC

Table 1. Baseline Characteristics of the Contributing Cohorts

Characteristics	FHS: Gen 2 (n=3103)	FHS: Gen 3 (n=3757)	KORA (n=1786)	SHIP (n=3125)	SUVIMAX (n=1518)	PREVEND (n=6487)
Age, y	59 (10)	40 (9)	61 (9)	54 (15)	51 (6)	50 (13)
Women, %	53.2%	53.3%	51.3%	51.2%	60.4%	50.5%
SBP, mm Hg	128 (19)	117 (14)	125 (19)	133 (20)	121 (12)	130 (21)
DBP, mm Hg	75 (9)	75 (10)	76 (10)	82 (11)	78 (8)	74 (10)
Hypertension, %	30.4%	16.5%	45.8%	50.7%	18.5%	34.7%
Diabetes mellitus, %	10.9%	3.0%	10.4%	10.9%	0.3%	3.7%
Prevalent CVD, %	10.8%	0.9%	3.6%	7.9%	0.0%	4.0%
eGFR, mL/min per 1.73 m^2	91 (78)	99 (18)	82 (18)	85 (21)	NA	80 (14)
eGFR, <60 mL/min per 1.73 m^2, %	8.4%	0.5%	7.4%	9.8%	NA	6.3%
Aldosterone, ng/L	115.9 (73.9)	128.2 (73.3)	45.7 (31.8)	51.5 (38.2)	132 (89)	132.6 (67.7)
PRC, ng/L	28.5 (113.6)	NA	23.0 (50.3)	16.5 (35.0)	NA	24.8 (31.8)
PRA, ng (angiotensin)/mL per hour	NA	2.38 (3.56)	NA	NA	1.97 (1.30)	NA

Data are mean (SD) or percent. CVD indicates cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FHS, Framingham Heart Study; Gen 2, offspring cohort; Gen 3, third Generation cohort; KORA, Cooperative Health Research in the Region of Augsburg; NA, not available; PRA, plasma renin activity; PRC, plasma renin concentration; PREVEND, Prevention of REnal and Vascular ENd-stage Disease; SBP, systolic blood pressure; SHIP, Study of Health in Pomerania; and SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants.

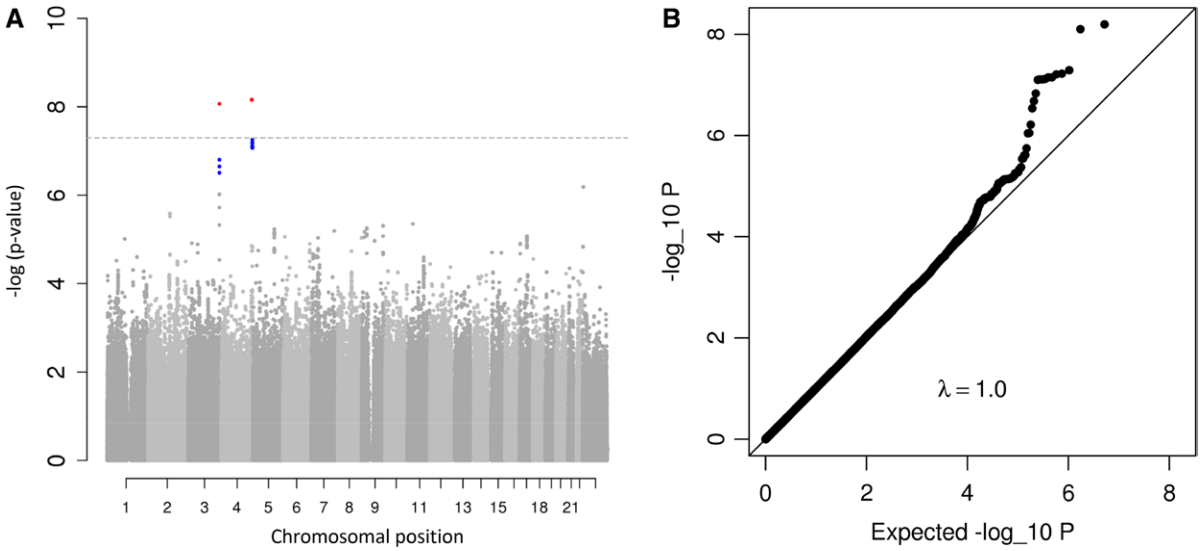

Figure 2. Manhattan plot (**A**) and quantile–quantile plot (**B**) for the genome-wide analysis for plasma renin activity.

in the discovery sample (top SNP rs3915911; meta-analytic $P=8.81\times10^{-9}$), but this observation was not replicated in the PREVEND cohort ($P=0.81$; Table IV in the Data Supplement).

No SNP reached genome-wide significance ($P\leq5\times10^{-8}$) for circulating aldosterone concentrations (Figure VA and VB in the Data Supplement). The top SNP associated with circulating aldosterone levels was SNP rs6986428 on chromosome 8 with a meta-analytic $P=4.01\times10^{-6}$. The top 4 loci associated with circulating aldosterone levels and the replication results are displayed in Table V in the Data Supplement.

Look-Up of Top SNPs in Blacks

In the Jackson Heart Study, rs5030062 (associated with PRA in our sample and in the replication sample PREVEND) was associated with PRC (β=0.100; $P=0.017$) and SNP rs3915911 (associated with PRC in our discovery sample, but not in the replication cohort; Table IV in the Data Supplement) displayed evidence for association with PRA in blacks (β=0.14; $P=0.003$). Furthermore, rs3733402, highly correlated with rs4253311 ($r^2=0.91$), and also located in the *KLKB1* gene, was associated with circulating B-type natriuretic peptide levels in blacks (companion article by Dr Musani et al[27]).

Association of Top Loci With Systolic and Diastolic BP, Renal Traits, and Left Ventricular Mass

In secondary analyses, rs5030062 and rs4253311 (the 2 SNPs that reached or were close to genome-wide significance for PRA in the discovery sample and replicated in PREVEND) were assessed for their association with systolic and diastolic BP. In the International Consortium for Blood Pressure Genome-Wide Association Studies,[19] neither SNP was associated with systolic or diastolic BP (Table 4). Furthermore, no statistically significant associations could be observed for these 2 SNPs with 3 renal traits (ie, estimated glomerular filtration rate, urinary albumin:creatinine ratio, and chronic kidney disease; Table 4). SNP rs4253311 displayed some evidence for association with left ventricular mass ($P=0.03$; Table 4).

Association of the Genetic Variation at the Renin Locus and at the CYP11B2 Locus With RAAS Biomarkers

Our genome-wide data set included 13 genetic variants in or close to the *REN* gene (encoding renin) and 7 SNPs at the *CYP11B2* locus (encoding the aldosterone synthase). Genetic variants in the *REN* gene were not associated with PRA or PRC, nor were SNPs at the *CYP11B2* locus associated with circulating aldosterone concentrations (Table VIA–VIC in the Data Supplement). Additional analyses based on the 1000 genome imputation (restricted to variants with an MAF, ≥1%) revealed rs72745753 ($P=0.0022$; MAF, 1%), rs189709785 ($P=0.001687$; MAF, 4%), and rs34617726 ($P=0.00128$; MAF, 14%) as the SNPs in the candidate regions most significantly associated PRC, PRA, and aldosterone, respectively. However, given the number of SNPs tested in the *CYP11B2*

Table 2. Lead SNPs of the Top Loci Associated With PRA in the Discovery Cohorts

			Meta-Analysis of the Discovery Samples (FHS Gen 3+SUVIMAX)					
SNP	Position	Trait	Chr	MAF	Coded Allele	Direction	*P* Value	Gene
rs12374220	183677641	PRA	4	0.0651	T	…	6.92×10^{-9}*	*TENM3*
rs5030062	187936874	PRA	3	0.3889	A	…	8.57×10^{-9}*	*KNG1*
rs4253311	187411677	PRA	4	0.4828	A	…	5.51×10^{-8}*	*KLKB1*

Chr indicates chromosome; FHS, Framingham Heart Study; Gen 3, Third Generation cohort; *KLKB1*, kallikrein B; *KNG1*, kininogen 1; MAF, minor allele frequency; PRA, plasma renin activity; SNP, single-nucleotide polymorphism; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants; and TENM3, teneurin transmembrane protein 3.
* indicates P≤5 x 10-8.

Table 3. ***Replication of top PRA-SNPs With Respect to Circulating Aldosterone Levels and PRC in the PREVEND Sample***

			Replication Sample (PREVEND)							
SNP	Trait	n	Coded Allele	β	*P* Value	Trait	n	Coded Allele	β	*P* Value
rs12374220	Aldosterone	5894	T	–0.026	0.126	PRC	6325	T	0.0046	0.886
rs5030062	Aldosterone	5865	A	0.017	0.024*	PRC	6296	A	–0.046	0.001*
rs4253311	Aldosterone	5875	A	0.028	<0.001*	PRC	6308	A	–0.065	<0.001*

PRA indicates plasma renin activity; PRC, plasma renin concentration; PREVEND, Prevention of REnal and Vascular ENd-stage Disease; and SNP, single-nucleotide polymorphism.

* indicates P<0.05.

(n=4275) and the *REN* (n=3167 [PRC-related analyses] and n=2979 [PCA-related analyses]) regions, these associations were not considered to be statistically significant.

Pathway Analyses

Our pathway analyses revealed that 2 canonical pathways were significantly enriched ($P<1\times10^{-3}$) with RAAS-related genes, including the G-protein α_s-subunit ($G\alpha_s$) signaling pathway ($P=1.2\times10^{-4}$), and the protein kinase A (PKA) signaling pathway ($P=5.5\times10^{-4}$; Table 5; Figure 4).

Discussion

Using genome-wide association data from ≤4 population-based cohorts with replication in an independent large fifth cohort, we identified 2 genetic loci that displayed statistically significant associations with clinically relevant hormones of the RAAS. The main findings of our analyses are summarized below. First, genetic variations in the *kininogen 1* and in the *kallikrein B* genes were associated with PRA in the discovery sample and with PRC and circulating aldosterone concentrations in the replication sample (where PRA was not available). These variants, however, were not associated with PRC or aldosterone levels in the individual discovery cohorts. SNP rs5030062 (in the *kininogen 1* gene) was also associated with PRC in blacks. Second, the top SNPs in these genes were not related to BP or renal traits. Third, pathway analyses identified 2 canonical pathways that were significantly enriched for RAAS-related genes: the $G\alpha_s$ signaling pathway and the PKA signaling pathway. Fourth, no genetic variant was associated with circulating aldosterone levels in a genome-wide significant fashion. Fifth, genetic variation in genes encoding renin and the aldosterone synthase, respectively, was not related to PRC or activity or to aldosterone levels.

In the Context of the Published Literature

Possible Mechanism for the Observed Association Between SNPs in the Kallikrein–Kinin System and RAAS Biomarkers

As a key finding, we observed that genetic variation within the kallikrein–kinin system (in the *kininogen 1* gene and in the *kallikrein* B gene) was associated with key biomarkers of the RAAS. The *kininogen 1* gene encodes both high and low molecular weight kininogen, the precursors of bradykinin and kallidin (Lys-Bradykinin), respectively.[28] The *kallikrein B* gene encodes plasma prekallikrein, a serine protease that, on transformation to kallikrein, catalyzes the conversion of high molecular weight kininogen to bradykinin (Figure 5)[27] and possibly other factors, such as adrenomedullin and endothelin-1.[29]

Thus, genetic variation in the precursor substance of bradykinin and in the precursor substance of the enzyme (kallikrein) that catalyzes the conversion of high molecular weight kininogen to bradykinin was related to biomarkers of the RAAS in our genome-wide analysis.

These observations are consistent with the concept that the kallikrein–kinin system and the RAAS are tightly interrelated.[30] A prime example for this interaction is the angiotensin-converting enzyme (ACE), which catalyzes on one hand the conversion of angiotensin I to II, and on the other hand, degrades bradykinin.[30,31] In other words, the ACE affects the concentrations of key effectors of the RAAS (ie, angiotensin II) and the kallikrein–kinin system, (ie, bradykinin)

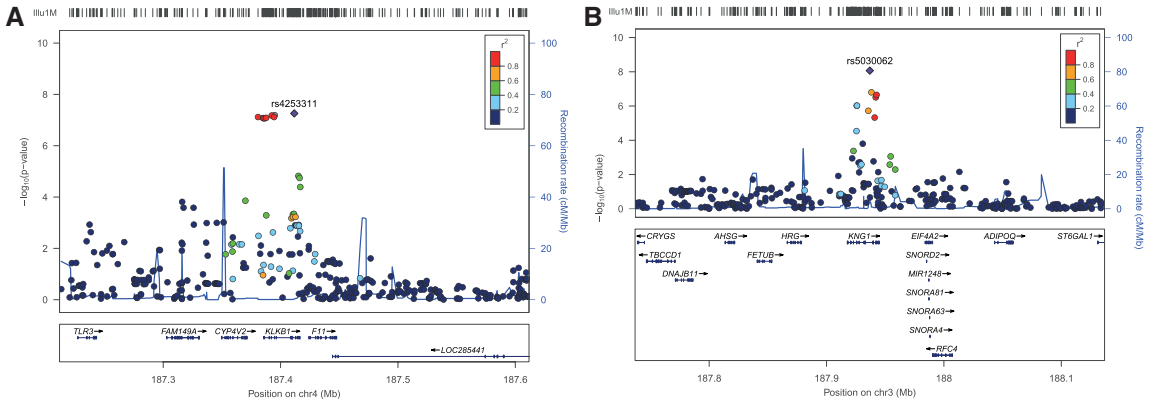

Figure 3. Regional plot of rs4253311 in exon 11 of the *kallikrein B* gene (**A**) and of rs5030062 in intron 6 of the *kininogen 1* gene (**B**). CEU indicates Utah residents with ancestry from northern and western Europe.

Table 4. Association of rs5030062 and rs4253311 With BP in the ICBP[19]; With Renal Traits in the CKDGen Consortium[21]; and With Echocardiographically Determined Left Ventricular Mass in the EchoGen Consortium[20]

			rs5030062_A		rs4253311_A	
Study	Ethnicity	Trait	β (SE)	*P* Value	β (SE)	*P* Value
ICBP[19]	European descent	SBP	−0.165 (0.101)	0.10	0.059 (0.097)	0.54
ICBP[19]	European descent	DBP	−0.077 (0.064)	0.23	0.082 (0.061)	0.18
EchoGen[20]	European descent	LV mass	−0.662 (0.444)	0.14	−0.966 (0.440)	0.03
CKDGen[21]	European descent	eGFR	0.0001 (0.001)	0.96	−0.002 (0.001)	0.19
CKDGen[21]	European descent	UACR	0.013 (0.009)	0.16	−0.002 (0.009)	0.80
CKDGen[21]	European descent	CKD	−0.014 (0.022)	0.53	0.023 (0.021)	0.29

BP indicates blood pressure; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate, based on serum creatinine measurements; ICBP, International Consortium for Blood Pressure Genome-Wide Association Studies; LV mass, left ventricular mass, echocardiographically determined; SBP, systolic blood pressure; and UACR, urinary albumin:creatinine ratio.

in opposite directions. Also both the systems interact at the receptor level. The AT1 receptor and the bradykinin 2 receptor have been shown to interact by forming heterodimers physically, and this heterodimerization affects downstream signaling.[30] Rodents lacking the bradykinin 2 receptor gene display reduced renin mRNA expression as compared with wild-type animals, underscoring that kinins influence renin synthesis through activation of the bradykinin 2 receptor.[30,32] Our genetic-epidemiological data add support to this concept by indicating that genetic variation in the kallikrein–kinin system influences interindividual variation of PRA.

Table 5. Top Results From the Pathway Analysis: Top 5 Enriched Canonical Pathways

Name	*P* Value	Ratio
Gαs signaling	1.2×10^{-4}*	10/116 (0.086)
Protein kinase A signaling	5.5×10^{-4}*	19/383 (0.05)
GNRH signaling	1.8×10^{-3}	9/140 (0.064)
Corticotropin-releasing hormone signaling	2.8×10^{-3}	8/122 (0.066)
CCR5 signaling in macrophages	3.7×10^{-3}	6/87 (0.069)

CCR5 indicates C-C chemokine receptor type 5; $G\alpha_s$ indicates G-protein α_s-subunit; and GNRH, gonadotropin-releasing hormone.
* indicates P<1 x 10-3.

Our analytic sample was restricted to participants of European descent. However, our top SNPs associated with PRA on a genome-wide scale also displayed some evidence for association with related traits in participants of black ancestry: SNP rs5030062 (in the *kininogen 1* gene) displayed evidence for association with renin concentration in participants of the

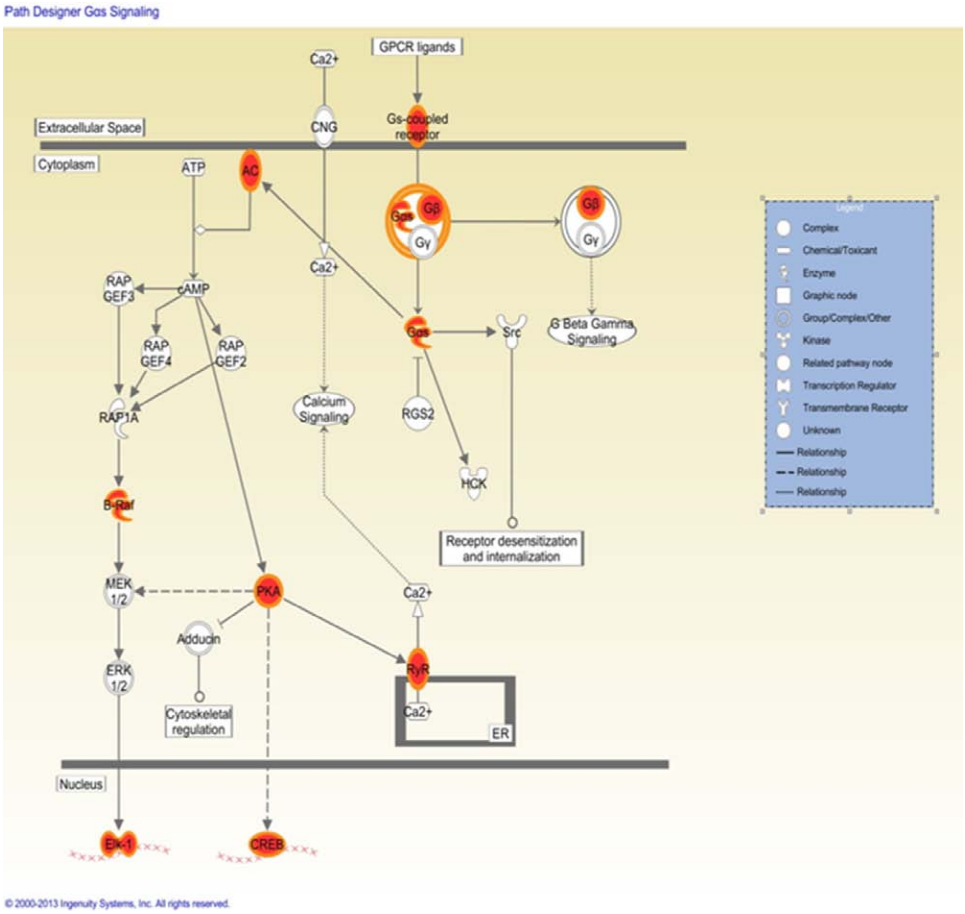

Figure 4. G-protein α_s-subunit ($G\alpha_s$) signaling pathway. Red nodes are genes related to renin–angiotensin–aldosterone system traits.

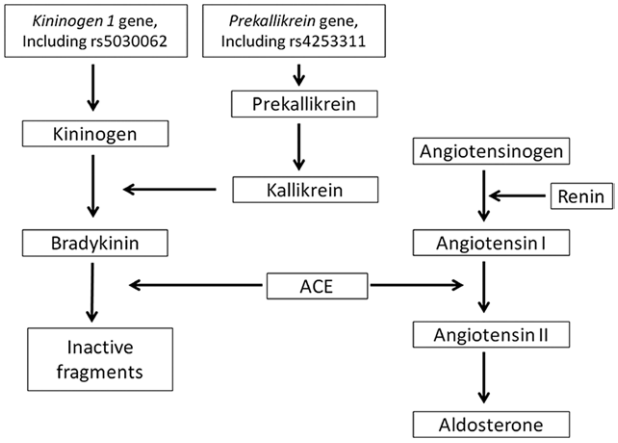

Figure 5. Graphical display of some key elements of the kallikrein–kinin system and the renin–angiotensin–aldosterone system.[4,27]

Jackson Heart Study; and in an companion article, Dr Musani et al report that a close proxy of SNP rs4253311 (rs3733402; r^2=0.91) in the *kallikrein B* gene was associated with circulating B-type natriuretic peptide levels in blacks in a genome-wide significant fashion. Overall, these data lend support to the concept that the kallikrein–kinin system is involved in regulating pathways with high relevance for the cardiovascular system, including the RAAS and the B-type natriuretic peptide pathway. Furthermore, these interrelations seem to be relevant in individuals of both European and African descent, even though the association findings are not identical in these 2 ethnicities (but display important overlap). Experimental data further support the significance of the 2 successfully replicated SNPs for the cardiovascular system. SNP rs5030062 (in the kininogen 1 gene) was predicted to disrupt the binding site of a forkhead box transcription factor,[33] which has been shown to be relevant for several cardiovascular traits, including the development of atherosclerotic plaque lesions.[34] SNP rs4253311 is in high linkage disequilibrium (r^2=0.91) with a missense mutation, rs3733402, which is associated with plasma prekallikrein deficiency.[35]

Association of Top SNPs With BP, Renal Traits, and Left Ventricular Mass

Because the RAAS is an important modulator of acute and chronic changes in BP and a key determinant of renal function, we assessed the association of the top (genome-wide significant) SNPs in the *kininogen 1* gene and in the *kallikrein B* gene (rs5030062 and rs4253311) with BP and renal traits. Both the SNPs were related to neither systolic or diastolic BP nor renal traits, including urinary albumin:creatinine ratio, estimated glomerular filtration rate, or chronic kidney disease (as a binary trait) in large international consortia.[19,21,22] SNP rs4253311 provided evidence for association with left ventricular mass (*P*=0.03), but this association would not remain statistically significant on correction for the multiple look-ups of several intermediate cardiovascular traits. Thus, in our sample, genetic variation associated with RAAS biomarkers did not translate into observable associations with BP or renal traits. We submit, though, that BP and renal function are complex traits that are regulated by multiple genetic, environmental, and lifestyle factors. Thus, the effect of single genetic variants primarily identified through modest associations with the RAAS pathway, on intermediate cardiovascular disease traits (such as BP or renal function), would not be expected to be prominent.

Association of Genetic Variation in REN and CYP11B2 With Circulating Renin and Aldosterone Levels

In previous studies, genetic variation in the *renin (REN)* gene itself has been associated with plasma renin activity[36,37] and the risk of incident hypertension.[36,37] On a parallel note, genetic variation in the *CYP11B2* gene, which encodes the aldosterone synthase (MIM 124080), has been related to the ratio of plasma aldosterone concentration/PRA.[38] In our analyses, however, a limited number of SNPs in these 2 genes were not associated with PRC, PRA, or circulating aldosterone levels, respectively. This is consistent with results from a previous GWAS in Japanese individuals (n=936).[39]

Pathway Analyses

Pathway analyses identified the $G\alpha_s$ signaling pathway and the PKA signaling pathway as being over-represented with RAAS-related genes. G-proteins play a central role in signal transduction. $G\alpha_s$ is a stimulatory G-protein subunit activating the adenylate cyclase, which—on such activation—increases intracellular cAMP levels.[40–42] cAMP is an important second messenger binding, for example, to PKA, which in turn, influences the transcription of multiple genes.[41,42] As reviewed in detail by Kim et al[43], the $G\alpha_s$/cAMP/PKA pathway plays a central role in the regulation of renin secretion, one of the key biomarkers of the RAAS. Mice with targeted deletion of $G\alpha_s$ in juxtaglomerular cells display lower basal renin secretion and expression[43] and a blunted response to chronic ACE inhibition or AT1 receptor blockade (impaired "feedback loop"),[44] indicating that $G\alpha_s$ signaling is highly relevant to renin secretion and expression under steady-state and dynamic conditions.[43]

Coadministration of ACE inhibitors or AT1 antagonists with inhibitors of the adenylate cyclase likewise substantially reduces renin expression as compared with ACE inhibitors or AT1 antagonists alone.[43,44] Thus, cell-specific disruption of the $G\alpha_s$ gene in juxtaglomerular cells and inhibition of the adenylate cyclase leads to impaired renin secretion in response to chronic blockade of the RAAS.[43,44] In addition, it is well established that the sympathetic nervous system is an important determinant of renin secretion and these effects are likewise mediated via the cAMP/PKA pathway.[43] Thus, multiple lines of evidence link the RAAS to the $G\alpha_s$/cAMP/PKA axis and our pathway analyses are in agreement with these experimental data, supporting the significance of $G\alpha_s$/PKA signaling for the regulation of the RAAS.

Strengths and Limitations

To our knowledge, this analysis is the first comprehensive GWAS analyzing the main circulating biomarkers of the RAAS conjointly. Additional strengths include the considerable samples size, the careful phenotyping, and genotyping in the contributing cohorts as well as their population-based character. As an apparent limitation, PRA was measured in only 2 cohorts (total n=5275) of the discovery sample, and not in the replication sample. Therefore, the genome-wide significant hits for PRA from the discovery sample had to be

tested for association with PRCs and circulating aldosterone levels in the replication sample. However, in the absence of large changes in plasma angiotensinogen, measurements of PRA and PRC investigate the same biomarker, circulating active renin, almost exclusively of renal origin, and both biomarkers (renin concentration and renin activity) are strongly correlated.[45] In population studies, the control of the physiological factors, which influence plasma renin, mainly posture and sodium intake, are less well controlled than in metabolic studies, and this may lead to an underestimation of the genetic association. However, we do not expect major changes in angiotensinogen in an epidemiological setting. Furthermore, because these 3 biomarkers are tightly interrelated and part of the same pathway, the key finding of our investigation, that genetic variation in the kallikrein–kinin systems is associated with RAAS biomarker levels, is supported by the replication analyses. As an additional limitation, the top SNPs associated with PRA in the discovery cohorts were associated with circulating renin concentrations and aldosterone levels in an independent sample (PREVEND) but not in the individual discovery cohorts themselves. Potential explanations for this discrepancy include the smaller sample sizes and the larger coefficients of variation for renin and aldosterone measurements in some individual discovery cohorts as compared with the PREVEND sample, resulting in larger measurement errors and reduced statistical power to detect modest associations in the discovery as compared with the replication cohort.

In conclusion, we present data from a meta-analysis of large genome-wide association studies for 3 biomarkers of the RAAS in ≤13 289 Europeans and European Americans. We observed that genetic variation in the kallikrein–kinin system was associated with PRA at a genome-wide significant level. This is consistent with experimental data linking the RAAS to the kallikrein–kinin system. Furthermore, pathway analyses identified 2 canonical pathways that were significantly enriched for RAAS-related genes: the $G\alpha_s$ signaling pathway and the PKA signaling pathway, which influence renal renin release. Our observations require replication in other independent cohorts, including samples from other ethnicities and age groups, to address the generalizability of our findings. Furthermore, the significant SNPs warrant further experimental exploration to elucidate in detail the molecular mechanisms underlying the observed associations.

Sources of Funding

A detailed description of funding sources is provided in the Data Supplement.

Disclosures

Drs Wang, Vasan, and Wilson received National Institutes of Health funding related to this article. Dr de Boer received lecture fees from Novartis and Medcon. The other authors report no conflicts.

Appendix

From The Framingham Heart Study, MA (W.L., M.-H.C., H.L., T.J.W., R.S.V.); Institute of Epidemiology, Christian-Albrechts-University, Kiel, Germany (W.L., S.S.); Section of Preventive Medicine and Epidemiology (R.S.V.) and Department of Neurology (M.-H.C.), Boston University School of Medicine, MA; Interfaculty Institute for Genetics and Functional Genomics (A.T., U.V.), Institute for Community Medicine, Section Study of Health in Pomerania - Clinical-Epidemiological Research (A.T., H.V.), Institute of Clinical Chemistry and Laboratory Medicine (M.N., A.H., H.W.), Department of Internal Medicine B (M.D.), and Institute of Physiology (R.R.), University Medicine Greifswald, Greifswald, Germany; Departments of Cardiology (R.A.d.B., W.H.v.G., D.J.v.V., P.v.d.H.), Internal Medicine (S.J.L.B.), Nephrology (G.N.), and University Medical Center Groningen (S.J.L.B., G.N.), University of Groningen, Groningen, The Netherlands; Department of Medicine, University of Mississippi Medical Center, Jackson (E.R.F., S.K.M., J.G.W.); Institute of Epidemiology I (H.-E.W.), Institute of Epidemiology II (C.M.), and Research Unit Molecular Epidemiology (A.-K.P., R.R), Helmholtz Zentrum München, München, Germany; Department of Internal Medicine IV, University Hospital Freiburg, Freiburg, Germany (Y.L.); Inserm, Clinical Investigation Centre (CIC1418), Paris, France (J.M.); Faculté de Médecine, Université Paris-Descartes, Paris, France (J.M.); Institut National de la Santé et de la Recherche Médicale, U1153, University Paris 13, Bobigny, France (S.H.); Medizinische Klinik und Poliklinik IV, Ludwig Maximilians University Hospital, Munich, Germany (M.B., M.R.).

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CLINICAL PERSPECTIVE

The renin–angiotensin–aldosterone system (RAAS) is a central pathway in cardiovascular and renal physiology that is frequently targeted pharmacologically in a variety of clinical settings. Despite the clinical significance of the RAAS, environmental and genetic factors influencing circulating RAAS biomarkers are incompletely understood. We evaluated the genetic correlates of 3 important RAAS biomarkers by meta-analyzing genome-wide association data for plasma renin activity (n=5275), plasma renin concentrations (n=8014), and circulating aldosterone levels (n=13 289) from ≤4 population-based discovery cohorts, and assessed replication of the top associations in an independent sample (n=6487). No locus reached genome-wide significance for circulating aldosterone levels. Single-nucleotide polymorphisms in 2 independent loci displayed associations with plasma renin activity in a genome-wide significant fashion ($P<5\times10^{-8}$). A third locus demonstrated a borderline association that was close to this threshold (rs4253311 in the kallikrein B [KLKB1] gene, $P=5.5\times10^{-8}$). Associations for 2 of these loci were successfully replicated in relation to plasma renin concentration and circulating aldosterone levels (single-nucleotide polymorphism rs5030062 in the kininogen 1 gene; and rs4253311 in the *KLKB1* gene). Single-nucleotide polymorphisms in the *NEBL* gene demonstrated a genome-wide significant association with plasma renin concentration in the discovery sample, but the findings were not replicated in the independent sample. Our genetic analyses identified 2 loci (kininogen 1 and kallikrein B) encoding proteins of the kallikrein–kinin system that were associated with key components of the RAAS, providing further evidence for a close interrelation between the kallikrein–kinin system and the RAAS.

SUPPLEMENTAL MATERIAL

Supplementary Methods

RAAS measurements

Measurements of renin and aldosterone

In FHS, KORA and SUVIMAX, blood samples were taken in the early morning from seated participants after an overnight fast. In PREVEND, fasting blood samples were taken between 8 AM and 4 PM. In SHIP, blood was drawn throughout the day (between 8 AM and 8 PM) in non-fasting participants while they were taking their regular medication. Serum and plasma were stored at −80 °C in all cohorts. Aldosterone measurements were available in all cohorts (FHS Gen 2 and Gen 3, KORA, SHIP, SUVIMAX, PREVEND; **Figure 1**); plasma renin concentrations were measured in the FHS Gen 2, KORA, SHIP and PREVEND samples; plasma renin activity was determined in the FHS Gen 3 and SUVIMAX samples (**Figure 1)**. Details on the methods to determine RAAS biomarkers in the different samples are provided below.

In **FHS**, plasma renin concentration (Gen 2) and serum aldosterone concentrations were determined with an immunochemi-luminometric assay (Nichols assay, Quest Diagnostics, Cambridge, Mass) and a radioimmunoassay (Quest Diagnostics), respectively.[1] The mean interassay coefficients of variation were 10.0% (low concentration) and 2.0% (high concentrations) for renin and 9.8% (low concentrations) and 4.0% (high concentrations) for aldosterone.[1] Plasma renin activity (ng/mL/hr; in Gen 3) was determined using the GammaCoat Plasma Renin Activity RIA Kit (DiaSorin) with an interassay coefficient of variation of 12.6%.[2]

In **KORA**, plasma aldosterone concentrations and plasma renin concentrations were measured in EDTA plasma. Specifically, plasma aldosterone concentrations were determined after

extraction by an immunofluorescence in-house assay, as detailed elsewhere.[3] Interassay coefficients of variation were 15.2% (low concentrations) and 8% (high concentrations).[4] The plasma renin concentration was determined by an automated chemiluminescence immunoassay (LIAISON Direct Renin, DiaSorin).[5] The interassay coefficient of variation was 12.2%[6].

In **SHIP**, plasma renin concentration was measured using a radioimmunometric assay (Renin III generation, Cisbio Bioassay, Bagnols-sur-Cèze Cedex, France). Interassay coefficients of variation were 5.0% (low concentrations) and 4.0% (high concentrations), respectively.[7] The standards in the kits were calibrated against the international reference preparation (WHO 68 / 356).[7] Plasma aldosterone concentration was measured using a radioimmunometric assay (Coat-A-Count Aldosterone, Siemens Healthcare Diagnostics, Eschborn, Germany). Interassay coefficients of variation were 15.7% (low concentrations) and 3.8% (high concentrations), respectively.

In **SUVIMAX**, plasma renin activity was measured using an in-house assay, as described previously,[8] with interassay coefficients of variation of 31% (low concentrations) and 25% (high concentrations). Aldosterone was determined using a radioimmuno-assay with 125I-aldosterone (Coat-A-Count Aldosterone, Diagnostic Products Corporation). The interassay coefficients of variation were 15% (low concentrations) and 10% (high concentrations).

In **PREVEND**, plasma renin concentrations were determined by an automated chemiluminescence immunoassay (LIAISON Direct Renin, DiaSorin).[5, 9] Plasma aldosterone concentrations were measured with an ELISA kit (Alpco, Salem, NH, USA).[9] The interassay coefficients of variation were 10.9% for renin[9] and 9.6% for aldosterone.[9]

Genotyping details

In **FHS**, from a total of 534,982 genotyped SNPs (Affymetrix 500K and MIPS 50K combined), 378,163 SNPs were used in the imputation after filtering out 15,586 SNPs (Hardy-Weinberg p<1e-6), 6,4511 SNPs (missingness >0.03), 4,5361 SNPs (mishap p<1e-9), 4,857 SNPs (>100 Mendel errors), 6,7269 SNPs (frequency<0.01), 2 SNPs (due to strandedness issues upon merging data with HapMap), and a further 1,3394 SNPs (as they were not present on HapMap). MACH (version 1.0.15) was used to impute all 2,543,887 SNPs on HapMap, using the publicly available phased haplotypes from HapMap (release 22, build 26, CEU population) as a reference panel.

The **KORA F4 samples** were genotyped with the Affymetrix Human SNP Array 6.0. Hybridization of genomic DNA was done in accordance with the manufacturer's standard recommendations. Genotypes were determined using Birdseed2 clustering algorithm. For quality control purposes, we applied a positive control and a negative control DNA every 96 samples. On chip level only subjects with overall genotyping efficiencies of at least 93% were included resulting in an average genotyping efficiency of 98% per chip. In addition the called sex had to agree with the sex in the KORA study database. Imputation of genotypes was performed with the software IMPUTE v0.4.2 based on HapMap II.

The **SHIP** samples were genotyped using the Affymetrix Human SNP Array 6.0. Hybridisation of genomic DNA was done in accordance with the manufacturer's standard recommendations. The genetic data analysis workflow was created using the Software InforSense. Genetic data were stored using the database Caché (InterSystems). Genotypes were determined using the Birdseed2 clustering algorithm. For quality control purposes, several control samples were

added. On the chip level, only subjects with a genotyping rate on QC probesets (QC callrate) of at least 86% were included. Finally, all arrays had a sample call rate > 92%. The overall genotyping efficiency of the GWA was 98.55%. Imputation of genotypes in SHIP was performed with the software IMPUTE v0.5.0 based on HapMap II.

The **SUVIMAX sample** was genotyped using the Illumina 317K array. Genotypes were called using the Beadstudio algorithm. A sample call rate ≥ 95%, a minor allele frequency ≥1% and a Hardy-Weinberg equilibrium p-value ≥ 10^{-6} were required. Imputation of genotypes was performed with the software IMPUTE.

The **PREVEND** sample was genotyped using the Illumina Human CytoSNP array. SNPs with a minor allele frequency below 1%, a call rate below 95% or a Hardy-Weinberg equilibrium p-value < 10^{-6} were excluded. Genotypes were called with the GenomeStudio algorithm. Imputation of genotypes in PREVEND was done using the BEAGLE software.

Funding

FHS: This work was supported by the National Heart, Lung, and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278), and by grants from the National Heart, Lung, and Blood Institute 2K24HL04334, RO1HL080124, RO1HL077477, and R01HL093328 (all to Dr Vasan), R01-HL-086875 (to Dr. Wang).

KORA: The KORA research platform (KORA, Cooperative Health Research in the Region of Augsburg) was initiated and financed by the Helmholtz Zentrum München - German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education

and Research and by the State of Bavaria. M.R. is recipient of a grant by the Else Kröner-Fresenius Stiftung for the German Conn-Registry.

Study of Health in Pomerania (SHIP): SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network 'Greifswald Approach to Individualized Medicine (GANI_MED)' funded by the Federal Ministry of Education and Research (grant 03IS2061A). Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 03ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg- West Pomerania. The University of Greifswald is a member of the 'Center of Knowledge Interchange' program of the Siemens AG and the Caché Campus program of the InterSystems GmbH.

SUVIMAX: This study was funded by the Association Robert Debré pour la Recherche Médicale, the Institut National pour la Santé et la Recherche Médicale, the Conservatoire National des Arts et Métiers, the Institut National de la Recherche Agronomique and the Université Paris 13.

PREVEND: PREVEND genetics is supported by the Dutch Kidney Foundation (Grant E033), the EU (grant LSHM-CT 2006-037697, GENECURE), the National Institutes of Health (grant LM010098), The Netherlands Organisation for Health Research and Development (NWO VENI grant 916.761.70), and the Dutch Interuniversity Cardiology Institute Netherlands (ICIN); the renin and aldosterone assays were supported by grants from the Netherlands Heart Foundation (grant 2007T046) and The Netherlands Organisation for Health Research and Development (NWO VENI grant 916.10.117).

Jackson Heart Study: The Jackson Heart Study is supported by contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, HHSN268201300050C from the National Heart, Lung, and Blood Institute and the National Institute on Minority Health and Health Disparities.

Supplementary Tables

Supplementary Table 1. Association of top plasma renin activity SNPs with plasma renin concentration (PRC) in the discovery cohorts (FHS, KORA, SHIP)

FHS

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183677641	PRC	T	-0.10627	0.068231	0.119338	3103	Yes	0.620956
rs5030062	3	187936874	PRC	C	0.016785	0.025657	0.512973	3103	Yes	1.010671
rs4253311	4	187411677	PRC	A	-0.03595	0.025317	0.155642	3103	Yes	1.001227

KORA

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183677641	PRC	T	0.02587	0.075007	0.730196	1704	Yes	0.877704
rs5030062	3	187936874	PRC	C	-0.02036	0.038499	0.597169	1658	Yes	0.968982
rs4253311	4	187411677	PRC	G	0.005742	0.036517	0.875898	1782	Yes	0.996428

SHIP

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183677641	PRC	T	0.019226	0.053	0.716842	3125	Yes	0.864969
rs5030062	3	187936874	PRC	C	0.019734	0.023968	0.410681	3123	Yes	0.957087
rs4253311	4	187411677	PRC	G	0.038588	0.022507	0.086393	3122	Yes	0.996274

FHS, Framingham Heart Study; KORA, Cooperative Health Research in the Region of Augsburg; SHIP, Study of Health in Pomerania

SE, standard error; oevar_imp, observed divided by expected variance for imputed allele dosage

Supplementary Table 2. Association of top plasma renin activity SNPs with aldosterone concentrations in the discovery cohorts (FHS, KORA, SHIP, SUVIMAX)

FHS

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183677641	Aldo	T	0.10192	1.393061	0.941676	6884	Yes	0.620956
rs5030062	3	187936874	Aldo	C	0.380553	1.393061	0.784717	6884	Yes	1.010671
rs4253311	4	187411677	Aldo	A	1.327637	1.393061	0.340572	6884	Yes	1.001227

KORA

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183677641	Aldo	T	0.001014	0.044538	0.981819	1709	Yes	0.888954
rs5030062	3	187936874	Aldo	C	0.047866	0.02306	**0.038031**	1663	Yes	0.96675
rs4253311	4	187411677	Aldo	G	0.010353	0.021797	0.635213	1787	Yes	0.995957

SHIP

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183677641	Aldo	T	-0.00801	0.040005	0.841405	3124.765	Yes	0.880806
rs5030062	3	187936874	Aldo	C	0.003495	0.01826	0.848189	3123.196	Yes	0.956395
rs4253311	4	187411677	Aldo	G	-0.00705	0.017132	0.679223	3122.162	Yes	0.995835

SUVIMAX

SNP-ID	Chromosome	Position	Trait	Coded allele	Beta	SE	P-value	N	Imputed	Oevar_imp
rs12374220	4	183815796	Aldo	T	-0.08666	0.045513	0.057082	1518	Yes	0.896527

rs5030062	3	187936882	Aldo	C	-0.00279	0.024193	0.908164	1518	No	0.999971
rs4253311	4	187549832	Aldo	G	-0.02046	0.023552	0.385029	1518	Yes	0.98611

FHS, Framingham Heart Study; KORA, Cooperative Health Research in the Region of Augsburg; SHIP, Study of Health in Pomerania; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants study

SE, standard error; oevar_imp, observed divided by expected variance for imputed allele dosage

Supplementary Table 3. Top loci associated with plasma renin activity, stratified by cohort, i. e. in the FHS Generation 3 sample and in the SUVIMAX sample.

SNP	chr	coded allele (FHS)	coded allele (SUVIMAX)	beta (FHS)	beta (SUVIMAX)	SE (FHS)	SE (SUVIMAX)	P-value (FHS)	P-value (SUVIMAX)	N (FHS)	N (SUVIMAX)
rs12374220	4	T	T	-0.26	-0.12	0.05	0.04	3.03E-07	0.0044039	3757	1518
rs5030062	3	C	C	0.11	0.05	0.02	0.02	2.70E-08	0.0369734	3757	1518
rs4253311	4	A	A	-0.10	-0.03	0.02	0.02	3.54E-08	0.120666	3757	1518

FHS, Framingham Heart Study; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants SNP, single nucleotide polymorphism; Chr, chromosome; SE, standard error

Supplementary Table 4. Top loci associated with plasma renin concentration (PRC) in the discovery sample and in the replication sample

					Meta-analysis of the discovery samples (FHS+SHIP+KORA)		
SNP	**Trait**	**N**	**Chr**	**MAF**	**Direction**	**P-value**	**Locus**
rs3915911	PRC	7971	10	0.29	---	8.81×10^{-9}	*NEBL*
rs3758601	PRC	7988	10	0.40	---	1.78×10^{-7}	*NEBL*

					Replication sample (PREVEND)			
SNP	**Trait**	**N**	**Beta**	**P-value**	**Trait**	**N**	**Beta**	**P-value**
rs3915911	Aldosterone	5839	-0.0069	0.402	PRC	6266	-0.0039	0.805
rs3758601	Aldosterone	5855	0.0173	0.025	PRC	6283	-0.0076	0.602

FHS, Framingham Heart Study; KORA, Cooperative Health Research in the Region of Augsburg; SHIP, Study of Health in Pomerania; PREVEND, Prevention of REnal and Vascular ENd-stage Disease; SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency

Supplementary Table 5. Top loci associated with circulating aldosterone concentration in the discovery sample and in the replication sample

						Meta-analysis of the discovery samples (FHS+SHIP+KORA+SUVIMAX)	
SNP	**Trait**	**N**	**Chr**	**MAF**	**Direction**	**P-value**	**Locus**
rs6986428	Aldosterone	13176	8	0.06	++++	4.01×10^{-6}	*C8orf22*
rs8597	Aldosterone	13291	7	0.07	++++	4.20×10^{-6}	*CALU*
rs7917400*	Aldosterone	4909	10	0.41	?--?	5.05×10^{-6}	*CUBN*
rs6884962	Aldosterone	13315	5	0.32	++++	5.36×10^{-6}	*NKX2-5*

		Replication sample (PREVEND)						
SNP	**Trait**	**N**	**Beta**	**P**	**Trait**	**N**	**Beta**	**P**
rs6986428	Aldosterone	5881	-0.0002	0.992	PRC	6312	-0.0068	0.88
rs8597	Aldosterone	5870	-0.013	0.394	PRC	6296	-0.052	0.07
rs7917400	Aldosterone	5866	-0.006	0.432	PRC	6294	0.0016	0.91
rs6884962	Aldosterone	5832	0.0142	0.072	PRC	6260	0.014	0.35

*This SNP was available only in KORA and SHIP. FHS, Framingham Heart Study; KORA, Cooperative Health Research in the Region of Augsburg; SHIP, Study of Health in Pomerania; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants; PREVEND, Prevention of REnal and Vascular ENd-stage Disease; SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency

Supplementary Table 6a. Association of genetic variation at the renin (*REN*) locus with plasma renin activity (PRA)

SNP	Chr	MAF	Direction	P-value
rs11571079	1	0.063	++	0.18
rs11571078	1	0.130	+-	0.33
rs1464816	1	0.381	++	0.33
rs10900555	1	0.357	+-	0.38
rs11240688	1	0.187	--	0.43
rs6676670	1	0.187	--	0.43
rs6693954	1	0.262	++	0.58
rs2368564	1	0.271	++	0.62
rs11571082	1	0.132	-+	0.72
rs3795575	1	0.140	-+	0.72
rs5705	1	0.132	+-	0.73
rs7521667	1	0.139	-+	0.73
rs2887284	1	0.208	--	0.85

SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency

Supplementary Table 6b. Association of genetic variation at the renin (*REN*) locus with plasma renin concentration (PRC)

SNP	Chr	MAF	Direction	P-value
rs2368564	1	0.271	--+	0.14
rs11571078	1	0.135	+-+	0.16
rs2887284	1	0.207	-?+	0.19
rs6693954	1	0.266	--+	0.24
rs1464816	1	0.358	+--	0.30
rs10900555	1	0.343	++-	0.55
rs11240688	1	0.177	-+-	0.58
rs6676670	1	0.176	-+-	0.65
rs11571079	1	0.058	--+	0.68
rs7521667	1	0.133	--+	0.75
rs3795575	1	0.137	--+	0.85
rs5705	1	0.131	++-	0.93
rs11571082	1	0.131	--+	0.97

SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency

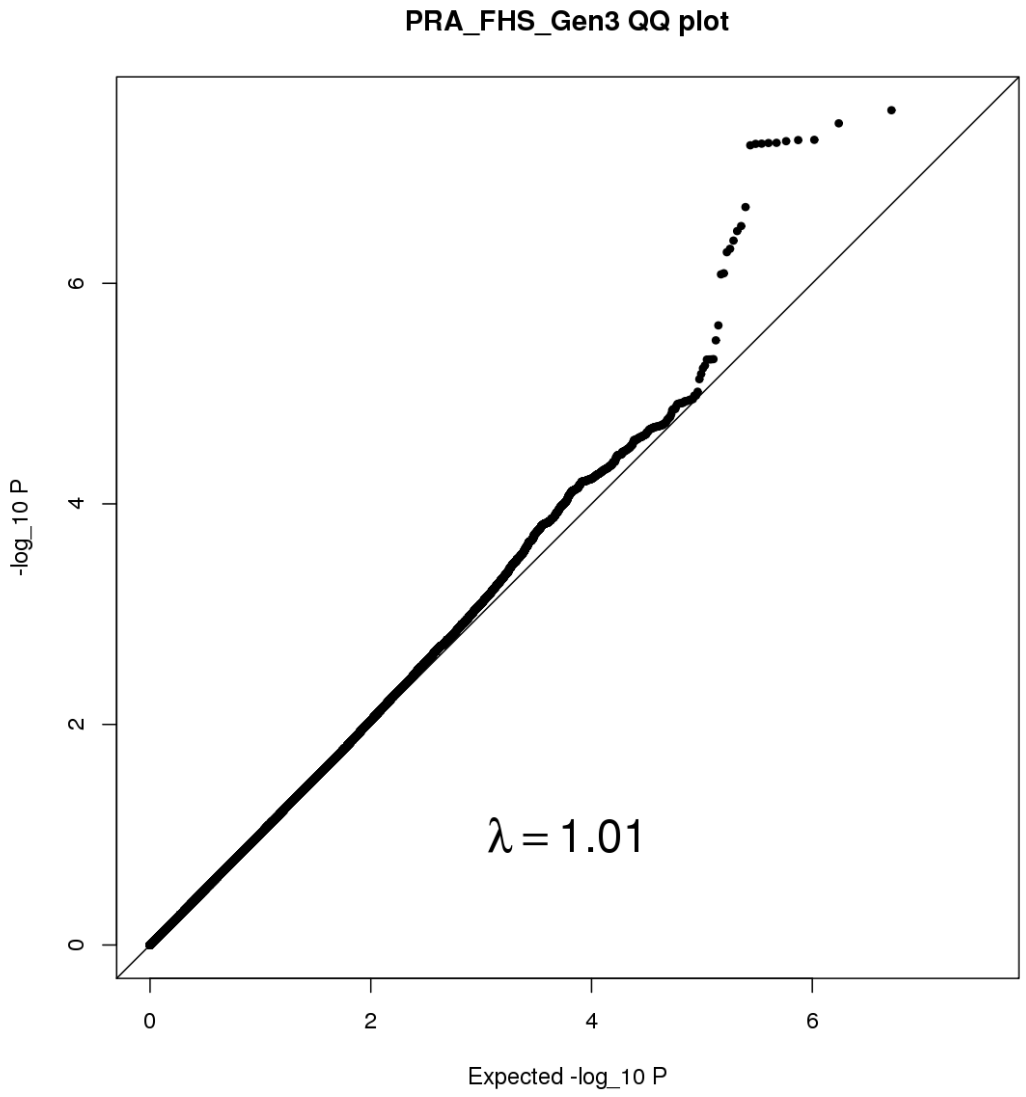
Supplementary Table 6c. Association of genetic variation at the *CYP11B2* locus with circulating aldosterone levels

SNP	Chr	MAF	Direction	P-value
rs4543	8	0.086	++++	0.15
rs6433	8	0.427	+---	0.57
rs6414	8	0.450	+---	0.63
rs11781816	8	0.426	-+++	0.66
rs4536	8	0.027	-+--	0.67
rs4545	8	0.018	-+++	0.78
rs3097	8	0.294	-+++	0.79

SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency

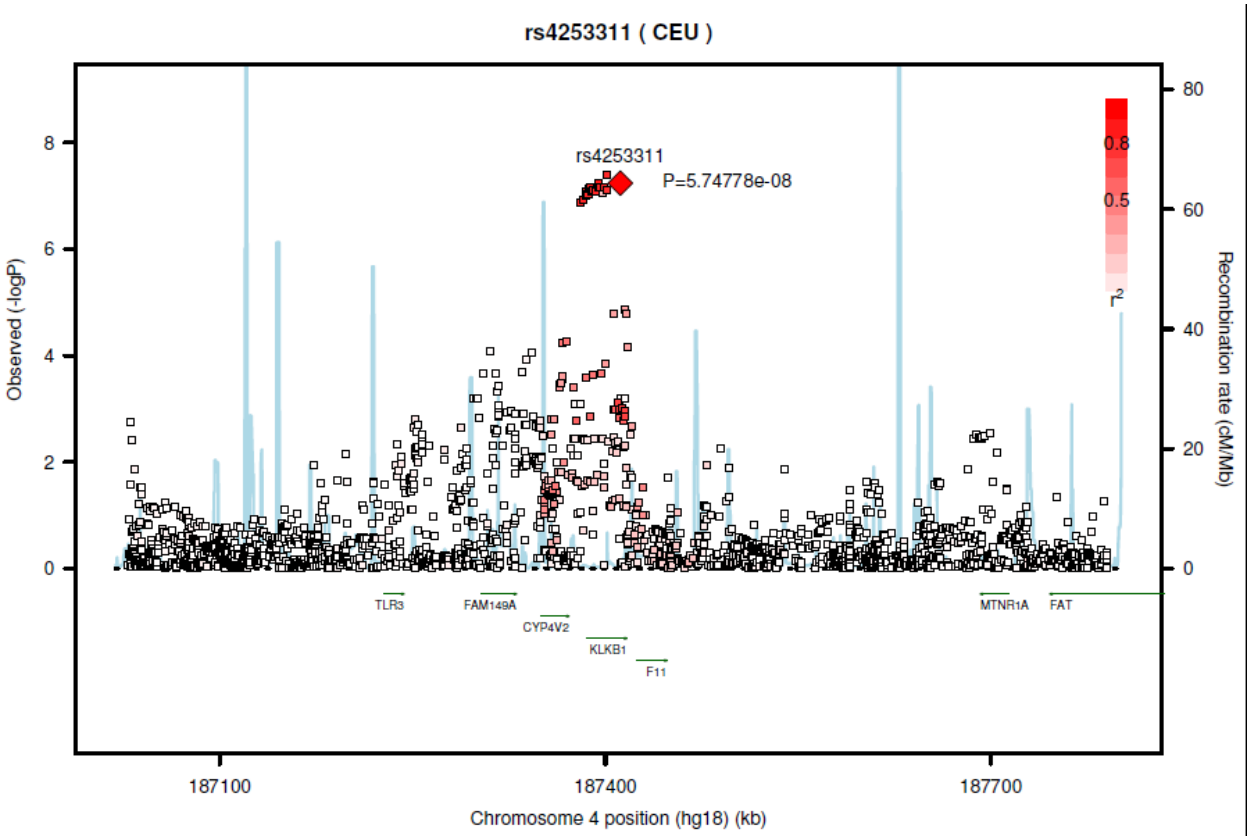
Supplementary Figures

Supplementary Figure 1. The quantile-quantile plot for plasma renin activity in the FHS Generation 3 sample only

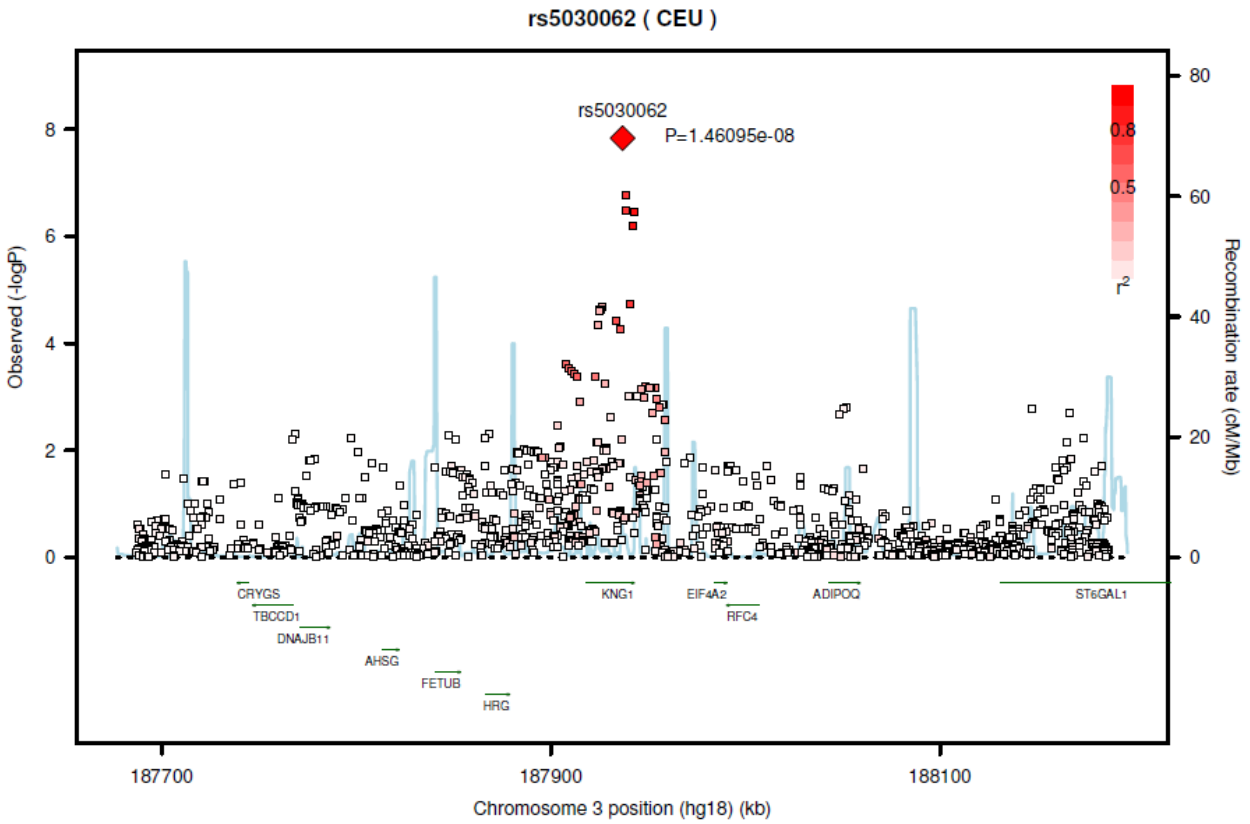


Supplementary Figure 2. Regional plot of rs4253311 in exon 11 of the *kallikrein B* gene (**Panel A**) and of rs5030062 in intron 6 of the *kininogen 1* gene (**Panel B)**; based on imputation to the 1000 genome dataset

Panel A

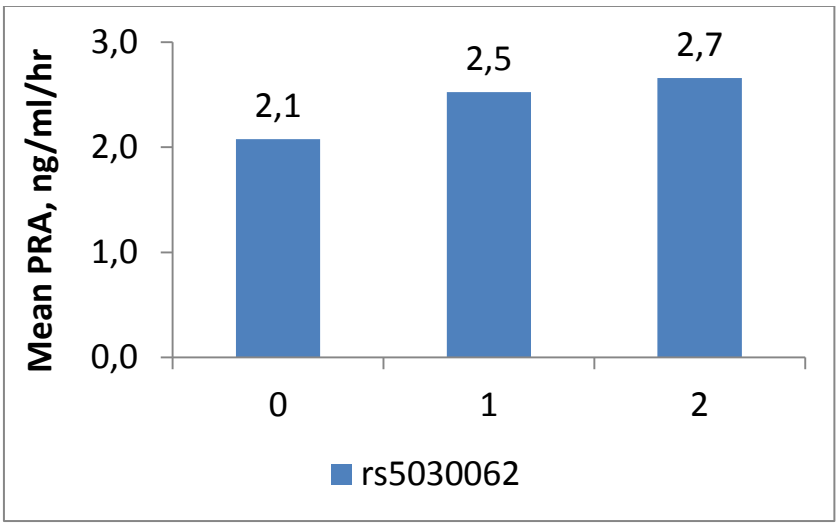


Panel B

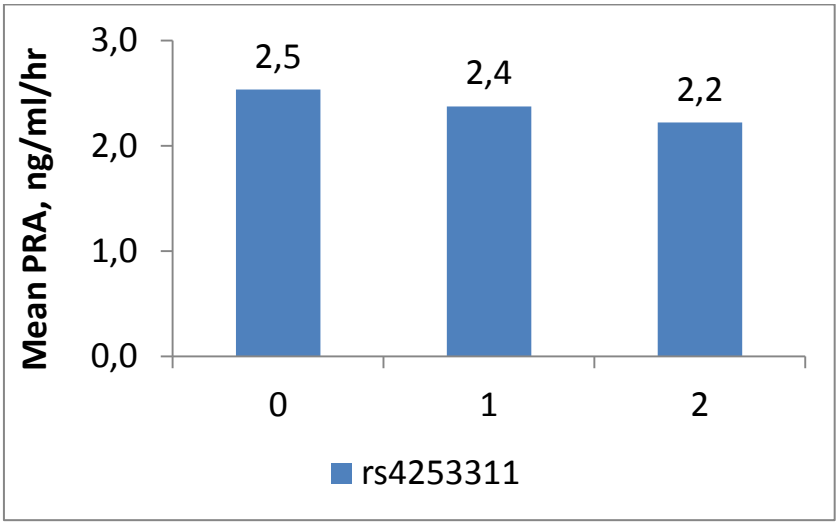

rs5030062 (CEU)
rs5030062
P=1.46095e-08
0.8
0.5
r²
Observed (-logP)
Recombination rate (cM/Mb)
CRYGS
TBCCD1
DNAJB11
AHSG
FETUB
HRG
KNG1
EIF4A2
RFC4
ADIPOQ
ST6GAL1
187700
187900
188100
Chromosome 3 position (hg18) (kb)

Supplementary Figure 3. Plasma renin activity by rs5030062 genotype (**Panel A**) and by rs4253311 genotype **(Panel B)**

Panel A

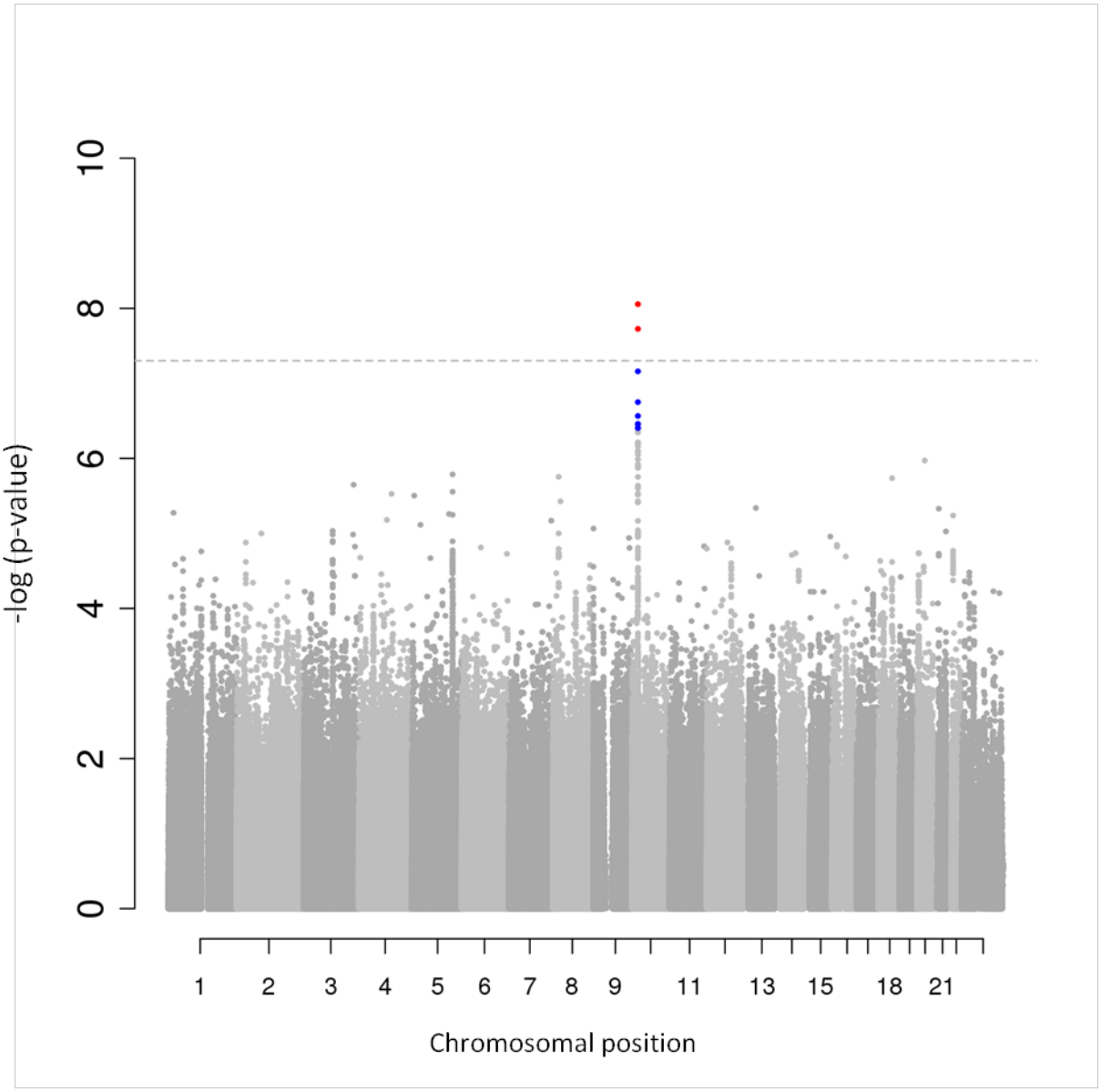


Panel B

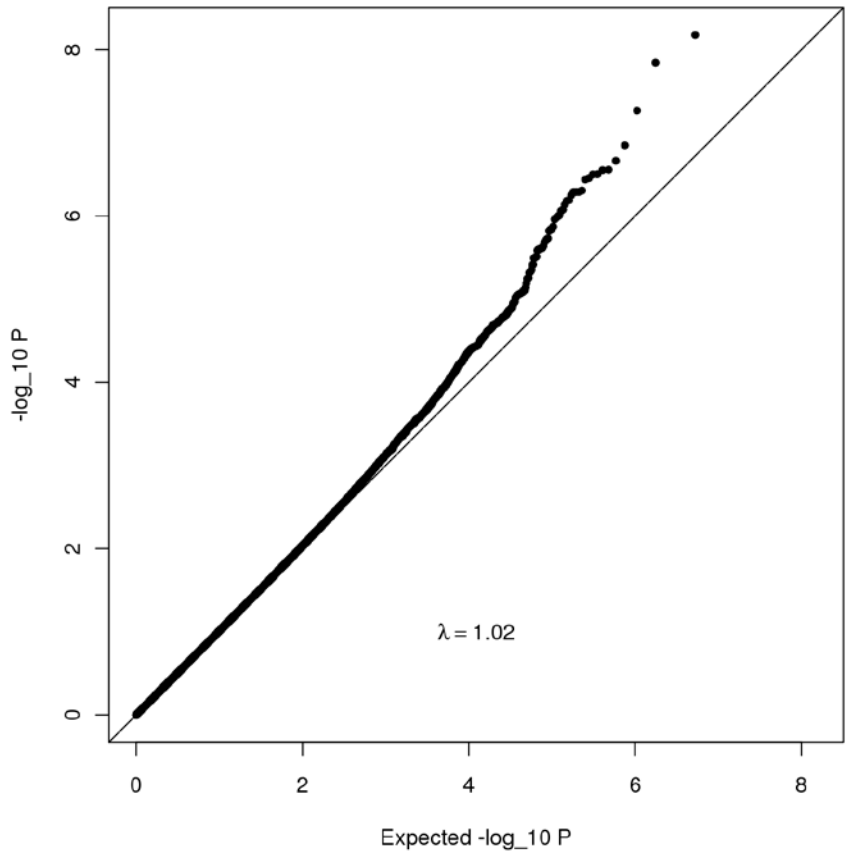


Supplementary Figure 4. Manhattan plot (**Panel A**) and quantile-quantile plot (**Panel B**) of the genome-wide analysis for plasma renin concentration

Panel A

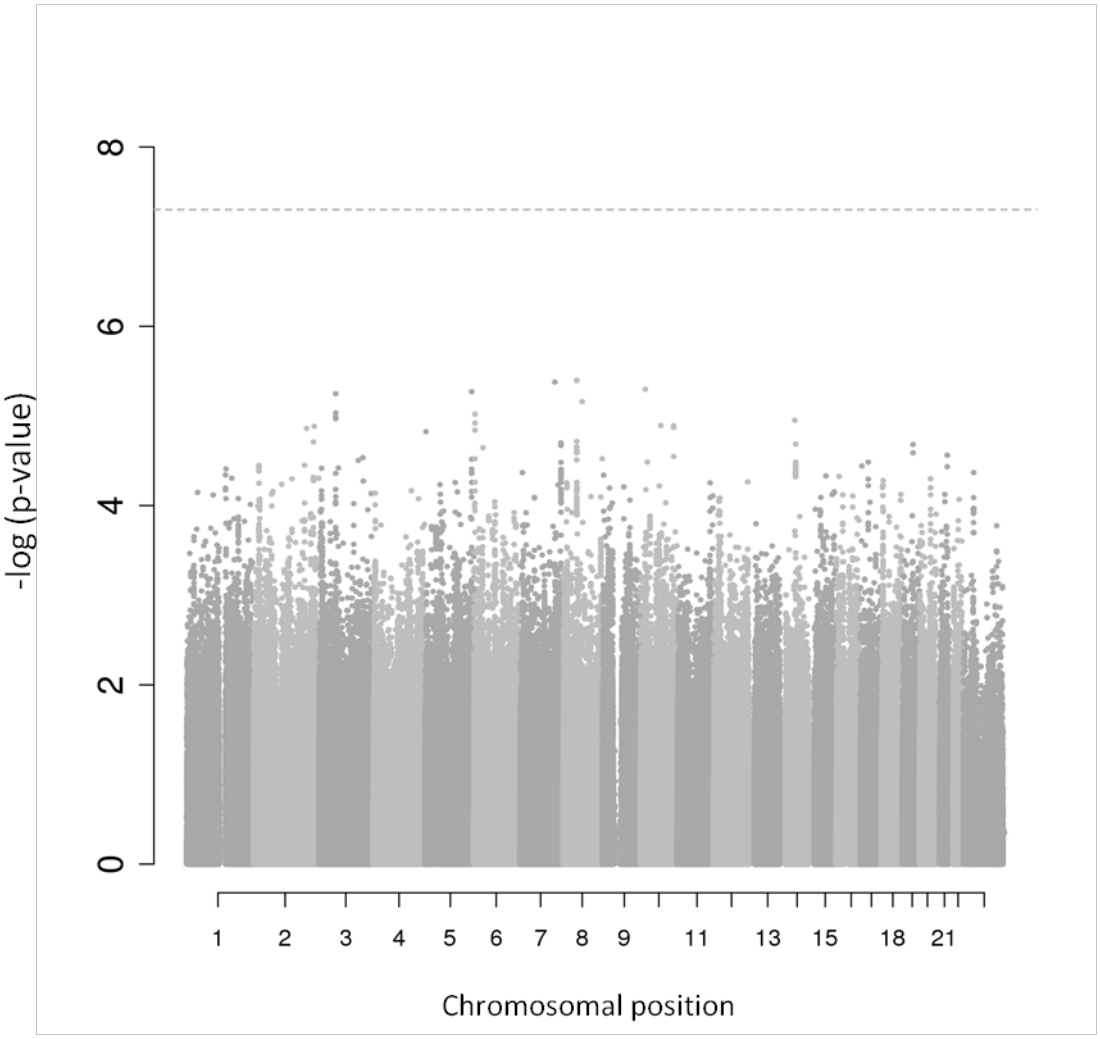


Panel B

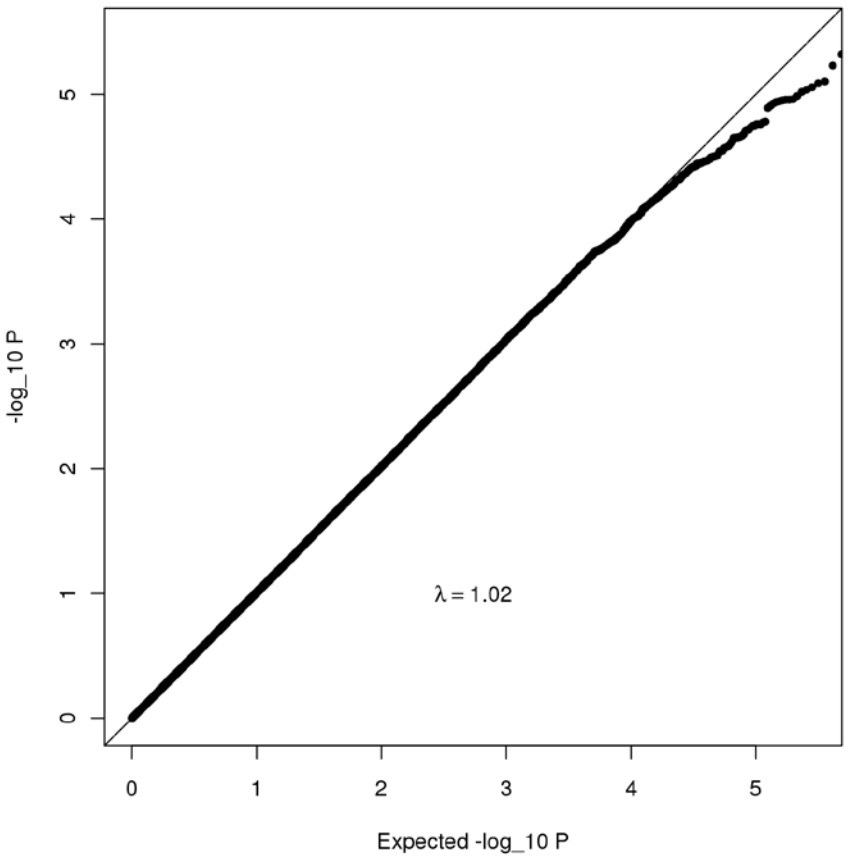


Supplementary Figure 5. Manhattan plot (**Panel A**) and quantile-quantile plot (**Panel B**) of the genome-wide analysis for circulating aldosterone concentration

Panel A



Panel B



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Genome-Wide Meta-Analyses of Plasma Renin Activity and Concentration Reveal Association With the Kininogen 1 and Prekallikrein Genes

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CKDGen Consortium; ICBP; EchoGen Consortium

Circ Cardiovasc Genet. 2015;8:131-140; originally published online December 4, 2014;
doi: 10.1161/CIRCGENETICS.114.000613
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1942-325X. Online ISSN: 1942-3268