UCLA

Title

Genome-wide meta-analysis of SNP-by9-ACEI/ARB and SNP-by-thiazide diuretic and effect on serum potassium in cohorts of European and African ancestry

Permalink

Journal

ISSN

Authors

Irvin, Marguerite R
Sitlani, Colleen M
Noordam, Raymond
et al.

Publication Date

DOI

Peer reviewed

Genome-wide meta-analysis of SNP-by-ACEI/ARB and SNP-by-thiazide diuretic and effect on serum potassium in cohorts of European and African ancestry

Marguerite R Irvin, PhD[*],

Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA

Colleen M Sitlani, PhD,

Department of Medicine, University of Washington, Seattle, WA, USA

Raymond Noordam, PhD,

Department of Epidemiology, Erasmus MC - University Medical Center Rotterdam, Rotterdam, the Netherlands and Department of Internal Medicine, Section Gerontology and Geriatrics, Leiden University Medical Center, Leiden, the Netherlands

Christie L Avery, PhD,

Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina, USA

Joshua C Bis, PhD,

Department of Medicine, University of Washington, Seattle, WA, USA

James S Floyd, MD,

Departments of Medicine and Epidemiology, University of Washington, Seattle, WA, USA

Jin Li, PhD,

Department of Medicine, Stanford University School of Medicine, Palo Alto, CA, USA

Nita A Limdi, PhD,

Department of Neurology, University of Alabama at Birmingham, Birmingham, AL, USA

Vinodh Srinivasasainagendra, MS,

Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL, USA

James Stewart, MA,

[*]Corresponding author: Marguerite R Irvin, RPHB 230P, 1720 2nd Ave S, Birmingham, AL 35294-0022 USA. irvinr@uab.edu. (205) 975-7672.

Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, and Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

Renée de Mutsert, PhD,
Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

Dennis O Mook-Kanamori, PhD,
Department of Clinical Epidemiology and Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, the Netherlands

Leonard Lipovich, PhD,
Department of Neurology, Wayne State University, Detroit, MI, USA

Erica L Kleinbrink, MS,
Center Molecular Medicine/Genetics, Wayne State University School of Medicine, Detroit, MI, USA

Albert Smith, PhD,
Icelandic Heart Association, Kopavogur, Iceland; University of Iceland, Reykjavik, Iceland

Traci M Bartz, MS,
Departments of Biostatistics and Medicine, University of Washington, Seattle, WA, USA

Eric A Whitsel, MD,
Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, and Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

Andre G Uitterlinden, PhD,
Department of Internal Medicine, Erasmus MC - University Medical Center Rotterdam, Rotterdam, the Netherlands

Kerri L Wiggins, MS,
Department of Medicine, University of Washington, Seattle, WA, USA

James G Wilson, MD,
Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

Degui Zhi, PhD,
School of Biomedical Informatics, University of Texas Health Sciences Center at Houston, Houston, TX, USA

Bruno H Stricker, PhD,
Department of Epidemiology, Erasmus MC - University Medical Center Rotterdam, Rotterdam, the Netherlands and Inspectorate of Health Care, Utrecht, the Netherlands

Jerome I Rotter, PhD,
Institute for Translational Genomics and Population Sciences and Departments of Pediatrics and Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA

Donna K Arnett, PhD,

College of Public Health, University of Kentucky, Lexington, KY, USA

Bruce M Psaty, PhD, and

Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, Health Services, University of Washington, Seattle, WA, USA; Group Health Research Institute, Group Health Cooperatives, Seattle, WA, USA

Leslie A Lange, PhD

Division of Biomedical Informatics and Personalized Medicine, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Abstract

We evaluated interactions of SNP-by-ACE-I/ARB and SNP-by-TD on serum potassium (K+) among users of antihypertensive treatments (anti-HTN). Our study included 7 European-ancestry (EA) (N=4835) and 4 African-ancestry (AA) cohorts (N=2016). We performed race-stratified, fixed-effect, inverse-variance-weighted meta-analyses of 2.5 million SNP-by-drug interaction estimates; race-combined meta-analysis; and trans-ethnic fine-mapping. Among EAs, we identified 11 significant SNPs ($P<5\times10^{-8}$) for SNP-ACE-I/ARB interactions on serum K+ that were located between *NR2F1-AS1* and *ARRDC3-AS1* on chromosome 5 (top SNP rs6878413 $P=1.7\times10^{-8}$; ratio of serum K+ in ACE-I/ARB exposed compared to unexposed is 1.0476, 1.0280, 1.0088 for the TT, AT and AA genotypes, respectively). Trans-ethnic fine mapping identified the same group of SNPs on chromosome 5 as genome-wide significant for the ACE-I/ARB analysis. In conclusion, SNP-by-ACE-I/ARB interaction analyses uncovered loci that, if replicated, could have future implications for the prevention of arrhythmias due to anti-HTN treatment-related hyperkalemia. Before these loci can be identified as clinically relevant, future validation studies of equal or greater size in comparison to our discovery effort are needed.

INTRODUCTION

Most people initiating treatment for hypertension (anti-HTN treatments) do not experience clinically meaningful changes in serum potassium (K^+). However, thiazide and thiazide-like diuretics (TDs), first-line treatments for hypertension, produce hypokalemia more frequently than other anti-HTN treatments.[1] Additionally, angiotensin-converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs) are associated with hyperkalemia. Though uncommon, altered serum K^+ concentration during anti-HTN treatment can be clinically serious as it is associated with adverse cardiovascular effects.[2] Hypokalemia during TD treatment has been implicated in the development and progression of coronary heart disease (CHD), new-onset diabetes mellitus[3] and myocardial infarction[4] as well as the provocation of cardiac arrhythmia and sudden death.[5] Of even greater clinical significance is hyperkalemia which is associated cardiac arrhythmia and cardiovascular disease (CVD) mortality.[2]

For example, in the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), hypokalemia ($K^+<3.5$ mmol/L) incidence was higher in the chlorthalidone (a TD) randomization group than in the amlodipine (a calcium channel blocker (CCB)) and

lisinopril (an ACE-I) randomization groups (12.9% vs. 2.1% and 1.0%, respectively).[6] Hyperkalemia (K^+>5.4 mmol/L) was more common for ACE-I (3.6%) than TD (1.2%) or CCB (1.9%). During more than 6 years of follow-up in ALLHAT, hypokalemia was associated with increased mortality and hyperkalemia was associated with increased risk of combined CVD (including fatal or non-fatal MI, coronary revascularization, stroke, angina, heart failure, and treated peripheral arterial disease).

Because serum K^+ is heritable[6–10] and inter-individual variations in serum K^+ during anti-HTN treatment have been observed, we hypothesized that genetic factors may modify serum K^+ levels during treatment. To test this hypothesis, we examined single-nucleotide polymorphism (SNP)-by-ACE-I/ARB and SNP-by-TD interaction effects on serum K^+ among observational epidemiology cohorts participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium.[11] There were few cases of hyperkalemia and hypokalemia in these epidemiological studies, therefore, our study considered K^+ measured as a continuous variable. Cohorts providing data on participants of European ancestry (EA) or African ancestry (AA) included the Cardiovascular Health Study (CHS); the Rotterdam Study (RS); the Atherosclerosis Risk in Communities Study (ARIC); the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES); the Heart and Vascular Health Study (HVH); the Hypertension Genetic Epidemiology Network Study (HyperGEN); and the Jackson Heart Study (JHS). The current study leveraged data from studies with available measures of serum K^+, genome-wide association study (GWAS) data, and comprehensive anti-HTN medication data for a total of 4835 EA and 2016 AA participants.

METHODS

Study populations

Inclusion criteria for this analysis entailed the following: treatment for hypertension, available GWAS data, and available serum K^+ data. Cohort participants were excluded if they were treated with a diuretic other than a thiazide or thiazidSEe e-like diuretic (other diuretics were loop, aldosterone antagonist, or K^+-sparing diuretics). We also excluded individuals who were treated concomitantly with single or combination preparations of TD plus ACE-I or ARB and individuals with renal failure (eGFR <30 mL/min/1.73m^2). Cohort-specific inclusions and/or exclusions can be found in the online-only Supplementary File Section 1 (Study Descriptions). Across the contributing cohorts, 7 studies contributed data on participants of European ancestry and 4 studies contributed data on participants of African ancestry.

Phenotype and other clinical data

Serum K^+ was measured using standard clinical chemistry methods in all cohorts. Information on K^+ supplementation was available in all cohorts except for the HyperGEN, JHS, AGES and Rotterdam studies. More detailed information on the serum K^+ assay and supplementation can be found in Supplementary File Section 1, Study Description. We also determined counts of clinically relevant cut points for high (>5.0 mmol/L), low (<3.5 mmol/L) and normal serum potassium 3.5 $K+$ 5.0 mmol/L by drug exposure group within each cohort.

Drug exposure information

Information on anti-HTN treatment use (yes/no) was assessed at the time of serum K^+ assay in all studies. Data was extracted at one point in time with the exception of the ARIC study which provided cross-sectional data at two time-points. See Supplementary File Section 1 (Study Descriptions). TD users were defined as those participants taking a thiazide or thiazide-like diuretic in a single or combination preparation without concomitant use of an ACE-I or ARB. For the purposes of the current study, we combined ACE-I and ARB treatment into one exposure category because both medications downregulate angiotensin II (Ang II) activity and can cause hyperkalemia. ACE-I/ARB users were defined as those participants taking an ACE-I or ARB in a single or combination preparation without concomitant use of a TD. The reference group for each drug exposure was treatment with anti-HTN medication belonging to one of the following classes and without concomitant use of TD, ACE-I, or ARB: calcium channel blocker, alpha blocker, beta blocker, central acting agent, and/or direct vasodilator (see Supplementary File Table 3 for counts of reference drug classes within each cohort). Drug exposures were assessed by medication inventory, self-report, or computerized databases. See Supplementary File Section 1 (Study Descriptions).

Genotype arrays and imputation

Genome-wide SNP genotyping was performed within each study using Illumina or Affymetrix genotyping arrays. Details of genotyping and quality control are provided in Supplementary File Table 1. General quality control procedures excluded participants based on sex mismatches, genotyping success rate <95%; and exclusion of SNPs failing genotyping call rate thresholds, typically between 90% and 99%. To increase coverage and facilitate evaluation of the same SNPs across cohorts, SNPs passing quality control were used to impute to the HapMap Phase 2 reference panels using MaCH,[12] BEAGLE,[13] or BIMBAM.[14]

Statistical analysis

General estimating equations (for repeated measures data in ARIC), and linear regression models (or mixed linear to accommodate family based designs) were used in each individual cohort where the outcome of interest was the natural log transform of serum K^+ level treated as a continuous variable. All models included a term for the SNP dosage and an indicator variable for the anti-HTN treatment exposure. The primary parameter of interest was the SNP-by-treatment interaction term (β_{GE}). Adjustment for age, sex, body mass index (BMI), and concomitant use of a K^+ supplement (if available), as well as study-specific covariates (e.g., principal components for ancestry and study site) was taken into account and, if applicable, correlation matrices to account for family relatedness. Robust standard error (SE) estimates were used for non-familial data.[15] Details of the models and software packages used to estimate cohort-specific results are shown in Supplementary File Table 2. In secondary analysis we considered clinically relevant cut points for serum K+ as a binary outcome variable for significant SNP findings from the primary analysis using logistic regression in cohorts with available data. Sensitivity analysis was performed removing participants with K+ supplementation for our top finding. Since beta blockers have been

associated with variation in serum K+[16] we conducted an additional sensitivity analysis in the HyperGEN EA cohort removing those exposed to beta blockers from the reference group for our top finding. Finally, since serum Na+ and K+ may behave in the opposite direction during anti-HTN treatment we also examined K+ significant drug-by-SNP interaction effects on serum Na+ in the HyperGEN EA cohort.

Study-specific interaction estimates (β_{GE}) and "corrected" SEs were combined by fixed effect inverse variance weighted meta-analysis using METAL (code available upon request).[17] To obtain "corrected" SEs, *P*-values were calculated using a t reference distribution for the ratio of the β_{GE} term to its SE; then corrected SEs were the SE values that would give the t-distribution-based p-values when assuming a normal distribution for the ratio of the β_{GE} term to its corrected SE. Such correction was necessary due to known underestimation of SEs by robust methods when any SNP-treatment stratum is small.[18] The degrees of freedom (DF) for the t reference distribution were estimated using Satterthwaite's method in cohorts with unrelated participants.[19, 20] In HyperGEN the DF was estimated as the Filter DF described below. To control for remaining inflation in the QQ-plots for variants with smaller minor allele frequency (MAF), due to a lower limit of accuracy of the t-distribution-based correction, a study-specific filter was calculated (Filter DF=2*imputation quality*MAF*$N_{exposed}$; where $N_{exposed}$ was the approximate number of independent observations exposed to the drug). SNPs within each study that had Filter DF value   10 were excluded from the meta-analysis.[18] The genome-wide threshold for significant drug-by-SNP interaction was $P<5.0\times10^{-8}$. SNPS with Cochran's *Q*-test for heterogeneity p-value <0.05 were excluded from the final result set. Both race-specific and race-combined meta-analyses were conducted. To illustrate the effect size of a statistically significant SNP-by-treatment interaction we used inverse-variance-weighted meta-analysis to get an estimate of the treatment coefficient and the treatment-SNP interaction coefficient along with the variances. We then calculated the expected difference between the treatment (of interest) exposed and unexposed by genotype, along with the 95% confidence interval (CI) and exponentiated those values.

We also performed trans-ethnic fine-mapping of significant results from the above analysis using MANTRA[21] combining fixed effect estimates from the EA and AA discovery. We considered an association to have reached genome-wide significance if the log10 Bayes Factor (BF) from MANTRA was greater than 5 as recommended by the authors.[21] A log10 Bayes Factor > 5 was estimated to approximate $P<8*10^{-7}$ and log10 Bayes Factor > 6.1 was estimated to approximate $P<5.0\times10^{-8}$ by Wang et al.[22]

Gene annotation

We used publicly available regional ENCyclopedia Of DNA Elements (ENCODE) annotations accessed June 28, 2016 to evaluate transcription factor binding sites, chromatin modifications, histone acetylation, long non-coding RNAs, previous GWAS findings, and micro RNAs in the region of our association results. We also used Bioconductor's FunciSNP to extract information on 1000 Genomes (1000G) database SNPs in linkage disequilibrium (LD) with our statistically significant findings. FunciSNP curates all 1000G database SNPs in LD to the trait-associated SNP and their overlap with genomic biological features from

ENCODE. We scanned for 1000G database SNPs within a 2-MB window of our index SNPs and required 1000G database SNPs reported on to have an R^2 value of >0.5 with our K^+-associated SNPs.[23] We used the online LDlink tool to investigate LD between SNPs belonging to association peaks.[24]

Replication

We attempted to replicate statistically significant findings from the discovery meta-analysis in the Netherlands Epidemiology of Obesity (NEO) Study. The inclusion and exclusion criteria, methods for medication use assessment and K^+ level measurement, and statistical analysis was the same as in the discovery analysis. A total of 817 NEO participants were included in the replication analysis. See Supplementary File section 1 for a description of the NEO cohort.

RESULTS

Characteristics of study participants are shown in Tables 1 and 2. The average age (SD) of the EA participants ranged from 54.6 (6) to 79.2 (4) in EAs and 51.3 (10) to 72.5 (5) in AAs. The average systolic blood pressure (SD) ranged from 125.3 (18) to 144.8 (22) mm Hg in EAs and 131.1 (18) to 145.4 (24) mm Hg in AAs. The average diastolic blood pressure ranged from 71.5 (12) to 82.6 (12) mm Hg in EAs and 75.8 (11) to 82.6 (12) mm Hg in AAs. Finally, the median value (interquartile range) of serum K^+ ranged 3.9 (0.3) to 4.4 (0.7) mmol/L in EAs and 3.9 (0.7) to 4.2 (0.5) mmol/L in AAs. See Supplementary File Tables 4 (EAs) and 5 (AAs) for counts of high, low and normal serum K+ levels by drug exposure group in each cohort.

Across the individual cohort GWAS analyses, there was not excessive evidence for the inflation of P-values for gene-by-drug interaction terms (range of genomic inflation factors 1.01–1.11, see Supplementary File Table 6). Manhattan plots for the ACE-I/ARB exposure discovery meta-analyses are presented in Figure 1 (race-combined, EA, and AA). QQ plots for the ACE-I/ARB exposure analysis in the individual EA cohorts are presented in Supplementary File Figure 1.

In the EA stratum, eleven SNPs with a statistically significant interaction effect were identified for the ACE-I/ARB exposure in an intergenic region between arrestin domain containing 3 antisense RNA (*ARRDC3-AS1*) and nuclear receptor subfamily 2, group F, member 1 antisense RNA (*NR2F1-AS1*) (smallest $P_{int}=1.7*10^{-8}$ for rs6878413) (Table 3). The SNPs were present in each of the 7 cohorts contributing EA data and had the same direction of effect for the interaction term across studies with the exception of the Rotterdam study. The 11 variants are in strong LD in the CEU population ($R^2>0.9$). Figure 2 shows that the ratio (95% CI) of serum K+ in ACE-I/ARB exposed compared to unexposed for genotypes TT, AT and AA at rs6878413 (the most significant SNP) is 1.0476 (1.038–1.057), 1.0280 (1.021–1.034) and 1.0088 (1.000–1.017), respectively. Sensitivity analysis removing those participants reporting K+ supplementation did not change individual cohort estimates for the top SNP (rs6878413) appreciably in the ARIC, CHS, HVH1 and HVH2 studies (Supplemental Table 7). In the HyperGEN EA cohort removing those treated with beta blockers did not substantially change the interaction effect estimate for the top SNP and the

SNP-ACE-I/ARB interaction term for these 11 SNPs was not associated with serum Na+ (P>0.1). We also calculated the OR for high serum K+ with ACE-I/ARB exposure versus other anti-HTN treatment using data from HVH1, HVH2, and ARIC and meta-analyzed those results using inverse variance weighted meta-analysis. For rs6878413 the OR (95% CI) for high K+ for genotypes TT, AT, and AA comparing those exposed to ACE-I/ARB versus other anti-HTN treatments was 3.7 (0.9–14.0), 2.4 (1.0–6.0), and 1.6 (1.0–2.6), respectively.

These findings on chromosome 5 for the ACE-I/ARB exposure were not consistent in the AA stratum and not strengthened in the race-combined meta-analysis (Table 3). Ten of the top 11 SNPS were present in both ethnicities and the log10 BF was >5 (approximating a P< $8*10^{-7}$) for 8 of 10 SNPs after combining the results of both ethnic groups using MANTRA (Supplementary File Table 8). None of the 10 variants reached a significance threshold approximating P<$5*10^{-8}$ or log10 BF >6.1. This group of 10 SNPs represents the highest MANTRA peak on chromosome 5 and includes variants with heterogeneous allelic effects (posterior probabilities >0.5). There was a different lead SNP (rs6557075) in trans-ethnic meta-analysis also suggesting differences between the race groups.

Annotation of the region on chromosome 5 revealed that the 11 markers (highlighted in blue in Supplementary File Figure 2) are closest to the downstream gene *NR2F1-AS1*. Upstream, the nearest functionally characterized gene is >1.8Mbp away (*ARRCDC3-AS1*) and the region between *NR2F1-AS1* and *ARRDC3-AS1* is devoid of protein-coding genes (i.e., in a gene desert), but includes lincRNA expression reads and previous published GWAS findings. SNPs from previous GWAS in the region are associated with breast cancer,[25, 26] subcutaneous adipose tissue,[26] and obesity.[27] The region immediately surrounding the SNPs of interest is rich in transcription factor binding sites for *NR2F1*.[28] FunciSNP identified 44 1000G SNPs in LD with our statistically significant findings (index SNPs) on chromosome 5. All 44 of those variants were upstream of *NR2F1-AS1* and located in open chromatin regions as determined by formaldehyde-assisted isolation of regulatory elements (FAIRE). See Supplementary Table 9 for FunciSNP output on the 44 1000G SNPs. In the NEO study population the K+ range was 3.1–6.1 mmol/L and 457 of 817 (56%) participants were exposed to ACE-I or ARB. The results for the interaction terms for the 11 SNPs of interest from the discovery meta-analysis were not replicated in NEO. Supplementary File Table 10 shows that P_{int} ranged from 0.068 to 0.44, and that the direction of effect was opposite the direction in the discovery meta-analysis.

There were no other statistically significant SNP-by-ACE-I/ARB interactions in the AA stratum. Although a peak of marginal significance intronic to choline/ethanolamine phosphotransferase 1 (*CEPT1*) was found on chromosome 1 (top SNP, rs2490334; P=1.5×10^{-6}). In the race-combined analysis, the most significant finding (P=1.4×10^{-7}) was on chromosome 15 for rs4886544 between LOC645752 (~8kb) and LOC645752 (~60 Kb) (Supplementary File Table 11).

Top results for the TD exposure meta-analysis of serum K+ levels in EAs and AAs are presented in Supplementary File Table 12 and graphically in Figure 3. No SNP-by-TD interaction was statistically significant in the EA or AA meta-analysis. Among EAs the most

significant (P_{int} =6.9×10^{-7}) SNP-by-TD interaction was on chromosome 12 where the SNP (rs7313728) was intergenic between T-Box 3 (*TBX3*, distance 1 Mbp) and mediator complex subunit 13-like (*MED13L*, distance 0.26 Mbp). Three other SNPs (rs1816225, rs6490032, rs1352141) in the same region had marginal significance with P_{int} <1×10^{-5}. In each case, the SNP was present in 5 of 7 cohorts (missing in the HyperGEN and Rotterdam studies due to filter DF<10) and the interaction term had the same direction of effect across the cohorts. Among AAs the most significant (P_{int} =1.9×10^{-7}) SNP-by-TD interaction (for rs12133062) was on chromosome 1 upstream of immunoglobulin superfamily, member 2 (*IGSF2*). Another SNP (rs12759956) in this region was marginally significant P_{int}=1.3×10^{-5}. When the results for the EA and AA strata were combined, a marker intronic to retinoic acid receptor beta (*RARB*) almost reached the statistical significance threshold at P_{int}=9.9×10^{-8} (Figure 2A). The SNP-by-TD exposure interaction peak nearby *TBX3* was strengthened in the race combined analysis (top SNP rs7313728 with P_{int}=1.7×10^{-7}). Finally, a dense peak was identified ~700 kb upstream of the angiotensin II receptor type 1 (*AGTR1*) gene with smallest P_{int}= 1.3×10^{-6} for rs1462657. Because none of the discovery findings were statistically significant we did not seek replication for the TD findings.

DISCUSSION

The mechanisms through which TDs, ACE-Is, and ARBs lead to lower or higher serum K$^+$ concentrations in some users but not the majority of users are not well understood. To better understand the potential for a genetic contribution to the inter-individual variation in serum K$^+$ during anti-HTN treatment, we undertook two independent, genome-wide, gene-by-treatment meta-analyses of serum K$^+$ (SNP-by-TD and SNP-by-ACE-I/ARB, respectively) in the CHARGE consortium representing genotypes and relevant data on almost 5000 persons of European descent and just over 2000 persons of African descent. We uncovered 11 statistically significant SNPs for the ACE-I/ARB exposure analysis on chromosome 5 in between *NR2F1-AS1* and *ARRDC3-AS1* in the EA stratum. The result was not replicated in a smaller group of EAs from the Netherlands, though trans-ethnic meta-analysis of chromosome 5 helped to validate the importance of the finding as the lead peak.

The group of SNPs downstream of *ARRDC3-AS1* are biologically interesting. *ARRDC3-AS1* is the antisense RNA to *ARRDC3* which is 1 of 6 known human α-arrestins and has been implicated in the downregulation of the β2-adrenergic receptor (β2AR).[29] Interestingly catecholamines (such as adrenaline) induce K$^+$ loss via up-regulation of beta-adrenoceptors.[30] Unfortunately, we were unable to identify 1000G SNPs (in LD with our statistically significant SNPs) that tagged functional elements near *ARRDC3-AS1*. The region between our statistically significant SNPs and *ARRDC3-AS1* is an extensive gene desert with no identified protein coding genes. The 1000G SNPs identified by the FunciSNP program (Supplemental Table 9) were between 7kb upstream and 40kb downstream of the index SNPs but upstream of *NR2F1-AS1. NR2F1* on chromosome 5 (immediately downstream of *NR2F1-AS1*) codes for transcription factor coup 1 (TFCOUP1) which is a member of the steroid/thyroid hormone receptor superfamily. Aldosterone is a steroid hormone belonging to the renin angiotensin aldosterone system (RAAS) that promotes K$^+$ excretion; therefore, this gene's biological role cannot be ruled out. Our index SNPs did overlap with open chromatin regions upstream of *NR2F1-AS1* and could be linked to the promoter region for

that gene. One interesting transcription factor binding site, which directly overlaps with rs1545708, was for STAT3 which is a key regulator of cell-to-cell communication in the heart.[31]

Though none of our findings for the TD exposure were statistically significant, two gene regions are potentially biologically interesting in relation to serum K+ during TD treatment. *TBX3* is member of a family of phylogenetically conserved transcription factors that share a common DNA-binding domain, the T-box involved in the regulation of developmental processes. *TBX3* has been highlighted in previous genome-wide association studies of systolic and diastolic blood pressure in both EA and AA populations.[32, 33] Another report suggested *TBX3* mutant mice are at increased risk for sudden death due to arrhythmia and that arrhythmias induced by knockdown of *TBX3* in adult mice reveal its requirement for conduction system homeostasis.[34] The other biologically plausible finding was near *AGTR1*. AGTR1 regulates aldosterone which works in the kidney to increase water and sodium reabsorption and K$^+$ excretion. Larger sample sizes may be needed to verify the importance of these findings.

Other pharmacogenetic discovery efforts have investigated whether genetic factors contribute to metabolic side effects of common anti-HTN agents, including changes in lipids, fasting glucose, and uric acid.[35, 36] Most of the previous pharmacogenetic research has focused on metabolic response to the TD class of anti-HTN agents. We know of no studies that have considered how genetic factors may modify serum K$^+$ during RAAS inhibition (i.e., treatment with ACE-Is or ARBs). Among the TD response studies, a genome-wide, trans-ethnic meta-analysis in 718 EA and AA hypertensive participants from the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) and the Genetic Epidemiology of Responses to Antihypertensive (GERA) Trials suggested variants belonging to the heme pathway influenced hydrochlorathiazide- (HCTZ) induced K$^+$ loss.[37] Both rs10845697 on chromosome 12, near to the HEME binding protein 1 gene (*HEBP1*), and rs11135740 on chromosome 8, near to the Mitoferrin-1 gene (*SLC25A37*), reached the statistical significance threshold for change in K$^+$ level over 2–3 weeks of treatment. In our cross-sectional data, we did not replicate these findings (data not shown). Our study also did not cover the same variants as in a renal sodium transport candidate gene analysis (N~75) of K+ during HCTZ treatment.[38]

A limitation of the current study is the lack of replication of the primary finding on chromosome 5 in 817 participants from the NEO study. Our post-hoc power calculations suggested we had adequate power (~80%) to replicate the interaction effect in NEO (with variance explained 3% for the interaction term and MAF of 0.40, data not shown) using an effect size estimate from one of the discovery studies. However, this effect may be inflated due to the winner's curse phenomenon, and several reports warn replication of genetic associations require samples sizes at least as big or bigger than the discovery.[39–44] Ultimately, replication and further fine-mapping of the region would be necessary to fully understand how variation in this region on chromosome 5 may modify serum K$^+$ level during anti-HTN treatment. Given the mechanistic relationship between this finding and serum K$^+$, additional studies should be pursued. Other limitations included a focus on common variants from GWAS arrays; therefore, rare variants and protein-coding variants

were not represented. We studied cross-sectional serum K+ as a continuous trait outcome in our observational data; clinical trial data would facilitate the study of K+ response to anti-HTN treatment. Additionally, hyperkalemia, the clinical outcome of interest, was rare in our data. However, in secondary analysis we found the TT genotype at our top SNP may be associated with 3 fold increased risk of high serum K+ levels with ACE-I/ARB exposure versus other anti-HTN treatment among persons of European descent. Since this result was based off of only 70 cases, future larger SNP-ACE-I/ARB interaction studies of hyperkalemia are needed to better understand the clinical impact of our finding on chromosome 5. The sample size for the AA stratum was only about a third of the size of that for the EA stratum for both anti-HTN treatment exposures. However, AAs are underrepresented in the GWAS literature in general, and, that we know of, this still represents the largest study of gene-by-anti-HTN treatment effects on serum K^+ among AAs. Finally, K^+ supplementation information was missing on 4 of the cohorts included in this study.

Changes in serum K^+ with TD or ACE-I or ARB treatment represent a serious potential metabolic side effect of common anti-HTN treatments. Overall anti-HTN treatment is safe and the vast majority of users do not experience serious changes in serum K^+. However, in a minority of users these changes can be clinically significant. Genetic factors may underlie the between-person variation observed. This study used data from a large consortium of observational cardiovascular epidemiology studies to look for common SNPs that modify serum K^+ levels during treatment with 2 common anti-HTN treatment classes. Results suggest that SNPs intergenic to *ARRDC1-AS1* and *NR2F1-AS1* may modify serum K^+ level during treatment with ACE-Is or ARBs among EAs. The 11 statistically significant SNPs are closest in distance to *NR2F1-AS1*, but both genes may have relevance to serum K^+ level during treatment with ACE-I or ARB. This region warrants further study using additional external data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Papademetriou V. Diuretics, hypokalemia, and cardiac arrhythmia: a 20-year controversy. J Clin Hypertens (Greenwich). 2006; 8:86–92. [PubMed: 16470076]

2. Cohen HW, Madhavan S, Alderman MH. High and low serum potassium associated with cardiovascular events in diuretic-treated patients. J Hypertens. 2001; 19:1315–1323. [PubMed: 11446723]

3. Salvetti A, Ghiadoni L. Thiazide diuretics in the treatment of hypertension: an update. J Am Soc Nephrol. 2006; 17:S25–29. [PubMed: 16565243]

4. Freis ED. The efficacy and safety of diuretics in treating hypertension. Ann Intern Med. 1995; 122:223–226. [PubMed: 7810942]

5. Franse LV, Pahor M, Di Bari M, Somes GW, Cushman WC, Applegate WB. Hypokalemia associated with diuretic use and cardiovascular events in the Systolic Hypertension in the Elderly Program. Hypertension. 2000; 35:1025–1030. [PubMed: 10818057]

6. Alderman MH, Piller LB, Ford CE, Probstfield JL, Oparil S, Cushman WC, et al. Clinical significance of incident hypokalemia and hyperkalemia in treated hypertensive patients in the antihypertensive and lipid-lowering treatment to prevent heart attack trial. Hypertension. 2012; 59:926–933. [PubMed: 22431578]

7. Bathum L, Fagnani C, Christiansen L, Christensen K. Heritability of biochemical kidney markers and relation to survival in the elderly--results from a Danish population-based twin study. Clin Chim Acta. 2004; 349:143–150. [PubMed: 15469867]

8. Marroni F, Grazio D, Pattaro C, Devoto M, Pramstaller P. Estimates of genetic and environmental contribution to 43 quantitative traits support sharing of a homogeneous environment in an isolated population from South Tyrol, Italy. Hum Hered. 2008; 65:175–182. [PubMed: 17934319]

9. Nilsson SE, Read S, Berg S, Johansson B. Heritabilities for fifteen routine biochemical values: findings in 215 Swedish twin pairs 82 years of age or older. Scandinavian journal of clinical and laboratory investigation. 2009; 69:562–569. [PubMed: 19343610]

10. Pilia G, Chen WM, Scuteri A, Orru M, Albai G, Dei M, et al. Heritability of cardiovascular and personality traits in 6,148 Sardinians. PLoS Genet. 2006; 2:e132. [PubMed: 16934002]

11. Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, et al. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: Design of prospective meta-analyses of genome-wide association studies from 5 cohorts. Circ Cardiovasc Genet. 2009; 2:73–80. [PubMed: 20031568]

12. Li Y, Abecasis GR. MACH 1. 0: Rapid Haplotype Reconstruction and Missing Genotype Inference. Am J Hum Genet. 2006; S79:2290.

13. Browning BL, Yu Z. Simultaneous genotype calling and haplotype phasing improves genotype accuracy and reduces false-positive associations for genome-wide association studies. Am J Hum Genet. 2009; 85:847–861. [PubMed: 19931040]

14. Servin B, Stephens M. Imputation-based analysis of association studies: candidate regions and quantitative traits. PLoS Genet. 2007; 3:e114. [PubMed: 17676998]

15. Voorman A, Lumley T, McKnight B, Rice K. Behavior of QQ-plots and genomic control in studies of gene-environment interaction. PLoS One. 2011; 6:e19416. [PubMed: 21589913]

16. Chang AR, Sang Y, Leddy J, Yahya T, Kirchner HL, Inker LA, et al. Antihypertensive Medications and the Prevalence of Hyperkalemia in a Large Health System. Hypertension. 2016; 67:1181–1188. [PubMed: 27067721]

17. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010; 26:2190–2191. [PubMed: 20616382]

18. Sitlani CM, Rice KM, Lumley T, McKnight B, Cupples LA, Avery CL, et al. Generalized estimating equations for genome-wide association studies using longitudinal phenotype data. Stat Med. 2015; 34:118–130. [PubMed: 25297442]

19. Satterthwaite FE. An approximate distribution of estimates of variance components. Biometrics. 1946; 2:110–114. [PubMed: 20287815]

20. Pan W, Wall MM. Small-sample adjustments in using the sandwich variance estimator in generalized estimating equations. Stat Med. 2002; 21:1429–1441. [PubMed: 12185894]

21. Morris AP. Transethnic meta-analysis of genomewide association studies. Genet Epidemiol. 2011; 35:809–822. [PubMed: 22125221]

22. Wang X, Chua HX, Chen P, Ong RT, Sim X, Zhang W, et al. Comparing methods for performing trans-ethnic meta-analysis of genome-wide association studies. Hum Mol Genet. 2013; 22:2303–2311. [PubMed: 23406875]

23. Coetzee SG, Rhie SK, Berman BP, Coetzee GA, Noushmehr H. FunciSNP: an R/bioconductor tool integrating functional non-coding data sets with genetic association studies to identify candidate regulatory SNPs. Nucleic Acids Res. 2012; 40:e139. [PubMed: 22684628]

24. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics. 2015; 31:3555–3557. [PubMed: 26139635]

25. Rafiq S, Khan S, Tapper W, Collins A, Upstill-Goddard R, Gerty S, et al. A genome wide meta-analysis study for identification of common variation associated with breast cancer prognosis. PLoS One. 2014; 9:e101488. [PubMed: 25526632]

26. Fox CS, Liu Y, White CC, Feitosa M, Smith AV, Heard-Costa N, et al. Genome-wide association for abdominal subcutaneous and visceral adipose reveals a novel locus for visceral fat in women. PLoS Genet. 2012; 8:e1002695. [PubMed: 22589738]

27. Comuzzie AG, Cole SA, Laston SL, Voruganti VS, Haack K, Gibbs RA, et al. Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One. 2012; 7:e51954. [PubMed: 23251661]

28. Daily K, Patel VR, Rigor P, Xie X, Baldi P. MotifMap: integrative genome-wide maps of regulatory motif sites for model species. BMC Bioinformatics. 2011; 12:495. [PubMed: 22208852]

29. Qi S, O'Hayre M, Gutkind JS, Hurley JH. Insights into beta2-adrenergic receptor binding from structures of the N-terminal lobe of ARRDC3. Protein science: a publication of the Protein Society. 2014; 23:1708–1716. [PubMed: 25220262]

30. Moratinos J, Reverte M. Effects of catecholamines on plasma potassium: the role of alpha- and beta-adrenoceptors. Fundam Clin Pharmacol. 1993; 7:143–153. [PubMed: 8388847]

31. Haghikia A, Ricke-Hoch M, Stapel B, Gorst I, Hilfiker-Kleiner D. STAT3, a key regulator of cell-to-cell communication in the heart. Cardiovasc Res. 2014; 102:281–289. [PubMed: 24518140]

32. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009; 41:677–687. [PubMed: 19430479]

33. Fox ER, Young JH, Li Y, Dreisbach AW, Keating BJ, Musani SK, et al. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum Mol Genet. 2011; 20:2273–2284. [PubMed: 21378095]

34. Frank DU, Carter KL, Thomas KR, Burr RM, Bakker ML, Coetzee WA, et al. Lethal arrhythmias in Tbx3-deficient mice reveal extreme dosage sensitivity of cardiac conduction system function and homeostasis. Proc Natl Acad Sci U S A. 2012; 109:E154–163. [PubMed: 22203979]

35. Vandell AG, McDonough CW, Gong Y, Langaee TY, Lucas AM, Chapman AB, et al. Hydrochlorothiazide-induced hyperuricaemia in the pharmacogenomic evaluation of antihypertensive responses study. J Intern Med. 2014; 276:486–497. [PubMed: 24612202]

36. Del-Aguila JL, Beitelshees AL, Cooper-Dehoff RM, Chapman AB, Gums JG, Bailey K, et al. Genome-wide association analyses suggest NELL1 influences adverse metabolic response to HCTZ in African Americans. Pharmacogenomics J. 2014; 14:35–40. [PubMed: 23400010]

37. Del-Aguila JL, Cooper-DeHoff RM, Chapman AB, Gums JG, Beitelshees AL, Bailey K, et al. Transethnic meta-analysis suggests genetic variation in the HEME pathway influences potassium response in patients treated with hydrochlorothiazide. Pharmacogenomics J. 2015; 15:153–157. [PubMed: 25201287]

38. Huang CC, Chung CM, Hung SI, Leu HB, Lin LY, Huang PH, et al. Genetic predictors of thiazide-induced serum potassium changes in nondiabetic hypertensive patients. Hypertens Res. 2014; 37:759–764. [PubMed: 24694645]

39. Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nature reviews Neuroscience. 2013; 14:365–376. [PubMed: 23571845]

40. Ashton JC. Experimental power comes from powerful theories - the real problem in null hypothesis testing. Nature reviews Neuroscience. 2013; 14:585.

41. Quinlan PT. Misuse of power: in defence of small-scale science. Nature reviews Neuroscience. 2013; 14:585.

42. Bacchetti P. Small sample size is not the real problem. Nature reviews Neuroscience. 2013; 14:585.

43. Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Confidence and precision increase with high statistical power. Nature reviews Neuroscience. 2013; 14:585–586.

44. Kraft P, Zeggini E, Ioannidis JP. Replication in genome-wide association studies. Stat Sci. 2009; 24:561–573. [PubMed: 20454541]
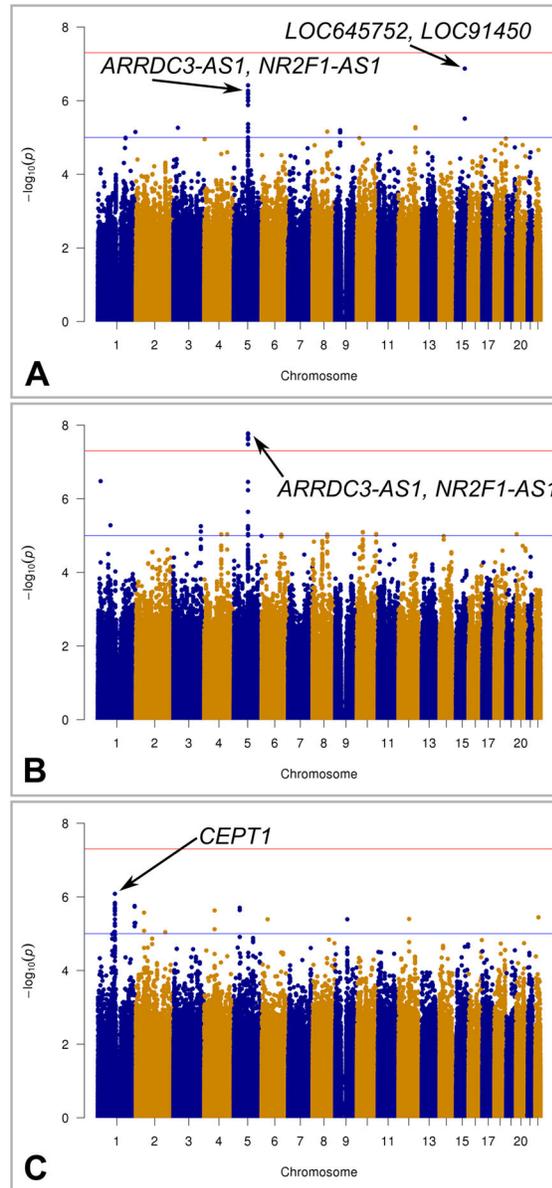
Figure 1.
Manhattan plot of SNP-by-ACE/ARB interaction term p-values for the (A) race combined meta-analysis, (B) EA meta-analysis, and (C) AA meta-analysis. Blue line is at 5 ($P=1\times10^{-5}$). Red line is at 7.30 ($P=5\times10^{-8}$).
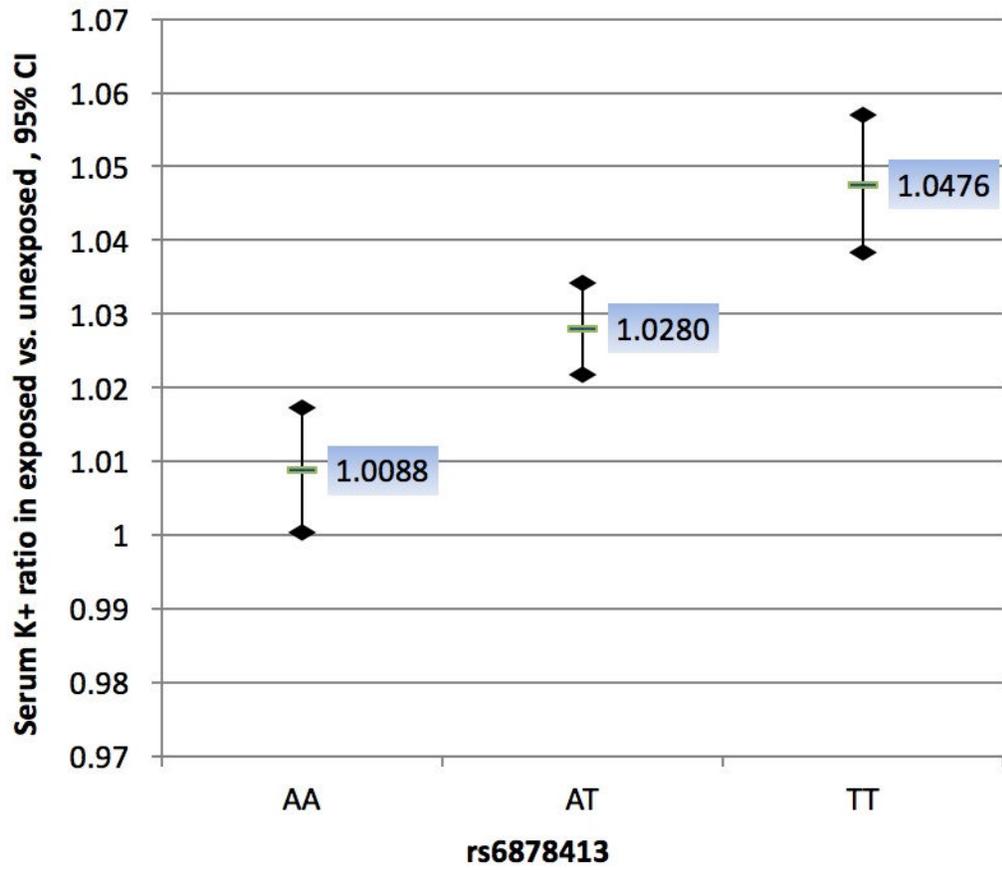
Figure 2.
Ratio (95% CI) of serum K+ in participants exposed to ACE-I/ARB versus the reference antihypertensive treatment group by genotype for rs6878413.
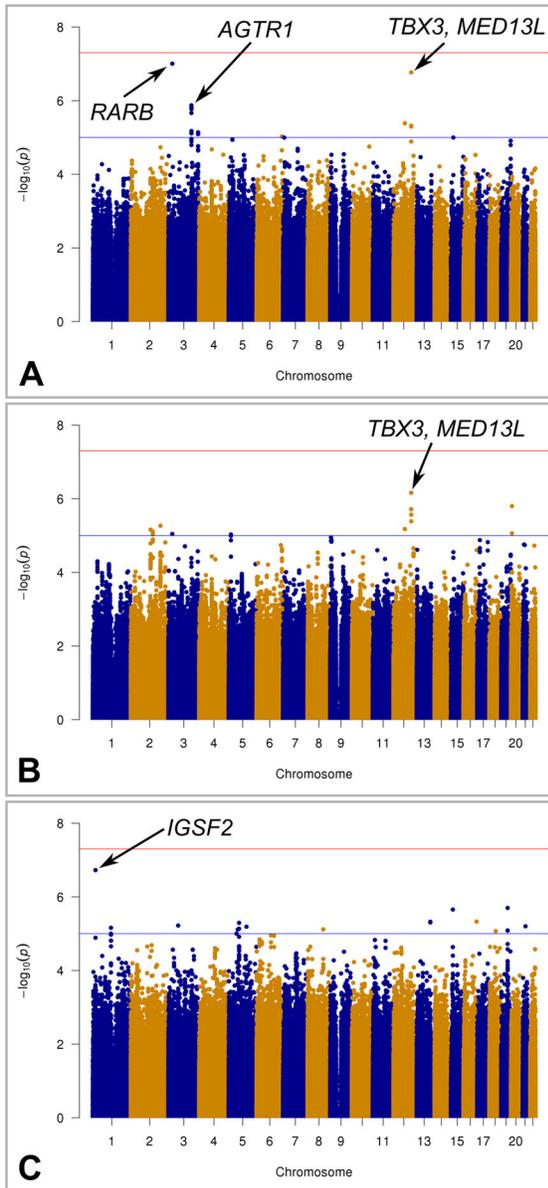
Figure 3.
Manhattan plot of SNP-by-TD interaction term *P*-values for the (A) race combined meta-analysis, (B) EA meta-analysis, and (C) AA meta-analysis. Blue line is at 5 ($P=1\times10^{-5}$). Red line is at 7.30 ($P=5\times10^{-8}$).

Table 1

Study characteristics of participants from 7 European ancestry subgroups.

Cohort	N % in drug group[3]	Age, yr (SD)	Sex. N Female (%)	BMI, kg/m² (SD)	SBP, mmHg (SD)	DBP,mmHg (SD)	K+,mmol/L[2] (IQ range)	K+ suppl, N yes (%)
ARIC[1]	1352 40/11/49	55.9 (5.6)	660 (48.8)	27.9 (5)	125.3 (18.4)	74 (10.7)	4.4 (0.7)	143 (10.6)
CHS	764 48/14/38	72.5 (5.1)	468 (61.3)	27.1 (4.7)	141.8 (22.2)	71.5 (11.7)	4.1 (0.5)	78 (10.2)
HVH1	618 28/39/33	66.3 (9.2)	215 (35.0)	30.3 (5.9)	140.0 (18.5)	80.9 (10.7)	4.3 (0.6)	56 (9.1)
HVH2	400 28/42/30	64.9 (9.1)	157 (39.3)	30.5 (6.0)	135.8 (17.5)	79.1 (11.0)	4.3 (0.7)	36 (9.0)
HyperGEN	331 10/43/47	58.9 (9.0)	149 (48.4)	30.9 (6.0)	130.4 (19.1)	71.9 (11.0)	4.1 (0.4)	NA
RS	360 11/34/55	70.9 (8.3)	156 (43.3)	26.2 (3.7)	144.8 (22.9)	75.4 (12.1)	4.1 (0.4)	NA
AGES	226 29/33/38	79.2 (3.9)	140 (61.9)	27.3 (3.9)	139.9 (16.5)	74.9 (9.8)	3.9 (0.3)	NA
Summary	4051	66.9 (7.2)	277.9 (48.3)	28.6 (5.0)	136.9 (19.3)	75.4 (11.0)	4.2 (0.5)	313

[1] ARIC data represents baseline participants only

[2] Median and Interquartile (IQ) Range

[3] % of total N in the thiazide diuretic/ACE inhibitor or angiotensin II receptor blocker/reference antihypertensive treatment groups

BMI, body mass index; DBP, diastolic blood pressure; K⁺, potassium; SBP, systolic blood pressure; SD, standard deviation; suppl, supplement; NA, Not available

Table 2

Study characteristics of participants from 4 African ancestry subgroups.

Cohort	N % in drug group[3]	Age, yr (SD)	Sex, N Female (%)	BMI, kg/m² (SD)	SBP, mmHg (SD)	DBP,mmHg (SD)	K+,mmol/L² (IQ range)	K+ suppl, N yes (%)
ARIC[1]	534 69/6/25	54.6 (5.6)	353 (66.1)	30.5 (6.0)	134.3 (21.6)	82.6 (12.3)	3.9 (0.7)	110 (20.6)
CHS	286 35/16/49	72.5 (5.4)	191 (66.8)	28.4 (4.8)	145.4 (24.0)	76.2 (12.0)	4.0 (0.5)	33 (11.5)
HyperGEN	394 24/25/51	51.3 (9.9)	259 (69.8)	32.0 (6.7)	133.4 (21.7)	75.8 (11.0)	4.0 (0.5)	NA
JHS	429 28/40/32	55.5 (11.6)	256 (59.7)	32.6 (7.2)	131.1 (18.2)	81.9 (10.7)	4.2(0.5)	NA
Summary	1643	58.4 (8.1)	265 (65.6)	30.8 (6.2)	136.1 (21.4)	79.1 (11.5)	4.0 (0.6)	143

[1]
ARIC data represents baseline participants only

[2]
Median and Interquartile (IQ) Range

[3]
% of total in the thiazide diuretic/ACE inhibitor or angiotensin II receptor blocker/reference antihypertensive treatment groups

BMI, body mass index; DBP, diastolic blood pressure; K+, potassium; SBP, systolic blood pressure; SD, standard deviation; suppl, supplement; NA, Not available

Table 3

Statistically significant findings from the discovery meta-analysis from the ACE-I/ARB exposure analysis among 7 cohorts of European Ancestry intergenic between *ARRDC3-AS1* (1.8 MB) and *NR2F1-AS1* (.14MB), corresponding results in the African Ancestry meta-analysis strata and race-combined results.

rs#	Chr:BP	A1/A2	AF	Effect	P-value	AF	Effect	P-value	Effect	P-value
		EA discovery				AA discovery			Race combined	
rs6878413	5:92634314	A/T	0.40	−0.021	$1.68*10^{-8}$	0.35	0.0019	0.758	−0.015	$5.45×10^{-6}$
rs6875717	5:92629704	A/G	0.60	0.021	$1.71*10^{-8}$	0.30	0.0033	0.5911	0.016	$5.40×10^{-7}$
rs6880218	5:92629998	A/G	0.60	0.021	$1.76*10^{-8}$	0.30	0.0022	0.7249	0.016	$9.75×10^{-7}$
rs1545708	5:92657862	A/C	0.60	0.021	$2.17*10^{-8}$	0.43	0.0031	0.5946	0.016	$8.24×10^{-7}$
rs11135569	5:92653247	T/C	0.40	−0.021	$2.19*10^{-8}$	0.45	−0.0038	0.517	−0.016	$6.34×10^{-7}$
rs6557075	5:92644553	A/G	0.60	0.021	$2.27*10^{-8}$	0.29	0.0046	0.4593	0.017	$3.84×10^{-8}$
rs6881337	5:92646945	T/C	0.60	0.021	$2.28*10^{-8}$	NA	-	-	-	-
rs4869421	5:92643571	A/G	0.40	−0.021	$2.32*10^{-8}$	0.30	−0.0036	0.5648	−0.016	$6.68×10^{-7}$
rs6888826	5:92649667	A/G	0.60	0.021	$2.34*10^{-8}$	0.30	0.0036	0.5619	0.016	$6.28×10^{-7}$
rs4242246	5:92619544	C/G	0.60	0.021	$2.42*10^{-8}$	0.31	0.0023	0.7017	0.016	$1.01×10^{-6}$
rs4541642	5:92614692	A/G	0.40	−0.021	$3.32*10^{-8}$	0.30	−0.0022	0.7244	−0.016	$1.32×10^{-6}$

A1(coded)/A2, alleles; AA, African ancestry cohorts; AF (coded), allele frequency; BP, base-pair position; Chr, chromosome; EA European ancestry cohorts