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

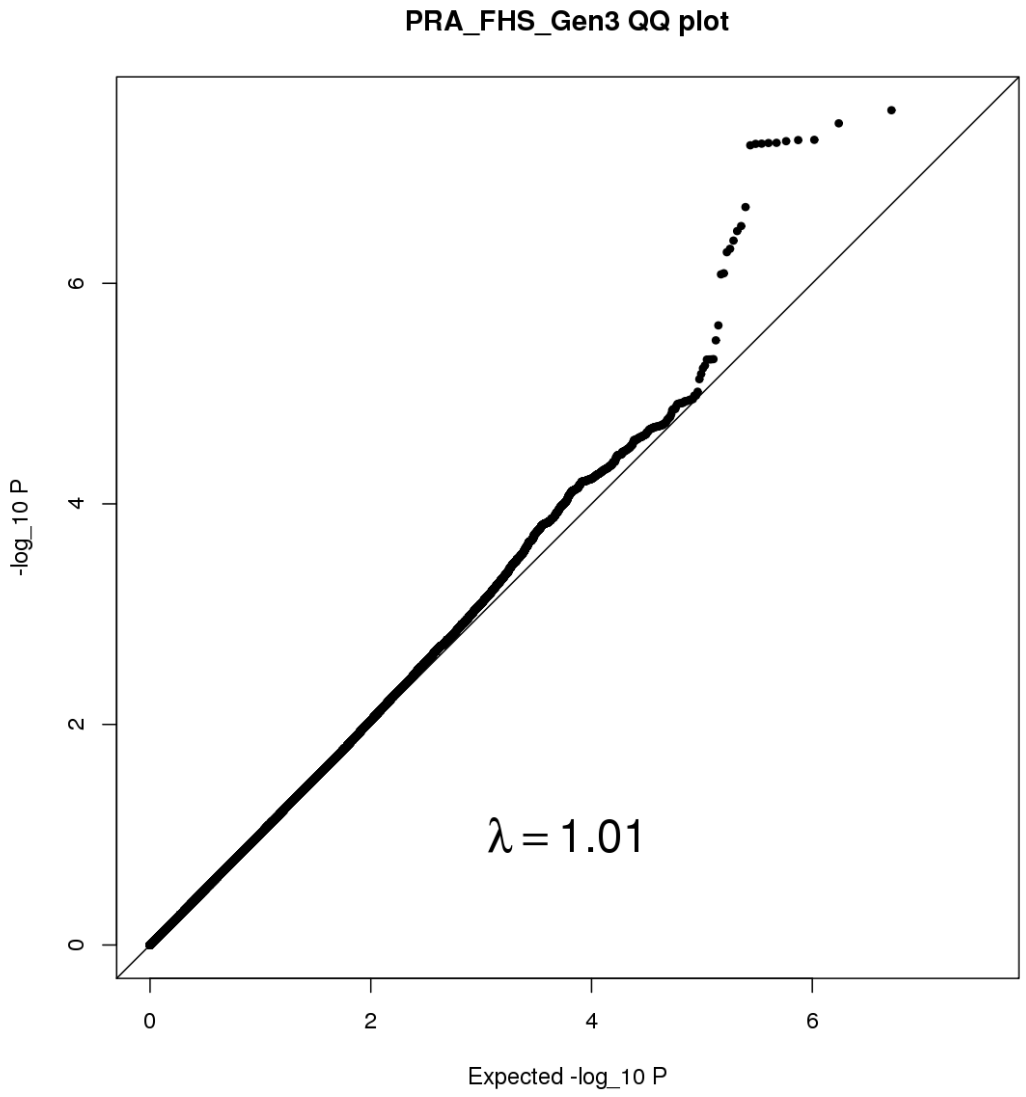
Supplementary Table 6c. Association of genetic variation at the *CYP11B2* locus with circulating aldosterone levels

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

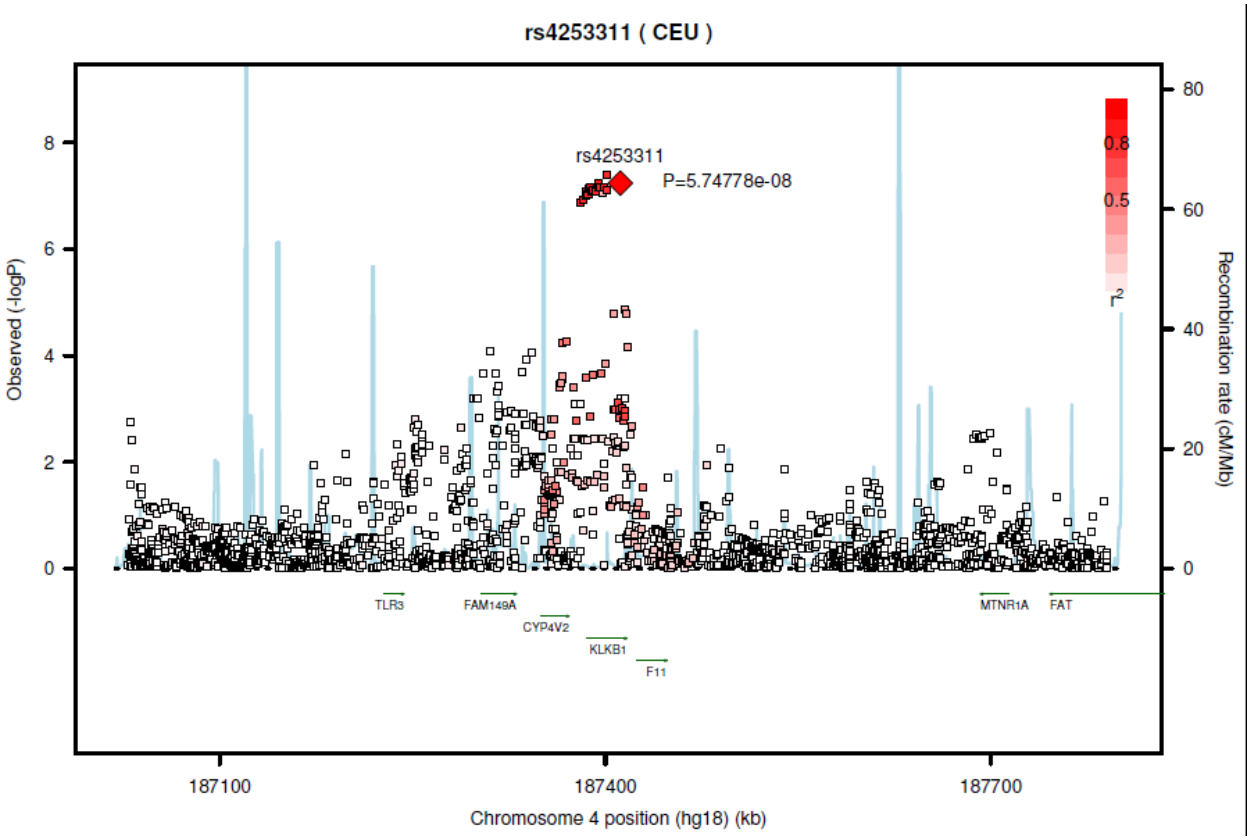
Supplementary Figures

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

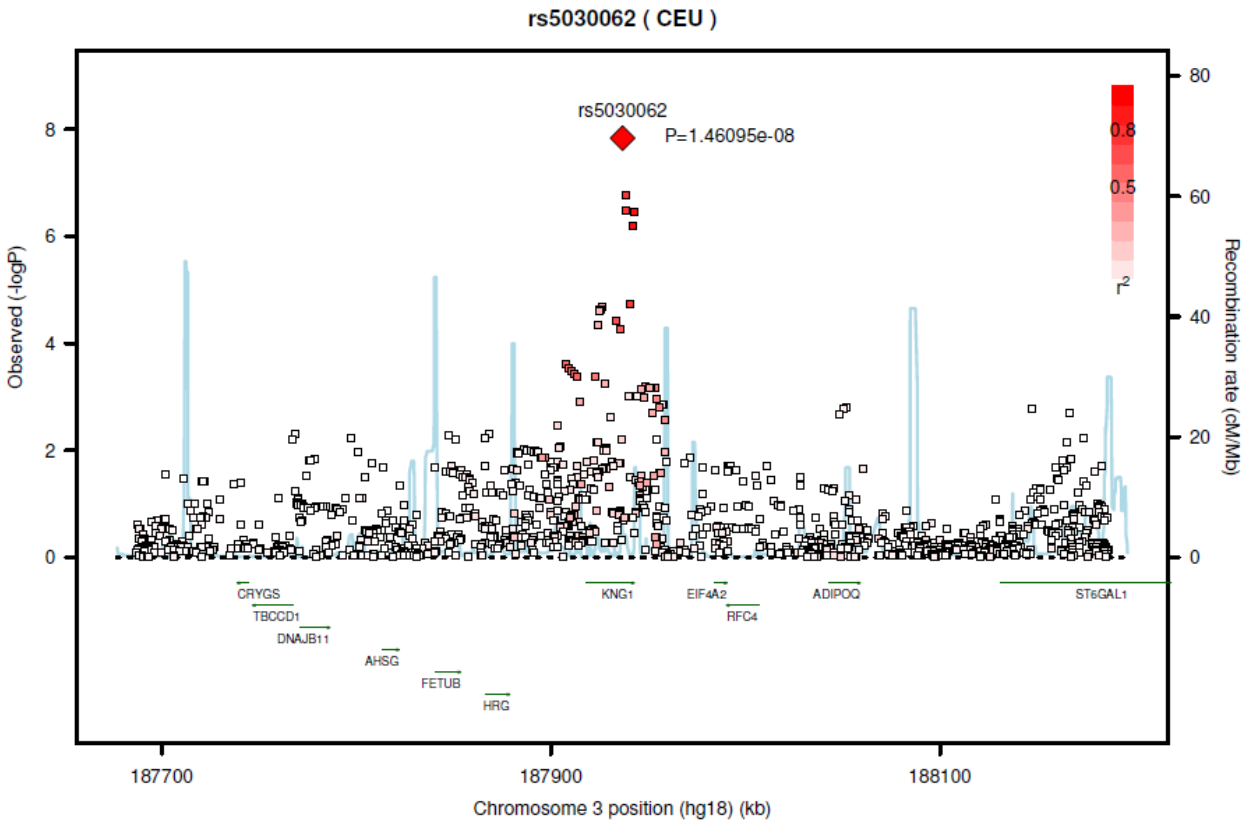


Supplementary Figure 2. Regional plot of rs4253311 in exon 11 of the *kallikrein B* gene (**Panel A**) and of rs5030062 in intron 6 of the *kininogen 1* gene (**Panel B)**; based on imputation to the 1000 genome dataset

Panel A

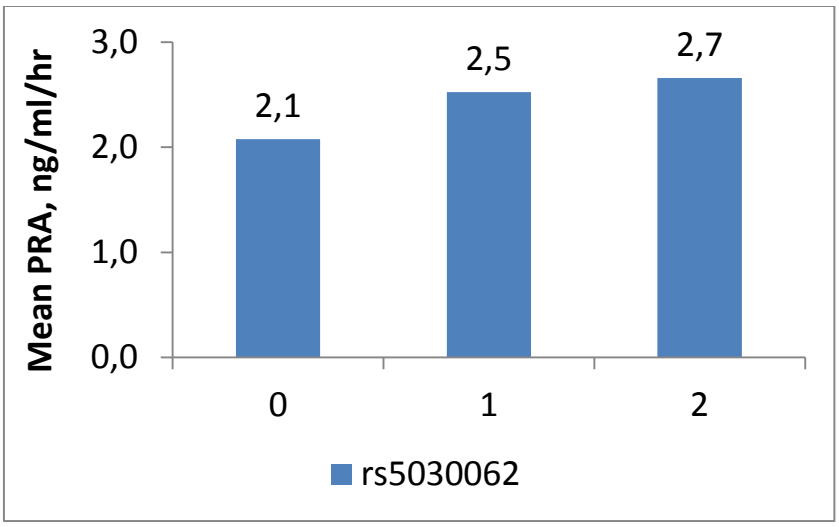


Panel B

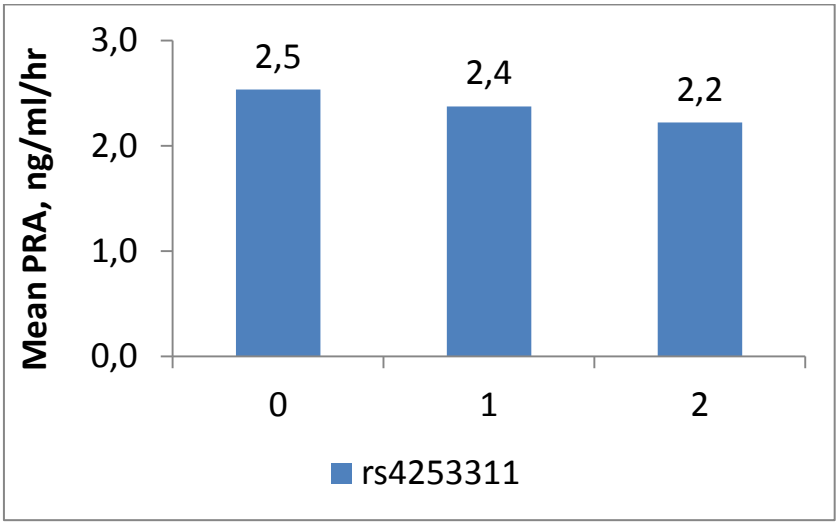

rs5030062 (CEU)
rs5030062
P=1.46095e-08
0.8
0.5
r²
Observed (-logP)
Recombination rate (cM/Mb)
CRYGS
TBCCD1
DNAJB11
AHSG
FETUB
HRG
KNG1
EIF4A2
RFC4
ADIPOQ
ST6GAL1
187700
187900
188100
Chromosome 3 position (hg18) (kb)

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

Panel A

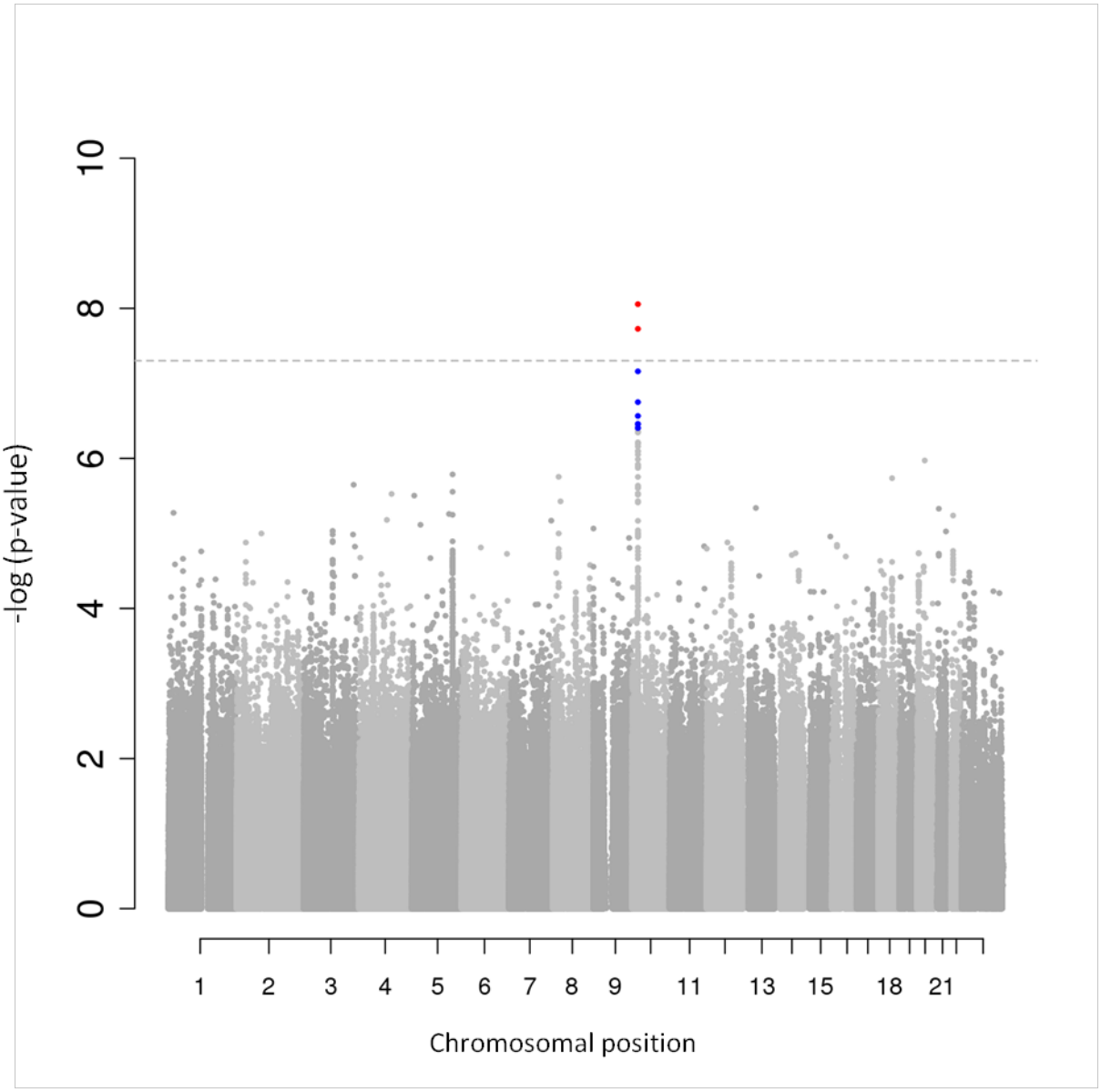


Panel B

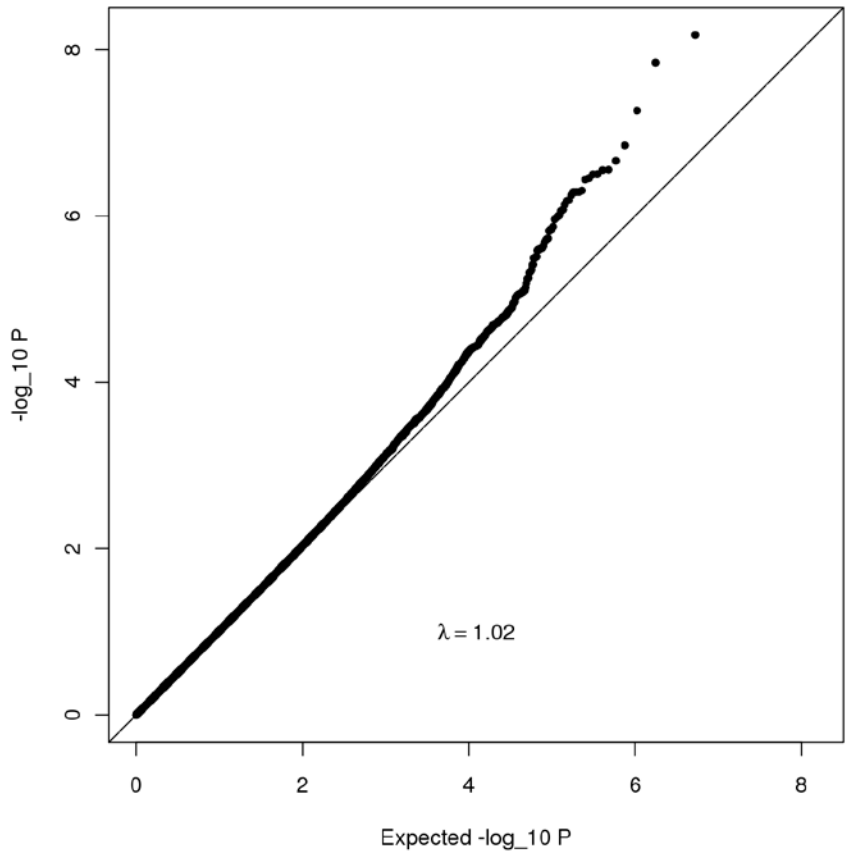


Supplementary Figure 4. Manhattan plot (**Panel A**) and quantile-quantile plot (**Panel B**) of the genome-wide analysis for plasma renin concentration

Panel A

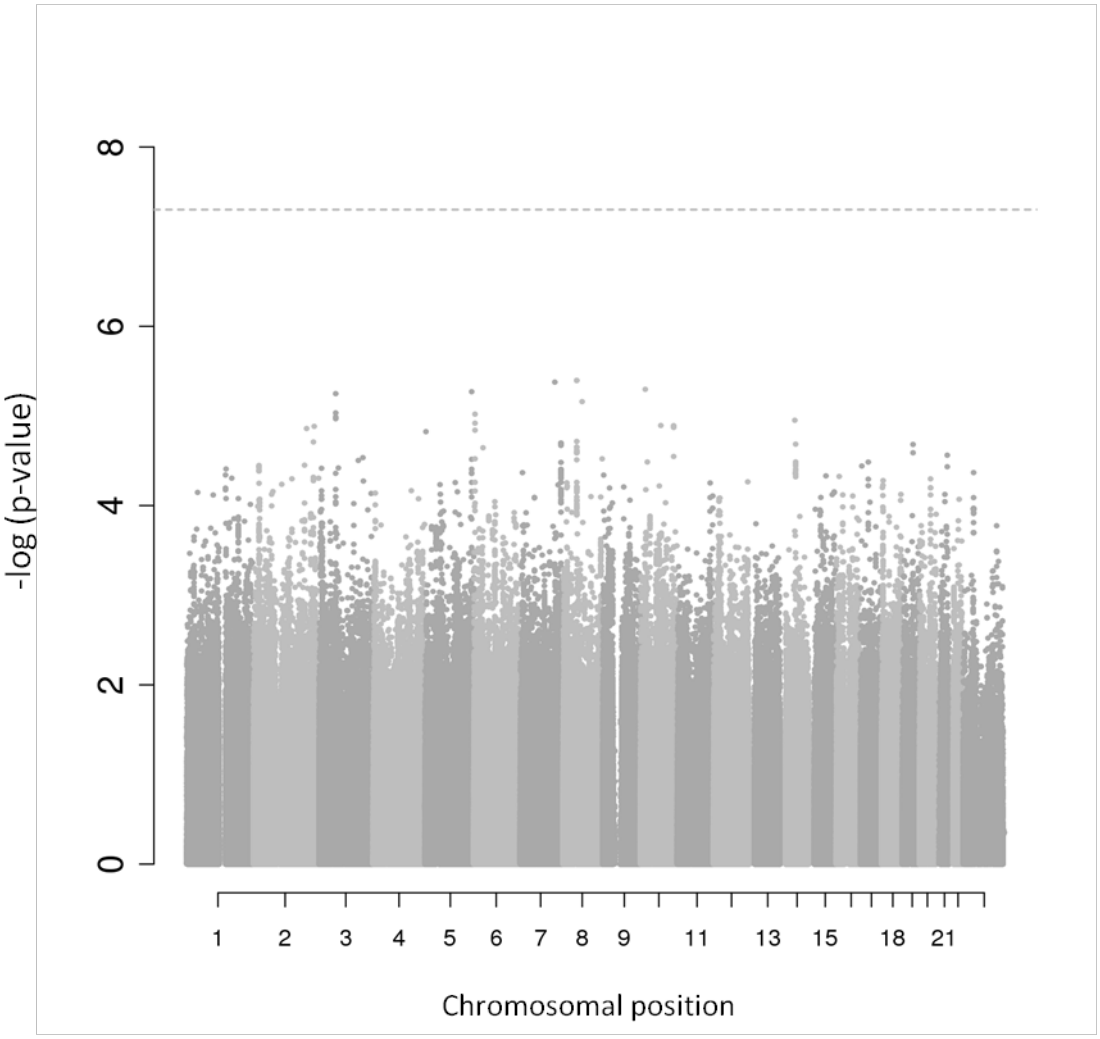


Panel B

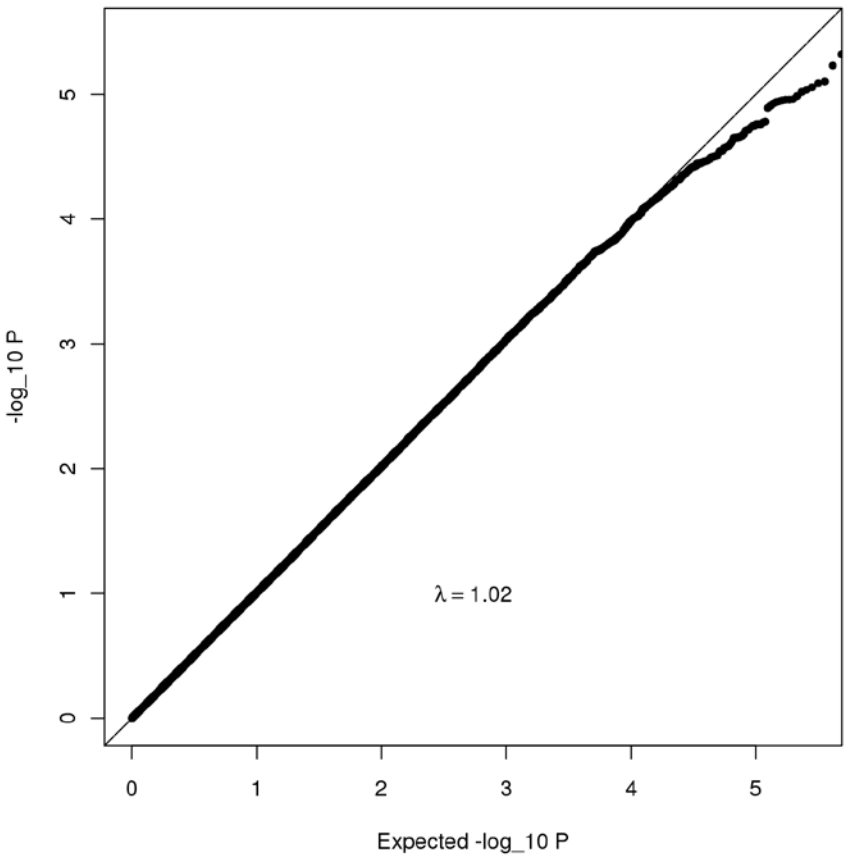


Supplementary Figure 5. Manhattan plot (**Panel A**) and quantile-quantile plot (**Panel B**) of the genome-wide analysis for circulating aldosterone concentration

Panel A



Panel B



References

1. Lieb W, Larson MG, Benjamin EJ, Yin X, Tofler GH, Selhub J, et al. Multimarker approach to evaluate correlates of vascular stiffness: The framingham heart study. *Circulation*. 2009;119:37-43.
2. O'Seaghdha CM, Hwang SJ, Vasan RS, Larson MG, Hoffmann U, Wang TJ, et al. Correlation of renin angiotensin and aldosterone system activity with subcutaneous and visceral adiposity: The framingham heart study. *BMC endocrine disorders*. 2012;12:3.
3. Manolopoulou J, Bielohuby M, Caton SJ, Gomez-Sanchez CE, Renner-Mueller I, Wolf E, et al. A highly sensitive immunofluorometric assay for the measurement of aldosterone in small sample volumes: Validation in mouse serum. *J Endocrinol*. 2008;196:215-224.
4. Hannemann A, Meisinger C, Bidlingmaier M, Doring A, Thorand B, Heier M, et al. Association of plasma aldosterone with the metabolic syndrome in two german populations. *Eur J Endocrinol*. 2011;164:751-758.
5. Morganti A. A comparative study on inter and intralaboratory reproducibility of renin measurement with a conventional enzymatic method and a new chemiluminescent assay of immunoreactive renin. *J Hypertens*. 2010;28:1307-1312.
6. Hafner S, Baumert J, Emeny RT, Lacruz ME, Bidlingmaier M, Reincke M, et al. Hypertension and depressed symptomatology: A cluster related to the activation of the renin-angiotensin-aldosterone system (raas). Findings from population based kora f4 study. *Psychoneuroendocrinology*. 2013;38:2065-2074.
7. Hannemann A, Friedrich N, Ludemann J, Volzke H, Rettig R, Peters J, et al. Reference intervals for aldosterone, renin, and the aldosterone-to-renin ratio in the population-based study of health in pomerania (ship-1). *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*. 2010;42:392-399.

8. Menard J, Guyenne TT, Corvol P, Pau B, Simon D, Roncucci R. Direct immunometric assay of active renin in human plasma. *J Hypertens Suppl*. 1985;3:S275-278.

9. de Boer RA, Schroten NF, Bakker SJ, Mahmud H, Szymanski MK, van der Harst P, et al. Plasma renin and outcome in the community: Data from prevend. *Eur Heart J*. 2012;33:2351-2359.