Genome-Wide Meta-analysis of Variant-by-Diuretic Interactions as Modulators of Lipid Traits in Persons of European and African Ancestry

Running Title: Diuretic-variant interaction on lipid traits

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Abstract

Hypertension (HTN) is a significant risk factor for cardiovascular morbidity and mortality. Metabolic abnormalities, including adverse cholesterol and triglycerides (TG) profiles, are frequent comorbid findings with HTN and contribute to cardiovascular disease. Diuretics, which are used to treat HTN and heart failure, have been associated with worsening of fasting lipid concentrations. A genome-wide meta-analyses with 39 710 European-ancestry (EA) individuals and 9 925 African-ancestry (AA) individuals was performed to identify genetic variants that modify the effect of loop or thiazide diuretic use on blood lipid concentrations. Both longitudinal and cross-sectional data were used to compute cohort-specific interaction results, which were then combined through meta-analysis in each ancestry. These ancestry-specific results were further combined through trans-ancestry meta-analysis. Analysis of EA data identified 6 genome-wide significant ($p < 5 \bullet 10^{-8}$) variants (in 2 loci) with loop diuretic-SNP interaction on TG concentrations (including 5 variants in ***COL11A1***). Analysis of AA data identified 9 genome-wide significant variants (in one locus adjacent to ***BMP2***) with loop diuretic-SNP interaction on TG concentrations. Trans-ancestry analysis strengthened evidence of association at two loci from suggestive to genome-wide significant (***KIAA1217*** and ***BAALC***). There were few significant diuretic-SNP interaction associations for thiazide diuretics on TG concentrations and for either diuretic on cholesterol concentrations. Several promising loci were identified that may implicate biologic pathways that contribute to adverse metabolic side effects from diuretic therapy.

Introduction

Hypertension (HTN) is a significant risk factor for cardiovascular morbidity and mortality. However, even after accounting for the beneficial effects of blood pressure reduction on cardiovascular events, cardiovascular risk remains elevated despite intensive blood pressure lowering.[1] Metabolic abnormalities, including adverse cholesterol and triglycerides (TG) profiles, are frequent comorbid findings with HTN and may contribute to the residual cardiovascular disease (CVD) risk associated with antihypertensive therapy. Thiazide diuretics are recommended as first-line therapy for the treatment of HTN.[2] Loop diuretics are a mainstay in the treatment of heart failure and are frequently used in patients with hypertension complicated by renal insufficiency to control volume retention. However, there is little evidence to support a survival benefit with the use of loop diuretics.[3] Both thiazide and loop diuretics have been associated with worsening fasting lipid concentrations,[4-6] which may dampen the salutary effects of antihypertensive therapy in some patients. While there have been studies identifying genetic sources of inter-individual blood pressure response to primarily thiazide diuretic therapy,[31-35] there is little existing literature regarding the interactions of diuretics with genetic variants on blood lipid concentrations.[36]

A meta-analysis of 56 randomized, placebo-controlled trials of diuretic monotherapy for the treatment of HTN, including both European-ancestry (EA) and African-ancestry (AA) individuals, showed that diuretic therapy was associated with average changes of 0.29 mmol/L for total cholesterol (TC), 0.24 mmol/L for low-density lipoprotein cholesterol (LDL), -0.02 mmol/L for high-density lipoprotein cholesterol (HDL), and 0.35 mmol/L for TG.[7] Some long-term studies have suggested that effects of diuretics on lipid

concentrations waned after one year.[8,9] A retrospective analysis of the Systolic Hypertension in the Elderly Program (SHEP) showed persistent, albeit more modest, increases in lipid concentrations (three-year change in TG: 0.28 ± 0.86 mmol/L with chlorthalidone vs. 0.09 ± 0.71 mmol/L with placebo, $p<0.001$) when a cohort was randomized to a thiazide-like diuretic or a placebo;[10] however, these results may have been affected by the use of non-diuretic antihypertensive drugs (atenolol and reserpine) in this study. Notably, the larger standard deviations suggest that an underlying genetic component may explain the high variability in individual's lipid response to diuretic therapy.

Although the genetic underpinnings of fasting cholesterol and TG concentrations have been well-described, single nucleotide polymorphisms (SNPs) identified through genome-wide association studies (GWAS) explain only ~10-12% of heritability.[11] Some studies have shown that the response of lipid concentrations to diuretics is significantly greater in AA than in EA individuals,[7] further suggesting the modulating effect of genetic architecture. Unfortunately, most long-term studies were based on either almost exclusively EA subjects[9] or studies including individuals of multiple ancestries where response by ancestry was not characterized.[8] The purpose of the present investigation was to determine whether common genetic variants modify the effect of diuretic use on blood lipid concentrations in persons of European and African ancestry. These analyses may identify biologic pathways that contribute to adverse metabolic side effects from diuretic therapy.

Materials and Methods

Study samples, phenotype, and genotype data

Data from 14 EA and 7 AA cohorts was used. Table 1 provides summary characteristics of these cohorts; a detailed description is provided in the Supplementary Materials. Each study obtained informed consent from participants and approval from the appropriate institutional review boards. Genotyping was performed using Illumina (San Diego, CA, USA) or Affymetrix (Santa Clara, CA, USA) genotyping arrays. Each study performed imputation using either HapMap Phase II data (release 22, build 36) or the 1000 Genomes Project[12] data with MACH,[13] Minimac,[14] IMPUTE2,[15] or BEAGLE[16] software. Information on genotype and imputation for each study is presented in Supplementary Table 1.

In total, 49 635 subjects over 18 years of age with genotype, phenotype, and covariate information were available in this analysis. Both longitudinal and cross-sectional studies were used, and all available data for each subject was used. In longitudinal cohorts that had multiple clinic visits for each subject, those multiple measurements (obtained across clinic visits) were used in the analysis. In cross-sectional studies that had only a single visit for each subject, a single measurement was used. Three fasting lipid traits were considered for analyses: TG, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol (all mmol/L). LDL was calculated via the Friedewald equation (LDL=TC-HDL- [TG/5]) for those with TG <4.52 mmol/L.[17] If TG >4.52 mmol/L, LDL was set to missing unless directly assayed. LDL concentrations

were adjusted for statin use as described elsewhere.[18] Triglyceride concentrations were natural log-transformed for analysis. Two patterns of diuretic exposure were used: 1) thiazide and thiazide-like diuretic use (yes/no) without use of loop; and 2) loop diuretic use (yes/no) without use of thiazide or thiazide-like diuretics. For each diuretic exposure, the unexposed group consisted of individuals taking neither diuretic. Drug use (yes/no) is defined as the use of any drug in the drug class, regardless of dosage, and with or without the use of a potassium supplement, as assessed by medication inventory at each clinic visit.

Statistical analyses

To determine the association of SNP-diuretic interactions on blood cholesterol and TG concentrations, the following regression model

$$\mathbb{E}[Y|D,G,C] = \beta_0 + \beta_D D + \beta_G G + \beta_{GD} G \times D + \beta_C C$$

was applied. Y is the fasting lipid value, D is the diuretic use (coded 0/1 for the absence/presence of the diuretic), G is the dosage of the imputed genetic variant coded additively (from 0 to 2), and C is the vector of all other covariates, which include age, sex, body mass index (BMI), principal components (to account for population stratification and admixture), and additional cohort-specific covariates (if any). Subjects with missing data for fasting (≥ 8 hours) lipid values, diuretic use, or any covariate were excluded from analysis. Subjects taking both thiazide and loop diuretics were also excluded from analysis.

Each study conducted GWAS analysis and provided the SNP-diuretic interaction effect β_{GD} and standard error. For longitudinal cohorts with repeated measures, generalized estimating equations (GEE) with an independent working correlation were used with an *R* package *boss*; for studies with cross-sectional data, linear regression was used. To account for relatedness in families, family studies used either GEE treating each family as a cluster using *R* software or the linear mixed effect model approach with a random polygenic component (for which the covariance matrix depends on the kinship matrix) using ProbABEL (Supplementary Table 1). Note the standardized interaction effect (β_{GD}/SE) is not normally distributed when minor allele counts of SNPs among participants on the diuretic were small (< 10). Following our earlier work,[19] cohort-specific *P*-values based on a Student's t reference distribution were computed. The degrees of freedom for the t-reference distribution depended on the number of drug-exposed participants (N_exposed), the SNP imputation quality, and the minor allele frequency. Before meta-analysis, SNPs were excluded if 2 • MAF • N_exposed • imputation quality measure < 10 to exclude unstable cohort-specific results that reflect small sample size, low MAF, or low imputation quality measures.

Meta-analysis for each ancestry group was performed to combine these cohort-specific *P*-values with weighted Z-statistics using METAL,[20] where weights were based on the number of drug-exposed subjects multiplied by the SNP imputation quality. As cohort-specific *P*-values were computed based on t distribution, inverse-variance weighted meta-analysis was not used. After meta-analysis, SNPs were excluded if they were available in fewer than three cohorts for EA results or two cohorts for AA results. The ancestry-specific results were further combined to perform trans-ancestry meta-analysis

using MANTRA (Meta-ANalysis of TRansethnic Association studies).[21] MANTRA accounts for similarity in allelic effects among closely-related populations, while allowing for heterogeneity across populations with more diverse ancestries. As MANTRA uses a Bayesian framework, a traditional fixed-effect meta-analysis with weighted Z-statistics was also performed using METAL.

Genome-wide significance was defined as $P < 5 \cdot 10^{-8}$ from METAL with a fixed-effect meta-analysis or Bayes Factor $>10^5$ from MANTRA, and suggestive evidence at $P < 1 \cdot 10^{-6}$ for association. To assess type I error due to population stratification and other factors, quantile-quantile (QQ) plots were examined for all cohort-specific GWAS results for each pair of lipid and diuretic exposure. In addition, during meta-analysis, genomic control correction[22] was applied to cohort-specific GWAS results if their genomic control lambda value was greater than 1. The gene locations referenced in the text and tables were obtained from the National Center for Biotechnology Information dbSNP database (reference assembly GRCh38.p2). Functional annotation information was sought using HaploReg[23] and RegulomeDB.[24]

Results

The European-ancestry (EA) group included 39 710 subjects from 14 cohorts; the African-ancestry (AA) group included 9 925 subjects from 7 cohorts (Table 1). The number of subjects exposed to loop diuretics was 2 117 (5.3%) in EA and 784 (7.9%) in AA; the number exposed to thiazide and thiazide-like diuretics was 6 878 (17.3%) in EA and 3 923 (39.5%) in AA.

The QQ plots (Supplementary Figures 1-6) showed moderate inflation, in particular for the loop diuretic-SNP interaction terms for TG, LDL, and HDL analyses, although the *P*-values based on a t-distribution (red crosses) have better genomic control values than the *P*-values based on a standard normal distribution (black circles). Manhattan plots show the adjusted -$\log_{10}(P)$ values for loop diuretics (Figures 1 and Supplementary Figures 7-8) and thiazide diuretics (Supplementary Figures 9-11) for TG, HDL cholesterol, and LDL cholesterol, respectively. Each figure includes plots of EA and AA separately, and for trans-ancestry meta-analysis using METAL and MANTRA. The SNPs reaching genome-wide significance ($P < 5 \cdot 10^{-8}$ from METAL or Bayes Factor $>10^5$ from MANTRA) for association with gene-medication interactions on TG concentration are shown in Table 2. Supplementary Table 2 shows the most highly ranked SNP-diuretic interactions by lipid trait (with $P < 10^{-6}$).

Loop diuretics with blood triglyceride (TG)

Analysis of the EA data identified 6 SNPs (2 loci) with genome-wide significant SNP-loop diuretic interaction effects ($P < 5 \cdot 10^{-8}$) on log-transformed TG concentrations (Figure 1). Another 33 SNPs (adding 13 loci) demonstrated a suggestive association ($P < 1 \cdot 10^{-6}$). The locus with the most promising evidence of association included a six-SNP cluster (most significant rs1463034, $P = 1.31 \cdot 10^{-9}$, $\beta_{GD} = 0.11 \pm 0.02$) spanning four introns (7 256 bp) in ***COL11A1*** on chromosome 1 (Figure 2, top). Another promising locus included a seven-SNP cluster spanning a single intron (18 804 bp) on chromosome 10 in ***KIAA1217*** (most significant rs7077598, $P = 7.48 \cdot 10^{-7}$). A suggestive locus of 12 SNPs was found at a distinct locus on chromosome 10 which is approximately 145 kb downstream of ***DKK1*** (most significant rs10762762, *P* =

$1.12 \cdot 10^{-7}$). Within this cluster of SNPs in tight linkage disequilibrium, rs1441122 (P = $9.33 \cdot 10^{-7}$) showed moderate evidence of altering the binding motif for the transcription factor ***TRIM28*** in a human embryonic kidney cell line (RegulomeDB Score 3a, http://www.regulomedb.org/).

Analysis of the AA data yielded 9 SNPs (one locus) with genome-wide significant interaction effects on TG concentrations, with three additional SNPs (adding 2 loci) with suggestive *P*-values (Figure 1). This 10-SNP locus (most significant rs6054018, P = $1.70 \cdot 10^{-10}$, $\beta_{GD} = 0.21 \pm 0.03$; Figure 2, middle) is located in an intergenic region on chromosome 20 approximately 500 kb upstream of ***BMP2*** and approximately 114 kb downstream of ***FERMT1***.

Trans-ancestry association tests strengthened evidence of association for the ***KIAA1217*** locus, elevating two of the eight SNPs in this locus from suggestive in EA to genome-wide significance. In particular, at rs10508671, the SNP-loop diuretic interaction effect (β_{GD}) was consistent in both ancestries (-0.10 vs -0.09, Supplementary Table 2), and therefore trans-ancestry test provided stronger evidence of association (EA $P = 9.4 \cdot 10^{-7}$, AA $P = 0.003$, trans-ancestry $P = 1.08 \cdot 10^{-8}$, MANTRA $\log_{10}$[BF] = 6.24, Figure 3, bottom). MANTRA also strengthened the association for a four-SNP locus on chromosome 8 (most significant rs6985929, trans-ancestry $P = 9.07 \cdot 10^{-6}$, MANTRA $\log_{10}$[BF] = 6.18) that overlaps an intron in ***BAALC*** and that is approximately 32 kb upstream of ***FZD6***.

Loop diuretics with blood HDL cholesterol and LDL cholesterol

Analysis of the EA data identified no SNP that met the threshold for genome-wide significance or suggestive association for SNP-loop diuretic interactions on HDL or LDL cholesterol concentrations. Three SNPs (two loci) showed suggestive association in analyses of AA data on HDL concentrations (Supplementary Figure 7). Trans-ancestry analyses newly identified two SNPs in two loci with suggestive interaction associations on HDL, including a SNP on chromosome 11 in ***TEAD1*** (rs7924536, $P = 5.79 \cdot 10^{-7}$, MANTRA $\log_{10}[BF] = 5.22$).

For LDL analyses in AA (Supplementary Figure 8), one SNP on chromosome 6 approximately 35 kb 5' of ***PHIP*** reached genome-wide significance (rs12208017, $P = 3.73 \cdot 10^{-8}$, $\beta_{GD} = 0.81 \pm 0.02$); this SNP is in an expression quantitative trait locus (eQTL) for both ***PHIP*** and the adjacent gene, ***IRAK1BP1***. An additional SNP with a suggestive association (rs1312663, $P = 4.06 \cdot 10^{-7}$) is located approximately 100 kb upstream of ***SLC24A4*** .

Thiazide diuretics with blood TG, HDL cholesterol and LDL cholesterol

For analyses of SNP-thiazide diuretic interactions in EA and AA, no loci reached genome-wide significance for TG, HDL or LDL (Supplementary Figures 9-11). In EA, suggestive association was noted with one SNP in ***MYO16*** (rs1926511, $P = 1.05 \cdot 10^{-7}$) for TG and three SNPs (in two loci) for LDL. In AA, 8 SNPs in two loci yielded suggestive associations with HDL including a seven-SNP locus on chromosome 14 in ***TRIP11*** (most significant rs10083510, $P = 3.20 \cdot 10^{-7}$). SNPs in this locus are eQTLs for ***FBLN5***.

In trans-ancestry analyses, a single intronic SNP on chromosome 20 in ***MACROD2*** (rs6043629, $P = 1.12 \bullet 10^{-8}$, $\beta_{GD} = 0.09 \pm 0.02$, MANTRA $\log_{10}$[BF] = 6.42) reached genome-wide significance for a thiazide diuretic interaction association on HDL; the strength of the association in EA ($P = 1.25 \bullet 10^{-5}$) and AA ($P = 1.82 \bullet 10^{-4}$) cohorts analyzed separately were much lower. Likewise, with analysis of SNP-thiazide interactions on LDL, two SNPs on chromosome 17 in ***KIAA0753*** reached genome-wide significance based on the MANTRA analysis (most significant rs2304976, MANTRA $\log_{10}$[BF] = 5.34) although neither SNP approached significance in separate EA and AA analyses.

Discussion

A genome-wide meta-analyses with 39 710 European-ancestry (EA) individuals and 9 925 African-ancestry (AA) individuals was performed to identify genetic variants that modify the effect of loop or thiazide diuretic use on blood lipid concentrations. Separate analyses of EA and AA data identified genome-wide significant variants in 3 loci with loop diuretic-SNP interaction on TG concentrations; trans-ancestry analysis strengthened evidence of association at two additional loci. The most significant associations were observed between loop diuretics and TG concentrations; intronic SNPs clustered in ***COL11A1*** (collagen, type XI, alpha 1) on chromosome 1 were significantly associated with TG concentrations in EA. ***COL11A1*** produces a structural/adhesion protein secreted by primary rat adipocytes.[37] The expression of this gene is up-regulated during human adipogenesis[38] which suggests a role in the modulation of lipid traits. Although it remains unclear how diuretics modulate the effect of ***COL11A1*** variants on lipid traits, a ***COL11A1*** mutation has been associated with

nephrogenic diabetes insipidus,[39] which is characterized by an inability of the kidney to concentrate urine, thus providing a possible mechanistic link between ***COL11A1***, diuretics, and lipids.

Trans-ancestry analysis combining European and African results provided evidence of association at intronic SNPs clustered in ***KIAA1217*** (an uncharacterized long coding DNA) which are just 5' to an intronic microRNA (***MIR603***) on chromosome 10. ***KIAA1217*** variants have been associated with obesity.[40] ***MIR603*** has been associated with benign and malignant tumors.[41-44] Of potential interest, SNPs in both ***KIAA1217*** and ***COL11A1*** have been associated with lumbar disk herniation which, in turn, has been associated with blood lipid concentrations[45] and cardiovascular risk traits and disease.[46]

Loci with genome-wide significant associations found in intragenic regions may impact the transcription of genes tens or hundreds of kilobases away from their target genes.[47] The cluster of SNPs on chromosome 20 reaching genome-wide significance for TG in AA lies between two genes. ***FERMT1*** (fermitin family member 1) is a membrane-associated protein that links intracellular structural proteins to the extracellular matrix. While mutations in this gene have been implicated in a heritable skin disorder,[48] connections to cardiovascular traits are sparse. Perhaps more relevant is this locus' proximity to ***BMP2*** (bone morphogenetic protein 2), the expression of which is regulated by angiotensin II,[49] a key regulator of blood pressure and fluid/electrolyte balance. Furthermore, BMP2, a member of the transforming growth factor (TGF)-β superfamily of cytokines, is expressed in endothelial cells, and has been shown to stimulate adjacent smooth muscle and endothelial cells to proliferate, differentiate, and deposit extra-

cellular matrix, thus affecting blood pressure and contributing to atherosclerosis by processes that also includes Wnt signaling.[50] BMP2 also participates in the differentiation of mesenchymal stem cells to mature adipocytes,[51] thus potentially affecting blood lipid concentrations.

A cluster of SNPs spanning the 3' end and intragenic region adjacent to ***BAALC*** and 5' of ***FZD6*** was nominally associated with TG response to loop diuretic therapy in both races; however, trans-ancestry meta-analysis by METAL and MANTRA strengthened the association. There is little evidence to link ***BAALC*** (brain and acute leukemia, cytoplasmic) with TG concentrations. However, both ***FZD6*** (frizzled class receptor 6), which is approximately 32 kb downstream from this locus on chromosome 8, and ***DKK1*** (dickkopf WNT signaling pathway inhibitor 1) on chromosome 10 are negative regulars of the β-catenin/Wnt signaling cascade.[52,53] Expression of ***DKK1*** has been noted in atherosclerotic plaques[54] and shown to induce PPARγ expression (a key regulator of adipogenesis),[55] further drawing a link between ***DKK1*** and blood lipid concentrations. Both ***FZD6*** and ***DKK1*** have also been associated with renal development,[56,57] glomerular damage, and proteinuria.[58,59] The ***DKK1*** locus may be of additional interest since rs1441122 shows moderate evidence of altering the binding motif for the transcription factor TRIM28 (also known as KAP1) in a human embryonic kidney cell line; TRIM28 expression has been shown to play a role in signaling pathways responsible for renal fibrosis,[60] a key risk factor for cardiovascular disease traits including hypertension and elevated TG concentrations.[61] Finally, not only does β-catenin/Wnt alter the expression of genes which regulate renal sodium, chloride and

potassium handling,[62] but the pathway is also inhibited by the loop diuretic ethacrynic acid.[63]

Evidence of SNP-loop diuretic interactions on HDL and LDL cholesterol was relatively sparse. Although no SNP met thresholds for genome-wide or suggestive association in EA, analyses in AA did identify potentially interesting associations. Loop diuretic-HDL analyses in AA identified a genome-wide significant association for a SNP adjacent to ***ROBO2*** (roundabout, axon guidance receptor, homolog 2).The ROBO2 receptor and its binding partner SLIT1 play a role in mediating axonal growth in the brain.[64] ROBO2 also plays a role in kidney development and function.[65,66] Trans-ancestry analyses identified SNPs in ***TEAD1*** (TEA domain family member 1 [SV40 transcriptional enhancer factor]) as significantly associated with HDL concentrations. This transcription factor is another β-catenin/Wnt pathway member[67] that has been shown to modulate adipocyte differentiation and proliferation[68] and play a role in regulating insulin sensitivity via skeletal muscle fiber type switching.[69,70]

In the analyses of AA data with LDL, a SNP reaching genome-wide significance adjacent to ***PHIP*** (pleckstrin homology domain interacting protein) is within 270 kb of another SNP (rs16890334) previously identified as having a genome-wide significant association with blood pressure response to a high-sodium diet intervention in Han Chinese.[71] Another SNP approximately 100 kb upstream of ***SLC24A4*** (solute carrier family 24 [sodium/potassium/calcium exchanger], member 4) may have relevant links to loop diuretic use. SNPs in ***SLC24A4*** have shown genome-wide significant association with systolic blood pressure in AA individuals[72] as well as with LDL and lipoprotein

particle concentrations following fenofibrate therapy in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study.[73]

A few loci from analyses of thiazide and thiazide-like diuretics with lipid traits were notable despite not reaching genome-wide significance. SNPs in ***MYO16*** (myosin XVI) have been previously associated with nephrotic syndrome in humans and mice;[74-77] nephrotic syndrome is a significant risk factor of lipid abnormalities.[61] SNPs in ***TRIP11*** (thyroid hormone receptor interactor 11), which are eQTLs for ***FBLN5*** (fibulin 5), are also of interest given prior suggestive association of ***FBLN5*** with HDL mean particle size.[78] Trans-ancestry analyses identified a ***MACROD2*** (MACRO domain containing 2) with genome-wide significance for a diuretic interaction effect on HDL. This gene was found to have a significant association with coronary artery disease and hypertension using a two-marker testing approach in a reanalysis of data from the Wellcome Trust Case Control Consortium,[79] and a suggestive association with brain infarcts in another meta-analysis;[80] however, the role of this gene in determining cardiovascular traits remain uncertain.

Limitations

Although this study identified interesting loop diuretic-SNP interactions as modulators of TG concentrations, there were limitations in this study. First, the study design assumed therapeutic class effects although individual drugs may have different effects on lipids.[7,81,82] Information regarding the dosage, duration, or adequacy of diuretic therapy may have also affected the traits but were not available in most studies. Second, because potassium-sparing diuretics were frequently co-administered with thiazide and

thiazide-like diuretics, it was not feasible to pursue the sole contributions of potassium-sparing diuretics which were therefore not considered in this study. Third, the potential contribution of co-morbidities such as diabetes and renal dysfunction on lipid traits was not considered, although diuretics have been shown to be less effective at controlling blood pressure and cause greater increases in LDL and TG concentrations in diabetics.[7] Fourth, the relatively smaller number of AA subjects exposed to diuretics may have reduced the power to detect significant association or increased the risk for spurious findings in this ancestry group. Finally, given the number of tests performed and the relatively small effect size for the interaction, this effort should be construed as a discovery analysis that warrants replication.

Conclusions

This genome-wide meta-analysis accounting for SNP-diuretic interactions on blood lipid concentrations used data from 39 710 European-ancestry individuals and 9 925 African-ancestry individuals. The results of the present study suggest stronger interaction effects for loop versus thiazide diuretics identifying several genome-wide significant loci of small effect sizes. The results of this medication interaction study may help identify biologic pathways that contribute to adverse metabolic side effects from diuretic therapy. These findings may also explain, at least in part, some of the residual CVD risk following reduction of blood pressure in patients treated with anti-hypertensive medications.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary information is available at *The Pharmacogenomics Journal's* website.

Figure Legends

Figure 1: Manhattan plots for analysis of loop diuretic-SNP interaction on triglyceride concentrations. The ancestry-sepcific meta-analysis used 11 cohorts of European ancestry (upper-left panel) and 6 cohorts of African ancestry (lower-left panel). Trans-ancestry meta-analysis used fixed-effect weighted Z-statistics with METAL (upper-right panel) and a Bayesian framework with MANTRA (lower-right panel). The $-\log_{10}(P)$ from METAL or $\log_{10}(\textit{Bayes Factor})$ from MANTRA was plotted at the chromosomal location of each variant. Manhattan plots for the remaining lipid-diuretic pairs are shown in Supplementary Figures 7-11.

Figure 2: Regional plots of significant SNP-loop diuretic interaction effects on triglyceride concentrations on chromosome 1 in European ancestry (top), chromosome 20 in African ancestry (middle), and chromosome 10 in trans-ancestry analyses of European and African ancestries (bottom). Plots were created using LocusZoom software (http://csg.sph.umich.edu/locuszoom/). Linkage disequilibrium (LD, r^2) was based on hg19/1000 Genomes Nov 2014 EUR for EA and AFR hg19/1000 Genomes Nov 2014 for AA. Because no LD information was available for trans-ancestry results combining EA and AA results, the bottom plot does not show LD.

Tables

Table 1. Baseline characteristics of 21 participating cohorts of European and African ancestry. 17 cohorts were used for loop diuretic analysis.

Ancestry	*Study*	*N*	*Loop Exposed Subjects*	*Thiazide Exposed Subjects*
European	AGES	1,677	153	466
	ARIC	8,963	387	1,471
	CHS	3,174	181	764
	FHS	7,680	177	540
	Health ABC	1,828	130	329
	HVH1	470	51	184
	HVH2	206	21	83
	HyperGEN	1,187	44	196
	MESA	2,359	NA	563
	PROSPER	4,592	618	1,402
	RS1	3,421	269	405
	RS2	2,096	86	147
	WHI CT GARNET Baseline	1,198	NA	142
	WHI CT GARNET Core	859	NA	186
African	ARIC	2,295	156	1,072
	CHS	709	95	235
	HyperGEN	1,110	86	278
	JHS	1,500	91	381
	WHI CT SHARe Baseline	3,486	150	752
	WHI CT SHARe Core	825	NA	362
	WHI OS SHARe Baseline	3,422	206	843

Abbreviations: AGES, Age, Gene/Environment Susceptibility-Reykjavik Study; ARIC, Atherosclerosis Risk in Communities study; CHS, Cardiovascular Health Study; CT, Clinical Trial, FHS, Framingham Heart Study; GARNET, Genomics and Randomized Trial Network; Health ABC, Health Aging, Body and Composition; HyperGEN, Hypertension Genetic Epidemiology Network; HVH, Heart and Vascular Health; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; OS, Observational Study; PROSPER, PROspective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SHARe, SNP Health Association Resource; WHI, Women's Health Initiative.

Table 2. Genome-wide significant SNPs from European-Ancestry, African-Ancestry, Transethnic, and MANTRA analyses for loop diuretic-SNP interactions on triglyceride concentrations.

Marker	*Chr*	*Position*	*Alleles*	*European Ancestry*		*African Ancestry*		*Transethnic*		*MANTRA log$_{10}$(BF)*	*Neighboring Genes*
				Freq	*P*	*Freq*	*P*	*Freq*	*P*		
rs1463034	1	103,227,805	t/c	0.92	1.31E-09	0.59	4.40E-01	0.83	1.78E-06	6.50	
rs1870958	1	103,229,744	t/g	0.92	1.76E-09	0.59	5.00E-01	0.82	3.16E-06	6.68	
rs2786125	1	103,235,061	a/g	0.06	1.24E-08	0.31	4.37E-01	0.14	1.65E-05	6.64	**COL11A1**
rs2622870	1	103,236,564	t/g	0.94	1.27E-08	0.69	3.40E-01	0.87	1.61E-05	7.11	
rs2622874	1	103,239,505	a/c	0.06	1.23E-08	0.31	3.26E-01	0.13	1.74E-05	6.80	
rs7544444	1	103,293,408	t/c	0.97	3.66E-06	0.86	6.27E-02	0.93	4.67E-03	5.26	
rs7607797	2	117,944,672	t/c	0.97	3.60E-08	0.66	4.62E-01	0.86	6.94E-05	3.78	DDX18
rs4554091	4	130,520,065	t/g	0.58	4.19E-01	0.16	5.65E-08	0.46	3.65E-02	5.44	SCLT1 / **LOC105377417**
rs11100093	4	158,213,492	a/t	0.15	3.67E-05	0.19	1.70E-02	0.16	2.32E-06	5.53	PDGFC / GLRB / GRIA2
rs7817074	8	104,300,634	t/g	0.05	6.72E-05	0.04	2.39E-02	0.05	6.31E-06	5.58	
rs7841861	8	104,329,002	t/c	0.06	4.38E-05	0.13	2.46E-02	0.08	3.92E-06	6.10	**BAALC** / FZD6
rs7002454	8	104,347,955	t/c	0.95	7.71E-05	0.87	3.58E-02	0.93	9.78E-06	6.01	
rs6985929	8	104,348,009	a/g	0.95	7.78E-05	0.87	3.29E-02	0.93	9.07E-06	6.18	
rs7899031	10	24,560,897	t/c	0.97	7.52E-07	0.86	2.34E-02	0.93	1.27E-07	5.22	**KIAA1217** / MIR603 /

rs10508671	10	24,574,062	t/c	0.03	9.43E-07	0.25	2.12E-03	0.11	1.08E-08	6.24	ARHGAP21
rs10508672	10	24,574,441	a/g	0.03	9.52E-07	0.24	2.32E-03	0.11	1.19E-08	6.21	
rs7071454	10	24,575,819	t/c	0.03	9.63E-07	0.25	1.18E-02	0.11	8.28E-08	5.52	
rs11013993	10	24,577,638	t/c	0.03	1.22E-06	0.25	1.26E-02	0.11	1.11E-07	5.39	
rs749140	10	130,259,880	t/c	0.96	5.52E-06	0.95	3.55E-01	0.96	1.94E-05	5.22	PTPRE / MKI67 / LINC01163
rs11016373	10	130,286,692	t/c	0.04	5.47E-07	0.08	6.91E-01	0.05	1.32E-05	5.24	
rs16921999	11	94,872,381	a/c	0.03	1.99E-04	0.04	1.11E-03	0.03	1.23E-06	5.10	ENDOD1 / SESN3 / FAM76B / CEP57 / MTMR2
rs3852940	20	6,165,600	a/g	0.79	7.10E-01	0.70	1.18E-08	0.77	9.80E-03	6.07	
rs6054016	20	6,174,368	t/c	0.06	6.18E-01	0.19	4.06E-10	0.10	2.05E-03	6.22	
rs6054018	20	6,175,236	t/g	0.94	6.24E-01	0.80	1.70E-10	0.90	1.75E-03	8.26	
rs8120588	20	6,178,849	t/c	0.94	6.36E-01	0.81	3.75E-10	0.90	1.79E-03	8.09	
rs7348828	20	6,182,498	a/g	0.94	6.50E-01	0.81	3.56E-10	0.90	1.67E-03	7.99	CRLS1 / LRRN4 / FERMT1 / CASC20 / BMP2
rs8122198	20	6,186,686	t/c	0.94	9.11E-01	0.85	9.53E-09	0.91	2.36E-03	6.44	
rs6054037	20	6,187,877	c/g	0.94	8.82E-01	0.85	9.94E-09	0.91	2.61E-03	6.22	
rs6038400	20	6,188,201	a/c	0.06	8.79E-01	0.15	1.03E-08	0.09	2.66E-03	6.45	
rs3852942	20	6,192,559	a/g	0.06	8.84E-01	0.17	3.36E-08	0.10	3.31E-03	5.15	
rs7262233	20	51,534,628	a/g	0.06	9.45E-06	0.09	4.75E-01	0.07	4.46E-05	5.43	TSHZ2 / ZNF217

Abbreviations: Chr, chromosome, Freq, frequency of allele 1. **Bolded** genes include intragenic SNPs.

References

1. Zanchetti A, Hansson L, Menard J, Leonetti G, Rahn KH, Warnold I *et al*. Risk assessment and treatment benefit in intensively treated hypertensive patients of the Hypertension Optimal Treatment (HOT) study. *J Hypertens* 2001; **19:** 819-825.

2. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J *et al*. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA* 2014; **311:** 507-520.

3. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr. *et al*. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289:** 2560-2572.

4. Brook RD. Mechanism of differential effects of antihypertensive agents on serum lipids. *Curr Hypertens Rep* 2000; **2:** 370-377.

5. Cutler R. Effect of antihypertensive agents on lipid metabolism. *Am J Cardiol* 1983; **51:** 628-631.

6. Ames RP. The influence of non-beta-blocking drugs on the lipid profile: are diuretics outclassed as initial therapy for hypertension? *Am Heart J* 1987; **114:** 998-1006.

7. Kasiske BL, Ma JZ, Kalil RS, Louis TA. Effects of antihypertensive therapy on serum lipids. *Ann Intern Med* 1995; **122:** 133-141.

8. Lakshman MR, Reda DJ, Materson BJ, Cushman WC, Freis ED. Diuretics and beta-blockers do not have adverse effects at 1 year on plasma lipid and lipoprotein profiles in men with hypertension.

Department of Veterans Affairs Cooperative Study Group on Antihypertensive Agents. *Arch Intern Med* 1999; **159:** 551-558.

9. Ott SM, LaCroix AZ, Ichikawa LE, Scholes D, Barlow WE. Effect of low-dose thiazide diuretics on plasma lipids: results from a double-blind, randomized clinical trial in older men and women. *J Am Geriatr Soc* 2003; **51:** 340-347.

10. Savage PJ, Pressel SL, Curb JD, Schron EB, Applegate WB, Black HR *et al*. Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension: The Systolic Hypertension in the Elderly Program. SHEP Cooperative Research Group. *Arch Intern Med* 1998; **158:** 741-751.

11. Maitland-van der Zee AH, Turner ST, Schwartz GL, Chapman AB, Klungel OH, Boerwinkle E. Demographic, environmental, and genetic predictors of metabolic side effects of hydrochlorothiazide treatment in hypertensive subjects. *Am J Hypertens* 2005; **18:** 1077-1083.

12. 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM *et al*. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; **491:** 56-65.

13. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* 2010; **34:** 816-834.

14. Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet* 2012; **44:** 955-959.

15. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009; **5:** e1000529.

16. Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet* 2009; **84:** 210-223.

17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18:** 499-502.

18. Peloso GM, Auer PL, Bis JC, Voorman A, Morrison AC, Stitziel NO *et al*. Association of low-frequency and rare coding-sequence variants with blood lipids and coronary heart disease in 56,000 whites and blacks. *Am J Hum Genet* 2014; **94:** 223-232.

19. Avery CL, Sitlani CM, Arking DE, Arnett DK, Bis JC, Boerwinkle E *et al*. Drug-gene interactions and the search for missing heritability: a cross-sectional pharmacogenomics study of the QT interval. *Pharmacogenomics J* 2014; **14:** 6-13.

20. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; **26:** 2190-2191.

21. Morris AP. Transethnic meta-analysis of genomewide association studies. *Genet Epidemiol* 2011; **35:** 809-822.

22. Devlin B, Roeder K. Genomic control for association studies. *Biometrics* 1999; **55:** 997-1004.

23. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012; **40:** D930-934.

24. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M *et al*. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 2012; **22:** 1790-1797.

25. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998; **97:** 1837-1847.

26. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. *JAMA* 1984; **251:** 351-364.

27. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* 1984; **251:** 365-374.

28. McGill HC, Jr., McMahan CA, Malcom GT, Oalmann MC, Strong JP. Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. The PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. *Arterioscler Thromb Vasc Biol* 1997; **17:** 95-106.

29. Keys A, Menotti A, Aravanis C, Blackburn H, Djordevic BS, Buzina R *et al*. The seven countries study: 2,289 deaths in 15 years. *Prev Med* 1984; **13:** 141-154.

30. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 1986; **256:** 2823-2828.

31. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M *et al*. Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. *Lancet* 1997; **349:** 1353-1357.

32. Citterio L, Lanzani C, Manunta P. Polymorphisms, hypertension and thiazide diuretics. *Pharmacogenomics* 2011; **12:** 1587-1604.

33. Gong Y, McDonough CW, Wang Z, Hou W, Cooper-DeHoff RM, Langaee TY *et al*. Hypertension susceptibility loci and blood pressure response to antihypertensives: results from the pharmacogenomic evaluation of antihypertensive responses study. *Circ Cardiovasc Genet* 2012; **5:** 686-691.

34. McDonough CW, Burbage SE, Duarte JD, Gong Y, Langaee TY, Turner ST *et al*. Association of variants in NEDD4L with blood pressure response and adverse cardiovascular outcomes in hypertensive patients treated with thiazide diuretics. *J Hypertens* 2013; **31:** 698-704.

35. Turner ST, Boerwinkle E, O'Connell JR, Bailey KR, Gong Y, Chapman AB *et al*. Genomic association analysis of common variants influencing antihypertensive response to hydrochlorothiazide. *Hypertension* 2013; **62:** 391-397.

36. Beeks E, Janssen RG, Kroon AA, Keulen ET, Geurts JM, de Leeuw PW *et al*. Association between the alpha-adducin Gly460Trp polymorphism and systolic blood pressure in familial combined hyperlipidemia. *Am J Hypertens* 2001; **14:** 1185-1190.

37. Lim JM, Sherling D, Teo CF, Hausman DB, Lin D, Wells L. Defining the regulated secreted proteome of rodent adipocytes upon the induction of insulin resistance. *J Proteome Res* 2008; **7:** 1251-1263.

38. Hung SC, Chang CF, Ma HL, Chen TH, Low-Tone Ho L. Gene expression profiles of early adipogenesis in human mesenchymal stem cells. *Gene* 2004; **340:** 141-150.

39. Kamsteeg EJ, Wormhoudt TA, Rijss JP, van Os CH, Deen PM. An impaired routing of wild-type aquaporin-2 after tetramerization with an aquaporin-2 mutant explains dominant nephrogenic diabetes insipidus. *EMBO J* 1999; **18:** 2394-2400.

40. Wang KS, Liu X, Zheng S, Zeng M, Pan Y, Callahan K. A novel locus for body mass index on 5p15.2: a meta-analysis of two genome-wide association studies. *Gene* 2012; **500:** 80-84.

41. Kushwaha D, Ramakrishnan V, Ng K, Steed T, Nguyen T, Futalan D *et al*. A genome-wide miRNA screen revealed miR-603 as a MGMT-regulating miRNA in glioblastomas. *Oncotarget* 2014; **5:** 4026-4039.

42. Mussnich P, D'Angelo D, Leone V, Croce CM, Fusco A. The High Mobility Group A proteins contribute to thyroid cell transformation by regulating miR-603 and miR-10b expression. *Mol Oncol* 2013; **7:** 531-542.

43. D'Angelo D, Palmieri D, Mussnich P, Roche M, Wierinckx A, Raverot G *et al*. Altered microRNA expression profile in human pituitary GH adenomas: down-regulation of miRNA targeting HMGA1, HMGA2, and E2F1. *J Clin Endocrinol Metab* 2012; **97:** E1128-1138.

44. Duttagupta R, DiRienzo S, Jiang R, Bowers J, Gollub J, Kao J *et al*. Genome-wide maps of circulating miRNA biomarkers for ulcerative colitis. *PLoS One* 2012; **7:** e31241.

45. Longo UG, Denaro L, Spiezia F, Forriol F, Maffulli N, Denaro V. Symptomatic disc herniation and serum lipid levels. *Eur Spine J* 2011; **20:** 1658-1662.

46. Jhawar BS, Fuchs CS, Colditz GA, Stampfer MJ. Cardiovascular risk factors for physician-diagnosed lumbar disc herniation. *Spine J* 2006; **6:** 684-691.

47. Heintzman ND, Ren B. Finding distal regulatory elements in the human genome. *Curr Opin Genet Dev* 2009; **19:** 541-549.

48. Siegel DH, Ashton GH, Penagos HG, Lee JV, Feiler HS, Wilhelmsen KC *et al*. Loss of kindlin-1, a human homolog of the Caenorhabditis elegans actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome. *Am J Hum Genet* 2003; **73:** 174-187.

49. Jaffe IZ, Mendelsohn ME. Angiotensin II and aldosterone regulate gene transcription via functional mineralocortocoid receptors in human coronary artery smooth muscle cells. *Circ Res* 2005; **96:** 643-650.

50. Al-Aly Z. Arterial calcification: a tumor necrosis factor-alpha mediated vascular Wnt-opathy. *Transl Res* 2008; **151:** 233-239.

51. Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM *et al*. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 2008; **454:** 1000-1004.

52. Golan T, Yaniv A, Bafico A, Liu G, Gazit A. The human Frizzled 6 (HFz6) acts as a negative regulator of the canonical Wnt. beta-catenin signaling cascade. *J Biol Chem* 2004; **279:** 14879-14888.

53. Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A *et al*. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* 2001; **411:** 321-325.

54. Ueland T, Otterdal K, Lekva T, Halvorsen B, Gabrielsen A, Sandberg WJ *et al*. Dickkopf-1 enhances inflammatory interaction between platelets and endothelial cells and shows increased expression in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2009; **29:** 1228-1234.

55. Cheng JH, She H, Han YP, Wang J, Xiong S, Asahina K *et al*. Wnt antagonism inhibits hepatic stellate cell activation and liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2008; **294:** G39-49.

56. Lyons JP, Miller RK, Zhou X, Weidinger G, Deroo T, Denayer T *et al*. Requirement of Wnt/beta-catenin signaling in pronephric kidney development. *Mech Dev* 2009; **126:** 142-159.

57. Pietila I, Ellwanger K, Railo A, Jokela T, Barrantes Idel B, Shan J *et al*. Secreted Wnt antagonist Dickkopf-1 controls kidney papilla development coordinated by Wnt-7b signalling. *Dev Biol* 2011; **353:** 50-60.

58. Dai C, Stolz DB, Kiss LP, Monga SP, Holzman LB, Liu Y. Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. *J Am Soc Nephrol* 2009; **20:** 1997-2008.

59. Kato H, Susztak K. Repair problems in podocytes: Wnt, Notch, and glomerulosclerosis. *Semin Nephrol* 2012; **32:** 350-356.

60. Uhlenhaut NH, Treier M. Transcriptional regulators in kidney disease: gatekeepers of renal homeostasis. *Trends Genet* 2008; **24:** 361-371.

61. Wheeler DC, Bernard DB. Lipid abnormalities in the nephrotic syndrome: causes, consequences, and treatment. *Am J Kidney Dis* 1994; **23:** 331-346.

62. Kahle KT, Wilson FH, Leng Q, Lalioti MD, O'Connell AD, Dong K *et al*. WNK4 regulates the balance between renal NaCl reabsorption and K+ secretion. *Nat Genet* 2003; **35:** 372-376.

63. Lu D, Liu JX, Endo T, Zhou H, Yao S, Willert K *et al*. Ethacrynic acid exhibits selective toxicity to chronic lymphocytic leukemia cells by inhibition of the Wnt/beta-catenin pathway. *PLoS One* 2009; **4:** e8294.

64. Long H, Sabatier C, Ma L, Plump A, Yuan W, Ornitz DM *et al*. Conserved roles for Slit and Robo proteins in midline commissural axon guidance. *Neuron* 2004; **42:** 213-223.

65. Brunskill EW, Georgas K, Rumballe B, Little MH, Potter SS. Defining the molecular character of the developing and adult kidney podocyte. *PLoS One* 2011; **6:** e24640.

66. Grieshammer U, Le M, Plump AS, Wang F, Tessier-Lavigne M, Martin GR. SLIT2-mediated ROBO2 signaling restricts kidney induction to a single site. *Dev Cell* 2004; **6:** 709-717.

67. Azzolin L, Panciera T, Soligo S, Enzo E, Bicciato S, Dupont S *et al*. YAP/TAZ incorporation in the beta-catenin destruction complex orchestrates the Wnt response. *Cell* 2014; **158:** 157-170.

68. An Y, Kang Q, Zhao Y, Hu X, Li N. Lats2 modulates adipocyte proliferation and differentiation via hippo signaling. *PLoS One* 2013; **8:** e72042.

69. Zhou D, Strakovsky RS, Zhang X, Pan YX. The skeletal muscle Wnt pathway may modulate insulin resistance and muscle development in a diet-induced obese rat model. *Obesity (Silver Spring)* 2012; **20:** 1577-1584.

70. Tsika RW, Schramm C, Simmer G, Fitzsimons DP, Moss RL, Ji J. Overexpression of TEAD-1 in transgenic mouse striated muscles produces a slower skeletal muscle contractile phenotype. *J Biol Chem* 2008; **283:** 36154-36167.

71. He J, Kelly TN, Zhao Q, Li H, Huang J, Wang L *et al*. Genome-wide association study identifies 8 novel loci associated with blood pressure responses to interventions in Han Chinese. *Circ Cardiovasc Genet* 2013; **6:** 598-607.

72. Adeyemo A, Gerry N, Chen G, Herbert A, Doumatey A, Huang H *et al*. A genome-wide association study of hypertension and blood pressure in African Americans. *PLoS Genet* 2009; **5:** e1000564.

73. Kraja AT, Borecki IB, Tsai MY, Ordovas JM, Hopkins PN, Lai CQ *et al*. Genetic analysis of 16 NMR-lipoprotein fractions in humans, the GOLDN study. *Lipids* 2013; **48:** 155-165.

74. McKnight AJ, Currie D, Patterson CC, Maxwell AP, Fogarty DG, Warren UKGSG. Targeted genome-wide investigation identifies novel SNPs associated with diabetic nephropathy. *Hugo J* 2009; **3:** 77-82.

75. Okamoto K, Tokunaga K, Doi K, Fujita T, Suzuki H, Katoh T *et al*. Common variation in GPC5 is associated with acquired nephrotic syndrome. *Nat Genet* 2011; **43:** 459-463.

76. Tsaih SW, Pezzolesi MG, Yuan R, Warram JH, Krolewski AS, Korstanje R. Genetic analysis of albuminuria in aging mice and concordance with loci for human diabetic nephropathy found in a genome-wide association scan. *Kidney Int* 2010; **77:** 201-210.

77. Pezzolesi MG, Poznik GD, Mychaleckyj JC, Paterson AD, Barati MT, Klein JB *et al*. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. *Diabetes* 2009; **58:** 1403-1410.

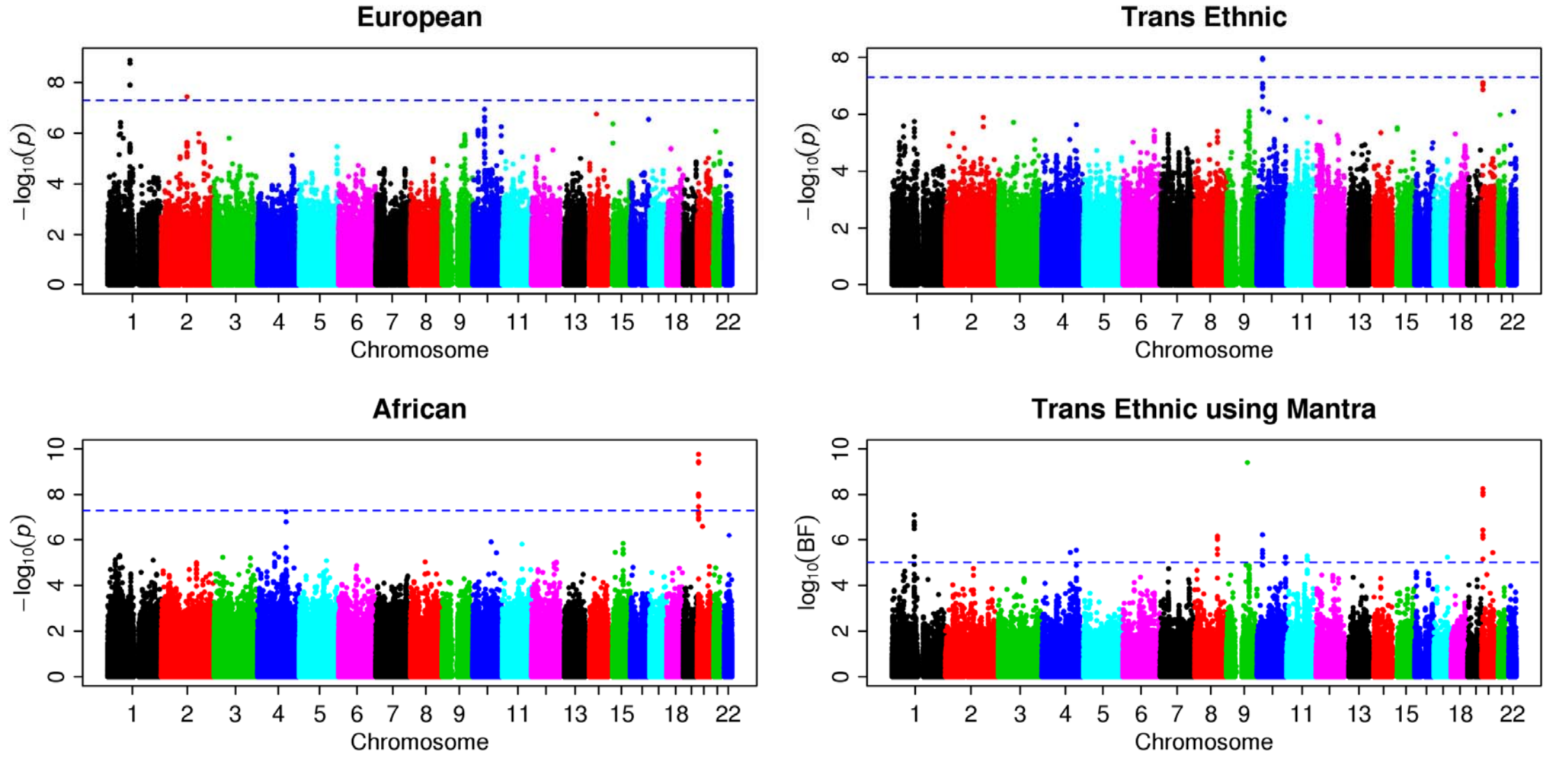
78. Kaess BM, Tomaszewski M, Braund PS, Stark K, Rafelt S, Fischer M *et al*. Large-scale candidate gene analysis of HDL particle features. *PLoS One* 2011; **6:** e14529.

79. Slavin TP, Feng T, Schnell A, Zhu X, Elston RC. Two-marker association tests yield new disease associations for coronary artery disease and hypertension. *Hum Genet* 2011; **130:** 725-733.

80. Debette S, Bis JC, Fornage M, Schmidt H, Ikram MA, Sigurdsson S *et al*. Genome-wide association studies of MRI-defined brain infarcts: meta-analysis from the CHARGE Consortium. *Stroke* 2010; **41:** 210-217.

81. Weidmann P, Uehlinger DE, Gerber A. Antihypertensive treatment and serum lipoproteins. *J Hypertens* 1985; **3:** 297-306.

82. Ferrari P, Weidmann P. Lipoproteins during antihypertensive therapy. Study supported by the Swiss National Science Foundation. *S Afr Med J* 1989; **Suppl:** 13-17.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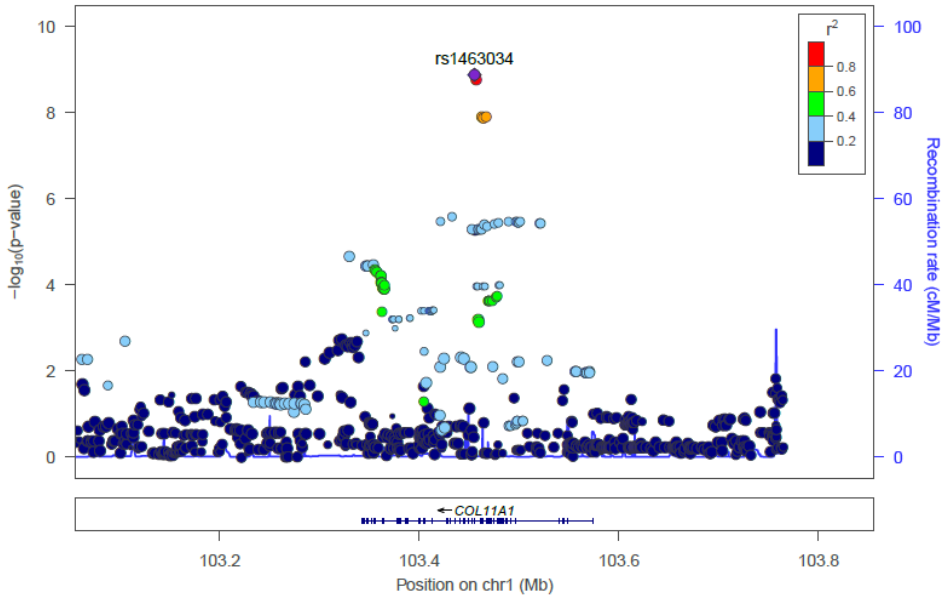

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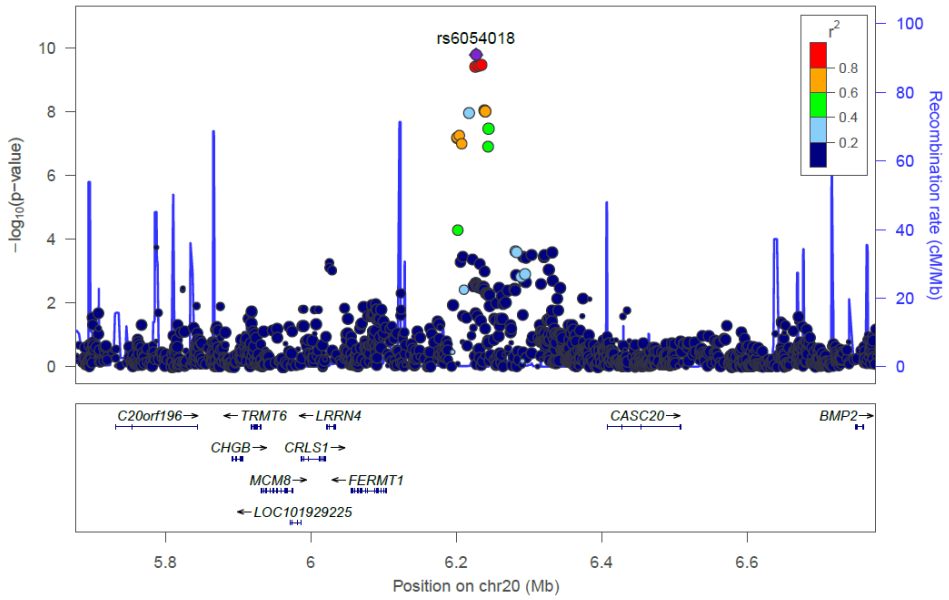
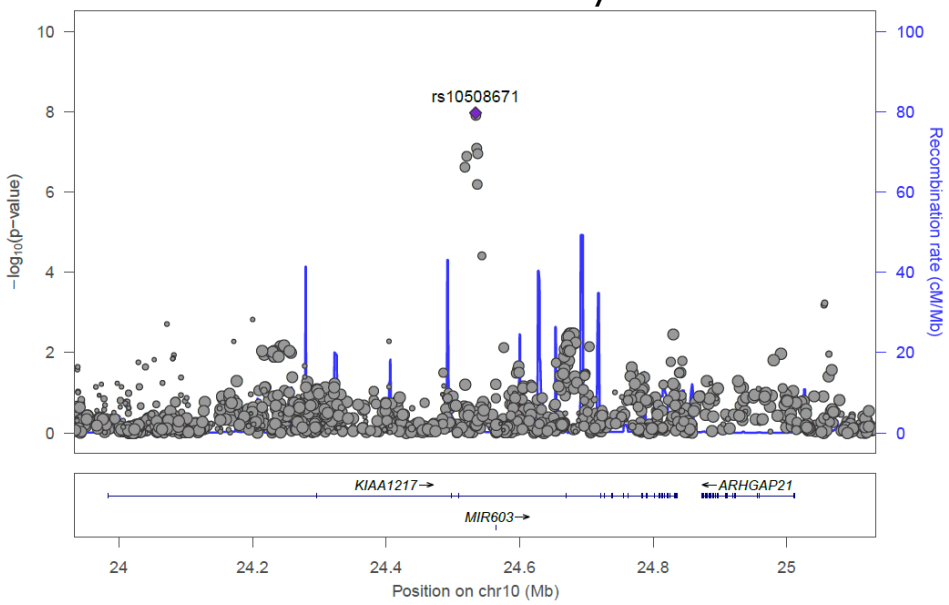


Trans-Ancestry



Genome-Wide Meta-analysis of Variant-by-Diuretic Interactions as Modulators of Lipid Levels in Persons of European and African Ancestry: Supplementary Materials

Table of Contents

Supplementary Tables

Supplementary Table 1. Genotyping, imputation, and statistical analysis for participating cohorts.

Ancestry	*Study*	*Genotyping Platform*	*Genotype Inclusion Criteria*	*Imputation Software*	*Association Test*
European	AGES	Illumina Hu370CNV	Call rates >97%, P HWE >1e-6	MACH	ProbABEL
	ARIC	Affymetrix 6.0	Call rates >90%, P HWE >1e-5	MACH	boss (R)
	CHS	Illumina 370CNV	Call rates >97%, P HWE >1e-5	BIMBAM	boss (R)
	FHS	Affymetrix 500K + 50 MIP	Call rates >97%, P HWE >1e-6	MACH	R
	Health ABC	Illumina 1M	Call rates >97%, P HWE >1e-6	MACH	ProbABEL
	HyperGEN	Affymetrix GeneChip SNP Array 5.0	Call rates >95%, P HWE >1e-6	MACH	ProbABEL
	HVH1	Illumina 370CNV	Call rates >97%, P HWE >1e-5	BIMBAM	R
	HVH2	Illumina Omni Express	Call rates >97%, P HWE >1e-5	MACH	R
	MESA	Affymetrix 6.0	Call rates >90%, P HWE >1e-4	IMPUTE	ProbABEL
	PROSPER	Illumina 660K	Call rates >97.5%, P HWE >1e-6	MACH	ProbABEL
	RS1	Illumina 550k	Call rates >98%, P HWE >1e-6	MACH	ProbABEL
	RS2	Illumina 550K Duo, 610KQuad	Call rates >95%, P HWE >1e-6	MACH	ProbABEL
	WHI CT GARNET Controls Baseline	Illumina Human Omni1-Quad v1-0 B	Call rates >98%, P HWE >1e-4	BEAGLE	boss (R)
	WHI CT GARNET Core	Illumina Human Omni1-Quad v1-0 B	Call rates >98%, P HWE >1e-4	BEAGLE	boss (R)

Ancestry	*Study*	*Genotyping Platform*	*Genotype Inclusion Criteria*	*Imputation Software*	*Association Test*
African	ARIC	Affymetrix GeneChip SNP Array 6.0	Call rates >90%, MAF ≥1%	MACH	boss (R)
	CHS	Illumina Human Omni1-Quad_v1 BeadChip	Call rates >97%, P HWE >1e-5	BEAGLE 3.2.1	boss (R)
	HyperGEN	Affymetrix GeneChip SNP Array 6.0	Call rates >95%, P HWE >1e-6	MACH	ProbABEL
	JHS	Affymetrix GeneChip SNP Array 6.0	Call rates >90%	MACH	boss (R)
	WHI CT SHARe Baseline	Affymetrix GeneChip SNP Array 6.0	Call rates >95%, P HWE >1e-6	MACH	boss (R)
	WHI CT SHARe Core	Affymetrix GeneChip SNP Array 6.0	Call rates >95%, P HWE >1e-6	MACH	boss (R)
	WHI OS SHARe Baseline	Affymetrix GeneChip SNP Array 6.0	Call rates >95%, P HWE >1e-6	MACH	boss (R)

Abbreviations: AGES, Age, Gene/Environment Susceptibility-Reykjavik Study; ARIC, Atherosclerosis Risk in Communities study; CHS, Cardiovascular Health Study; CT, Clinical Trial, FHS, Framingham Heart Study; GARNET, Genome-wide Association Research Network into Effects of Treatment; Health ABC, Health Aging, Body and Composition; HyperGEN, Hypertension Genetic Epidemiology Network; HVH, Heart and Vascular Health; JHS, Jackson Heart Study; MESA, Multi-Ethnic Study of Atherosclerosis; OS, Observational Study; PROSPER, PROspective Study of Pravastatin in the Elderly at Risk; RS, Rotterdam Study; SHARe, SNP Health Association Resource; WHI, Women's Health Initiative.

Supplementary Table 2. Genome-wide significant and suggestive SNPs-diuretic interaction ($P < 10^{-6}$) on lipid traits.

Marker	*Chr*	*Position*	*Alleles*	*European Ancestry*			*African Ancestry*			*Trans Ancestry*			*MANTRA*	*Neighboring Genes*
				Freq	*P*	*HetPVal*	*Freq*	*P*	*HetPVal*	*Freq*	*P*	*HetPVal*	$log_{10} BF$	
Loop Diuretic - Triglyceride														
rs12563198	1	59,718,894	a/g	0.96	5.48E-07	5.42E-01	0.91	9.85E-01	7.15E-01	0.95	2.15E-05	1.02E-02	3.90	
rs11808336	1	59,736,902	t/c	0.03	3.75E-07	4.46E-01	0.06	9.17E-01	9.73E-01	0.04	1.54E-05	9.68E-03	4.35	**FGGY**
rs17119531	1	59,740,182	a/g	0.97	3.82E-07	4.45E-01	0.94	9.01E-01	9.62E-01	0.96	1.29E-05	1.20E-02	4.62	
rs1463034	1	103,227,805	t/c	0.92	1.31E-09	3.25E-01	0.59	4.40E-01	7.68E-01	0.83	1.78E-06	1.92E-04	6.50	
rs1870958	1	103,229,744	t/g	0.92	1.76E-09	2.35E-01	0.59	5.00E-01	8.23E-01	0.82	3.16E-06	1.54E-04	6.68	
rs2786125	1	103,235,061	a/g	0.06	1.24E-08	1.21E-01	0.31	4.37E-01	8.66E-01	0.14	1.65E-05	1.93E-04	6.64	**COL11A1**
rs2622870	1	103,236,564	t/g	0.94	1.27E-08	1.22E-01	0.69	3.40E-01	8.28E-01	0.87	1.61E-05	1.72E-04	7.11	
rs2622874	1	103,239,505	a/c	0.06	1.23E-08	1.21E-01	0.31	3.26E-01	8.02E-01	0.13	1.74E-05	1.51E-04	6.80	
rs7544444	1	103,293,408	t/c	0.97	3.66E-06	1.82E-01	0.86	6.27E-02	9.06E-01	0.93	4.67E-03	4.99E-05	5.26	
rs7607797	2	117,944,672	t/c	0.97	3.60E-08	1.18E-01	0.66	4.62E-01	9.62E-01	0.86	6.94E-05	1.38E-04	3.78	DDX18
rs4554091	4	130,520,065	t/g	0.58	4.19E-01	6.18E-01	0.16	5.65E-08	6.03E-01	0.46	3.65E-02	5.23E-07	5.44	SCLT1 / **LOC105377417**
rs7691747	4	130,528,914	a/g	0.46	6.01E-01	6.33E-01	0.83	1.58E-07	9.96E-01	0.56	2.22E-02	2.74E-06	3.43	
rs11100093	4	158,213,492	a/t	0.15	3.67E-05	3.88E-01	0.19	1.70E-02	3.74E-01	0.16	2.32E-06	9.31E-01	5.53	PDGFC / GLRB / GRIA2
rs7817074	8	104,300,634	t/g	0.05	6.72E-05	6.74E-03	0.04	2.39E-02	6.90E-01	0.05	6.31E-06	6.38E-01	5.58	
rs7841861	8	104,329,002	t/c	0.06	4.38E-05	1.36E-02	0.13	2.46E-02	5.55E-01	0.08	3.92E-06	8.13E-01	6.10	**BAALC** / FZD6
rs7002454	8	104,347,955	t/c	0.95	7.71E-05	6.10E-03	0.87	3.58E-02	5.20E-01	0.93	9.78E-06	7.29E-01	6.01	
rs6985929	8	104,348,009	a/g	0.95	7.78E-05	6.03E-03	0.87	3.29E-02	5.35E-01	0.93	9.07E-06	7.51E-01	6.18	
rs12351434	9	103,449,707	a/g	0.05	1.84E-06	7.61E-01	0.16	7.51E-02	7.78E-01	0.08	7.95E-07	2.96E-01	4.40	**GRIN3A**
rs7077598	10	24,558,186	a/g	0.03	7.48E-07	2.55E-02	0.18	3.83E-02	8.29E-01	0.08	2.37E-07	2.12E-01	4.87	
rs7899031	10	24,560,897	t/c	0.97	7.52E-07	2.56E-02	0.86	2.34E-02	9.66E-01	0.93	1.27E-07	2.78E-01	5.22	
rs10508671	10	24,574,062	t/c	0.03	9.43E-07	2.67E-02	0.25	2.12E-03	6.38E-01	0.11	1.08E-08	6.69E-01	6.24	
rs10508672	10	24,574,441	a/g	0.03	9.52E-07	2.69E-02	0.24	2.32E-03	6.82E-01	0.11	1.19E-08	6.57E-01	6.21	**KIAA1217** / MIR603 / ARHGAP21
rs7071454	10	24,575,819	t/c	0.03	9.63E-07	2.69E-02	0.25	1.18E-02	4.19E-01	0.11	8.28E-08	3.05E-01	5.52	
rs12251962	10	24,576,990	a/g	0.03	9.76E-07	2.69E-02	0.11	7.79E-02	5.69E-01	0.06	6.59E-07	1.71E-01	4.88	
rs11013993	10	24,577,638	t/c	0.03	1.22E-06	2.51E-02	0.25	1.26E-02	4.17E-01	0.11	1.11E-07	3.04E-01	5.39	
rs1612115	10	53,893,261	t/c	0.97	2.31E-07	4.79E-01	0.84	4.41E-01	9.01E-01	0.93	1.31E-04	4.65E-04	1.92	
rs1441118	10	53,910,163	t/c	0.97	8.16E-07	2.05E-01	0.85	3.15E-01	4.36E-01	0.92	1.48E-03	1.21E-04	2.26	
rs10762762	10	53,923,822	a/g	0.03	1.12E-07	3.52E-01	0.13	3.19E-01	2.92E-01	0.07	2.71E-04	8.82E-05	2.25	
rs1620449	10	53,924,468	t/c	0.97	3.27E-07	2.16E-01	0.85	4.73E-01	3.70E-01	0.93	3.14E-04	2.88E-04	2.36	
rs1660756	10	53,929,701	t/c	0.03	6.66E-07	1.62E-01								
rs1441125	10	53,931,168	t/c	0.97	6.84E-07	1.60E-01	0.85	4.65E-01	2.70E-01	0.93	4.47E-04	4.23E-04	2.25	
rs1733732	10	53,932,187	t/c	0.97	9.49E-07	1.46E-01	0.85	3.48E-01	2.51E-01	0.92	9.14E-04	2.42E-04	2.19	PRKG1 / DKK1 / **LINC01468** / LOC105378305
rs1730463	10	53,933,172	a/g	0.03	9.19E-07	1.43E-01	0.15	6.66E-01	3.26E-01	0.07	2.05E-04	1.51E-03	2.22	
rs1730462	10	53,933,391	a/g	0.03	9.22E-07	1.43E-01	0.15	2.73E-01	2.67E-01	0.07	1.26E-03	1.45E-04	2.24	
rs1733735	10	53,936,820	t/c	0.97	9.39E-07	1.44E-01	0.85	3.33E-01	2.57E-01	0.92	9.72E-04	2.18E-04	2.14	
rs1441122	10	53,938,711	a/c	0.03	9.33E-07	1.44E-01	0.15	6.60E-01	3.25E-01	0.07	2.07E-04	1.52E-03	2.08	
rs1733701	10	53,944,852	a/t	0.97	9.32E-07	1.44E-01	0.86	7.16E-01	3.82E-01	0.93	1.75E-04	1.85E-03	2.35	
rs16930890	10	53,946,621	a/g	0.97	9.86E-07	1.41E-01	0.93	2.42E-01	3.20E-01	0.96	9.63E-05	1.87E-03	2.85	
rs749140	10	130,259,880	t/c	0.96	5.52E-06	6.56E-02	0.95	3.55E-01	1.47E-01	0.96	1.94E-05	8.96E-02	5.22	PTPRE / MKI67 / LINC01163
rs11016373	10	130,286,692	t/c	0.04	5.47E-07	9.05E-02	0.08	6.91E-01	6.37E-01	0.05	1.32E-05	1.58E-02	5.24	
rs16921999	11	94,872,381	a/c	0.03	1.99E-04	2.72E-01	0.04	1.11E-03	3.81E-01	0.03	1.23E-06	4.84E-01	5.10	ENDOD1 / SESN3 / FAM76B / CEP57 / MTMR2

Supplementary Table 2. Genome-wide significant and suggestive SNPs-diuretic interaction ($P < 10^{-6}$) on lipid traits – *Continued.*

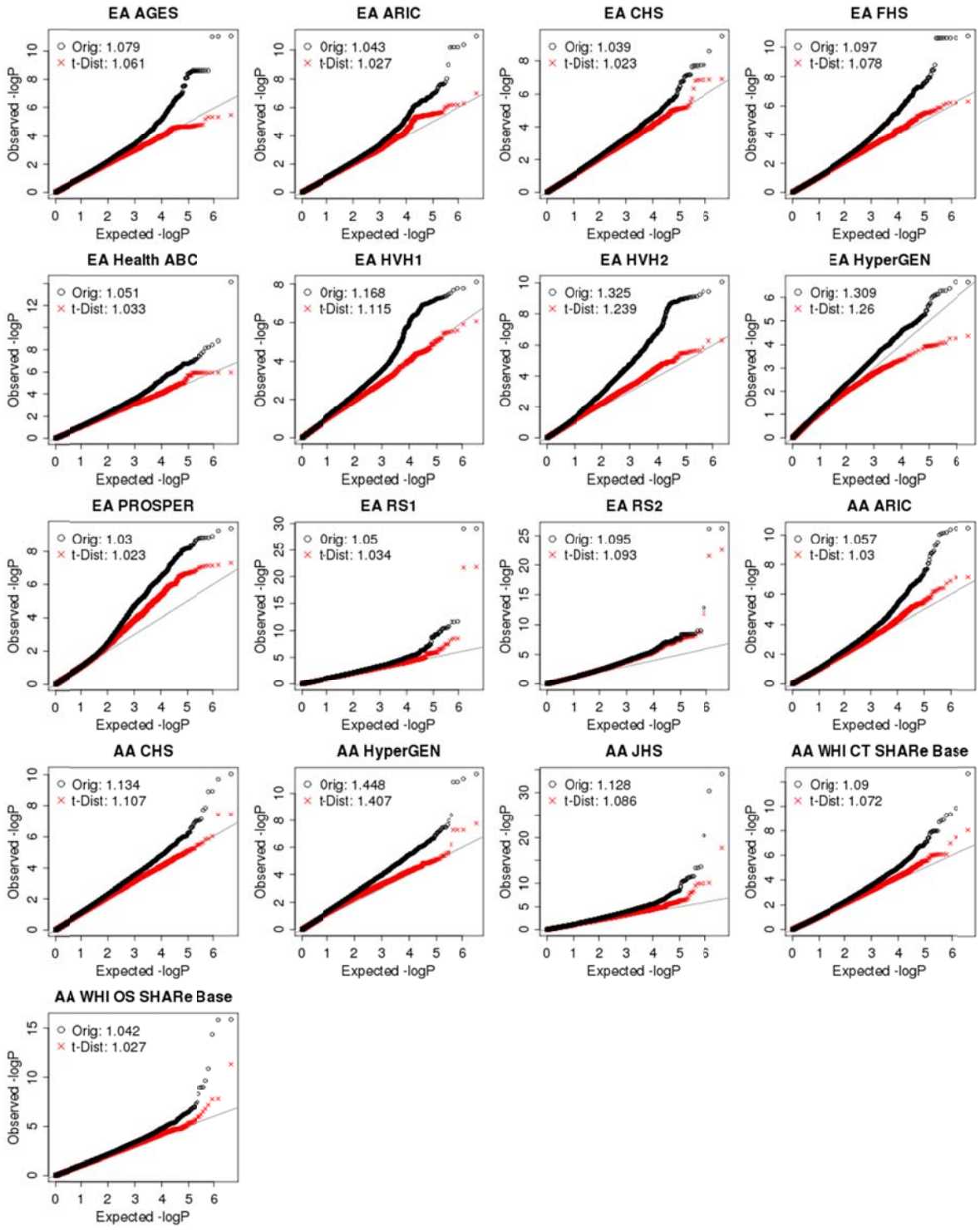
Marker	*Chr*	*Position*	*Alleles*	*European Ancestry*			*African Ancestry*			*Trans Ancestry*			*MANTRA*	*Neighboring Genes*
				Freq	*P*	*HetPVal(t)*	*Freq*	*P*	*HetPVal*	*Freq*	*P*	*HetPVal*	$log_{10} BF$	
Loop Diuretic - Triglyceride - continued														
rs2790503	14	51,378,963	a/c	0.96	1.74E-07	6.21E-01	0.72	6.58E-01	4.85E-01	0.89	4.43E-06	1.47E-02	3.90	FRMD6 / FRMD6-AS1 / GNG2 / LOC102723604
rs2278458	15	20,551,298	a/g	0.04	4.25E-07	4.67E-01	0.30	4.63E-01	7.63E-01	0.11	3.37E-06	4.39E-02	3.64	NIPA1 / NIPA2 / **CYFIP1** / TUBGCP5
rs11863116	16	83,876,060	a/g	0.84	2.84E-07	6.67E-01	0.72	3.40E-01	6.81E-01	0.82	9.89E-06	7.09E-03	3.84	LINC00311 / **GSE1** / GINS2 / FAM92B
rs3852940	20	6,165,600	a/g	0.79	7.10E-01	9.80E-01	0.70	1.18E-08	8.95E-01	0.77	9.80E-03	4.76E-07	6.07	CRLS1 / LRRN4 / FERMT1 / CASC20 / BMP2
rs6054016	20	6,174,368	t/c	0.06	6.18E-01	1.56E-01	0.19	4.06E-10	2.76E-01	0.10	2.05E-03	6.99E-08	6.22	
rs6054018	20	6,175,236	t/g	0.94	6.24E-01	1.54E-01	0.80	1.70E-10	3.14E-01	0.90	1.75E-03	3.46E-08	8.26	
rs8120588	20	6,178,849	t/c	0.94	6.36E-01	1.49E-01	0.81	3.75E-10	2.77E-01	0.90	1.79E-03	7.44E-08	8.09	
rs7348828	20	6,182,498	a/g	0.94	6.50E-01	1.29E-01	0.81	3.56E-10	2.78E-01	0.90	1.67E-03	7.65E-08	7.99	
rs8122198	20	6,186,686	t/c	0.94	9.11E-01	7.73E-02	0.85	9.53E-09	3.92E-01	0.91	2.36E-03	1.57E-06	6.44	
rs6054037	20	6,187,877	c/g	0.94	8.82E-01	7.25E-02	0.85	9.94E-09	3.90E-01	0.91	2.61E-03	1.48E-06	6.22	
rs6038400	20	6,188,201	a/c	0.06	8.79E-01	7.14E-02	0.15	1.03E-08	3.90E-01	0.09	2.66E-03	1.50E-06	6.45	
rs3885310	20	6,191,754	t/c	0.06	8.78E-01	7.39E-02	0.17	1.23E-07	2.89E-01	0.09	7.43E-03	6.64E-06	3.91	
rs3852942	20	6,192,559	a/g	0.06	8.84E-01	7.36E-02	0.17	3.36E-08	4.18E-01	0.10	3.31E-03	3.94E-06	5.15	
rs7508797	20	24,167,384	a/t	0.03	2.10E-01	1.97E-01	0.04	2.50E-07	6.33E-02	0.03	5.29E-04	7.73E-05	4.47	LINC01721
rs7262233	20	51,534,628	a/g	0.06	9.45E-06	2.48E-03	0.09	4.75E-01	5.95E-01	0.07	4.46E-05	7.77E-02	5.43	TSHZ2 / LOC101927770 / ZNF217 / LOC105372672
rs12627019	21	22,811,777	t/c	0.06	8.33E-07	6.37E-02								LINC01687 / LINC00308
Loop Diuretic - HDL														
rs17130785	1	68,928,375	t/c	0.93	7.67E-05	3.41E-01	0.91	5.32E-03	3.71E-01	0.92	1.35E-06	8.07E-01	5.06	DEPDC1 / DEPDC1-AS1
rs2139476	2	34,534,998	a/c	0.20	9.14E-02	3.47E-01	0.06	9.63E-07	1.08E-01	0.19	1.57E-03	4.03E-05	2.99	LINC01318 / LINC01317 / LINC01320
rs2887973	2	34,541,414	a/c	0.80	4.90E-02	3.11E-01	0.94	7.49E-07	9.33E-02	0.81	6.19E-04	4.51E-05	2.71	
rs11136986	8	6,060,930	t/c	0.94	1.31E-04	2.98E-01	0.85	1.93E-03	8.96E-01	0.92	9.71E-07	6.15E-01	4.59	LOC100287015 / MCPH1 / ANGPT2
rs7924536	11	12,874,326	t/c	0.98	1.53E-05	2.83E-01	0.77	1.06E-02	2.61E-01	0.90	5.79E-07	6.16E-01	5.22	**TEAD1** / LINC00958
rs2422862	20	3,147,703	a/g	0.86	7.33E-01	1.99E-01	0.96	2.83E-07	1.61E-05	0.87	1.62E-02	5.38E-06	6.28	DDRGK1 / **ITPA** / SLC4A11
Loop Diuretic - LDL														
rs12208017	6	79,880,090	t/g	0.78	4.16E-01	2.16E-01	0.95	3.73E-08	4.92E-01	0.80	2.69E-01	4.99E-08	2.03	IRAK1BP1/ PHIP / HMGN3
rs1312663	14	91,758,433	a/g	0.10	5.42E-01	2.55E-01	0.04	4.06E-07	9.08E-02	0.09	1.47E-01	9.96E-07	0.96	TRIP11 / ATXN3 / NDUFB1 / CPSF2 / SLC24A4
Thiazide Diuretic - Triglyceride														
rs4653061	1	34,844,885	a/g	0.48	4.16E-01	9.14E-01	0.19	6.15E-07	8.41E-01	0.38	2.16E-02	6.83E-06	4.33	GJB5 / GJB4 / GJB3 / GJA4 / SMIM12
rs1567793	6	67,180,515	t/c	0.63	7.60E-07	1.18E-01	0.62	5.76E-01	4.19E-01	0.63	2.82E-04	8.04E-04	2.89	EYS
rs1926511	13	108,153,937	t/c	0.27	1.05E-07	3.27E-01	0.18	2.76E-01	2.42E-01	0.24	4.17E-04	4.58E-05	3.71	**MYO16**
rs510438	18	55,084,025	a/c	0.19	6.45E-07	8.09E-01	0.09	6.05E-02	7.68E-01	0.16	9.84E-04	3.58E-05	4.54	GRP / RAX

Supplementary Table 2. Genome-wide significant and suggestive SNPs-diuretic interaction ($P < 10^{-6}$) on lipid traits – *Continued.*

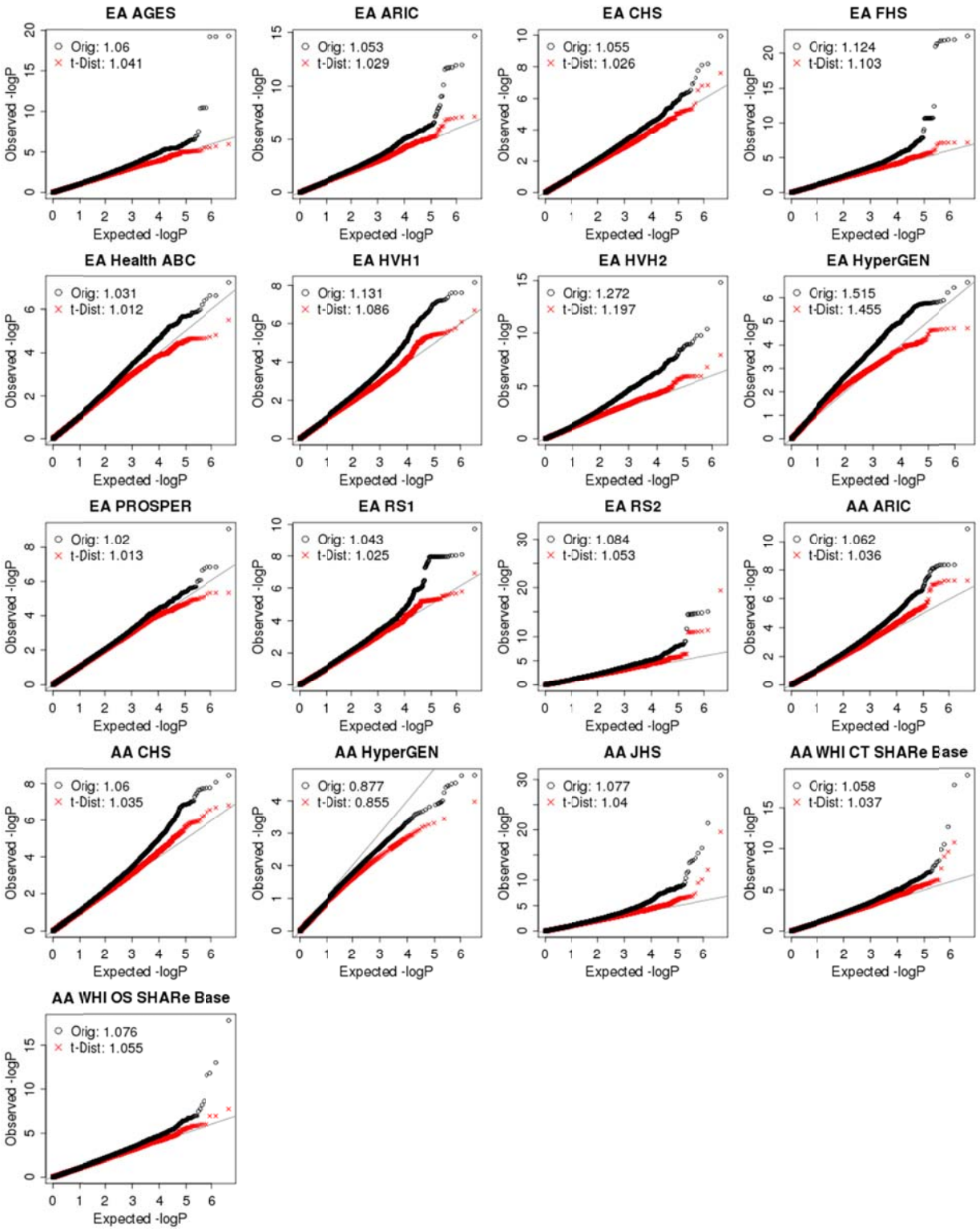
Marker	*Chr*	*Position*	*Alleles*	*European Ancestry*			*African Ancestry*			*Trans Ancestry*			*MANTRA*	*Neighboring Genes*
				Freq	*P*	*HetPVal(t)*	*Freq*	*P*	*HetPVal*	*Freq*	*P*	*HetPVal*	$log_{10} BF$	
Thiazide Diuretic - HDL														
rs1971692	9	124,904,313	t/g	0.86	1.29E-04	2.62E-01	0.54	1.19E-03	3.52E-01	0.73	5.87E-07	9.43E-01	4.23	GPR21 / **RABGAP1** / MIR600 / STRBP
rs10507678	13	62,067,267	t/c	0.08	5.47E-01	8.49E-01	0.17	3.58E-07	5.30E-02	0.11	8.93E-04	9.71E-05	3.40	PCDH20 / LOC101926951 / LINC00358 / LINC01075
rs7154514	14	91,496,952	a/t	0.45	5.60E-01	9.42E-01	0.68	8.03E-07	8.77E-01	0.53	1.44E-02	1.56E-05	4.02	
rs2295162	14	91,508,500	a/g	0.53	8.51E-01	9.60E-01	0.31	5.99E-07	8.66E-01	0.45	5.51E-03	3.34E-05	3.80	
rs2295166	14	91,523,099	t/g	0.53	9.38E-01	8.01E-01	0.31	5.96E-07	8.59E-01	0.45	4.15E-03	4.42E-05	3.48	
rs10083510	14	91,532,250	t/c	0.53	6.75E-01	9.59E-01	0.34	3.20E-07	9.99E-01	0.46	8.19E-03	1.13E-05	3.41	FBLN5 / **TRIP11/** ATXN3
rs10083447	14	91,534,180	a/g	0.47	6.96E-01	9.60E-01	0.69	5.75E-07	8.61E-01	0.55	8.31E-03	2.05E-05	3.13	
rs4904830	14	91,536,833	t/c	0.53	7.97E-01	9.14E-01	0.31	6.14E-07	8.60E-01	0.45	5.35E-03	3.47E-05	3.41	
rs4575474	14	91,539,034	c/g	0.53	9.55E-01	7.56E-01	0.31	8.77E-07	8.61E-01	0.46	4.22E-03	6.45E-05	3.67	
rs6043629	20	15,920,298	c/g	0.74	1.25E-05	7.19E-01	0.62	1.82E-04	8.69E-01	0.70	1.12E-08	6.41E-01	6.42	**MACROD2 / LOC613266**
rs9983495	21	30,118,644	t/g	0.04	2.73E-04	5.45E-01	0.26	2.80E-04	8.66E-01	0.13	3.63E-07	5.37E-01	3.88	**GRIK1**
Thiazide Diuretic - LDL														
rs12199608	6	743,361	c/g	0.11	7.55E-05	2.29E-01	0.03	2.99E-04	4.00E-01	0.10	4.14E-07	7.76E-02	4.41	EXOC2 / LOC101927691 / LINC01622
rs10840923	12	17,974,726	a/g	0.46	9.58E-07	2.02E-01	0.60	6.19E-01	6.14E-01	0.51	2.07E-04	1.20E-03	2.82	RERGL / PIK3C2G
rs10840928	12	18,010,451	a/g	0.52	3.67E-07	9.47E-02	0.62	9.15E-01	6.04E-01	0.56	7.28E-05	1.46E-03	2.35	
rs2359740	14	75,093,572	c/g	0.07	2.17E-07	2.62E-01	0.02	3.36E-01	2.44E-01	0.06	5.81E-05	6.46E-04	2.72	BATF / LOC102724153 / FLVCR2
rs4796525	17	6,456,407	t/c	0.05	1.48E-03	4.78E-02	0.19	9.53E-05	3.32E-01	0.10	8.98E-07	2.75E-01	4.91	PITPNM3 / **KIAA0753** / TXNDC17 / MED31 / SLC13A5
rs2304976	17	6,466,743	a/g	0.05	6.22E-04	5.29E-02	0.19	8.78E-05	3.35E-01	0.11	3.06E-07	3.48E-01	5.34	
rs2240275	17	6,484,840	a/g	0.05	1.27E-03	3.94E-02	0.19	7.70E-05	3.46E-01	0.11	5.83E-07	3.05E-01	5.15	

Abbreviations: Chr, chromosome, Freq, frequency of allele 1; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. **Bolded** genes include intragenic SNPs.

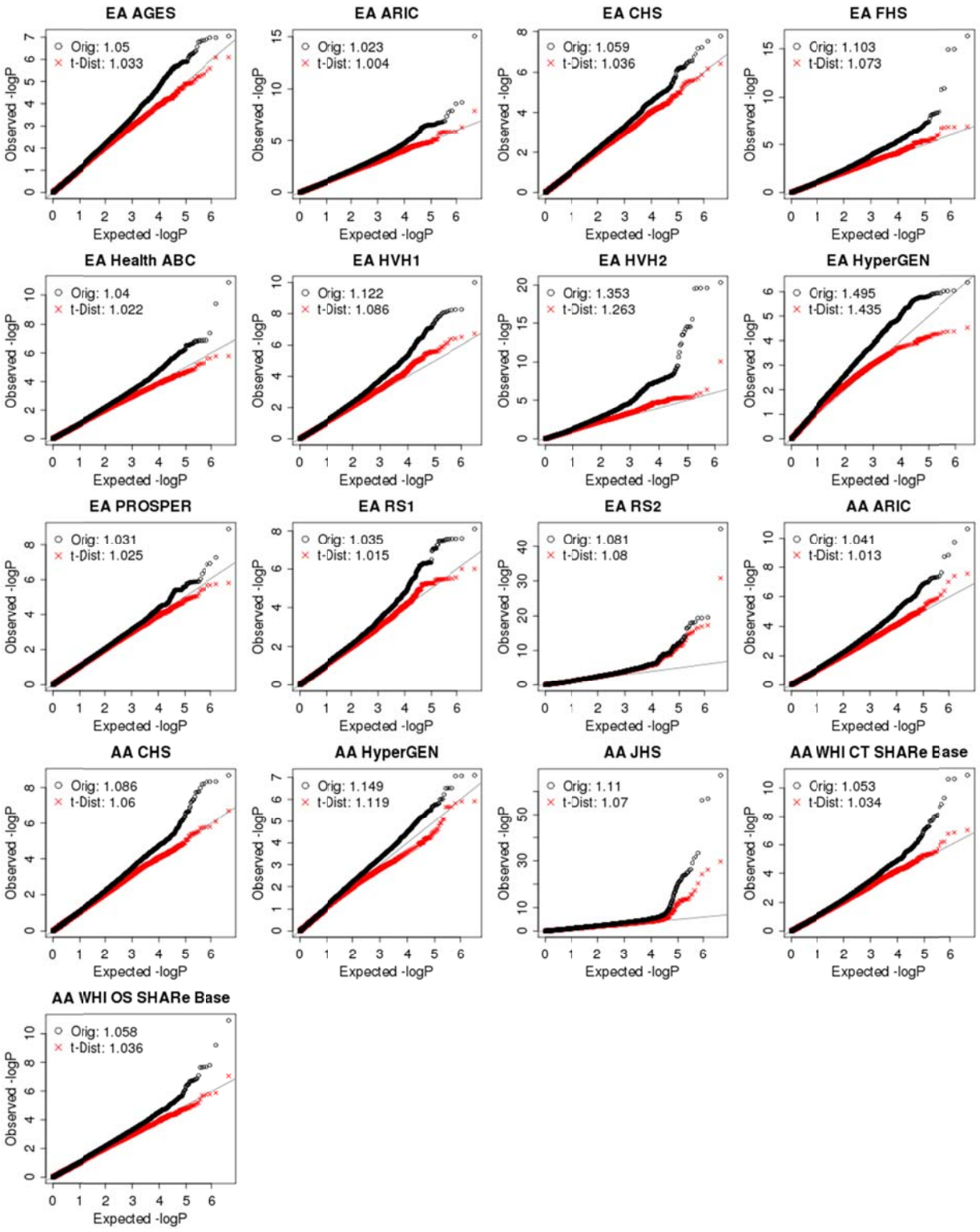
Supplementary Figures



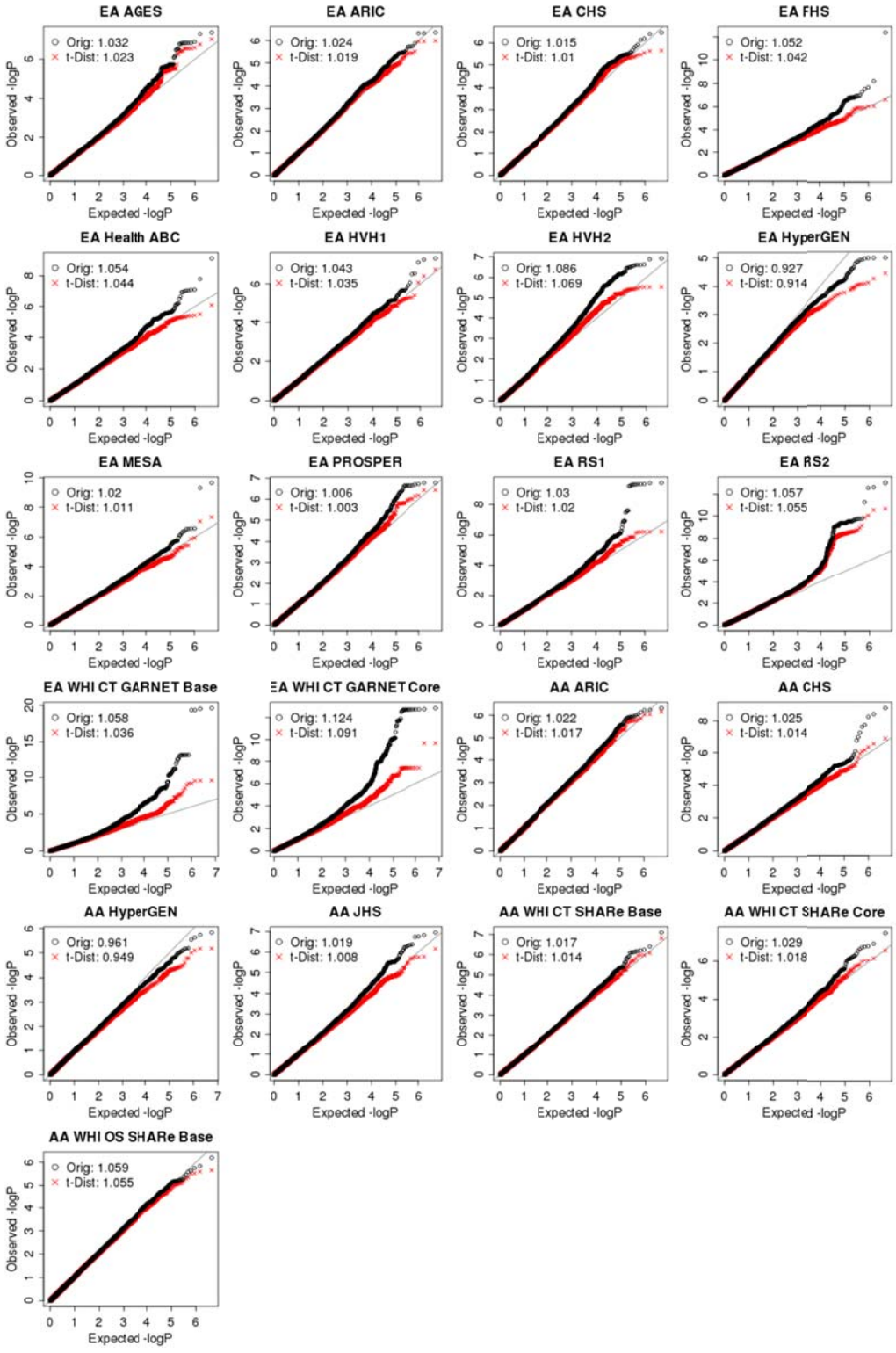
Supplementary Figure 1: QQ plots for cohort-specific GWAS results on TG-loop diuretic analysis. Each panel shows *P*-values based on a standard normal distribution (black circles), *P*-values based on a t-distribution (red crosses), and their genomic inflation factor lambda.



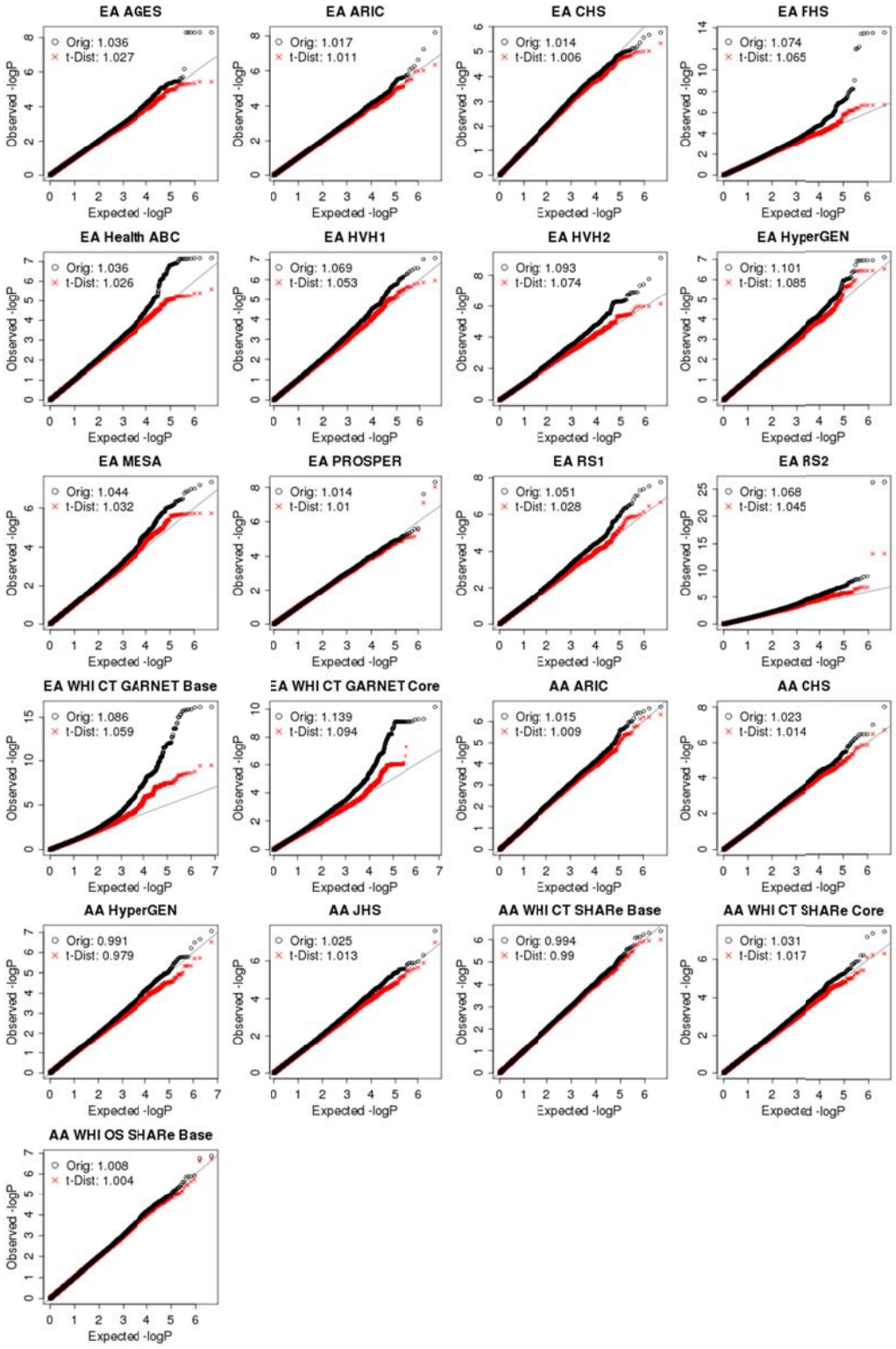
Supplementary Figure 2: QQ plots for cohort-specific GWAS results on HDL-loop diuretic analysis. Each panel shows *P*-values based on a standard normal distribution (black circles), *P*-values based on a t-distribution (red crosses), and their genomic inflation factor lambda.



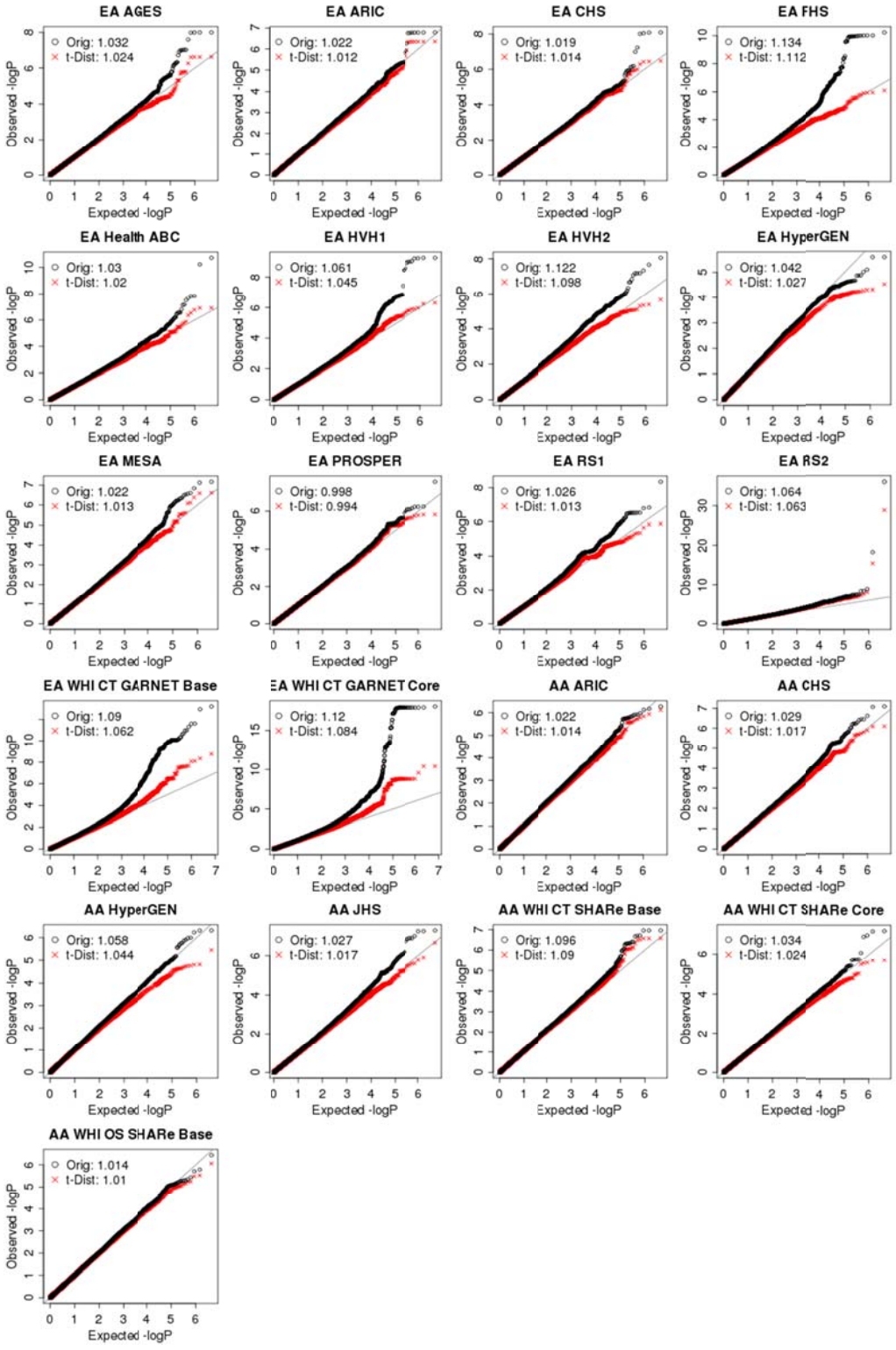
Supplementary Figure 3: QQ plots for cohort-specific GWAS results on LDL-loop diuretic analysis. Each panel shows *P*-values based on a standard normal distribution (black circles), *P*-values based on a t-distribution (red crosses), and their genomic inflation factor lambda.



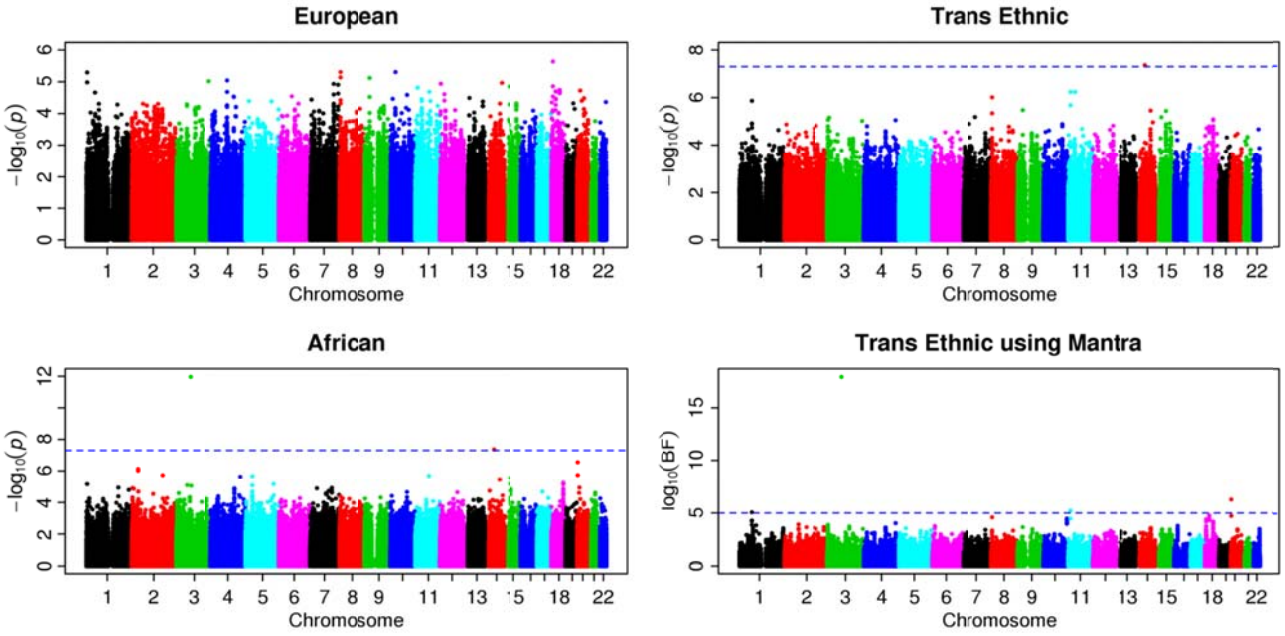
Supplementary Figure 4: QQ plots for cohort-specific GWAS results on TG-thiazide diuretic analysis. Each panel shows *P*-values based on a standard normal distribution (black circles), *P*-values based on a t-distribution (red crosses), and their genomic inflation factor lambda.



Supplementary Figure 5: QQ plots for cohort-specific GWAS results on HDL-thiazide diuretic analysis. Each panel shows *P*-values based on a standard normal distribution (black circles), *P*-values based on a t-distribution (red crosses), and their genomic inflation factor lambda.

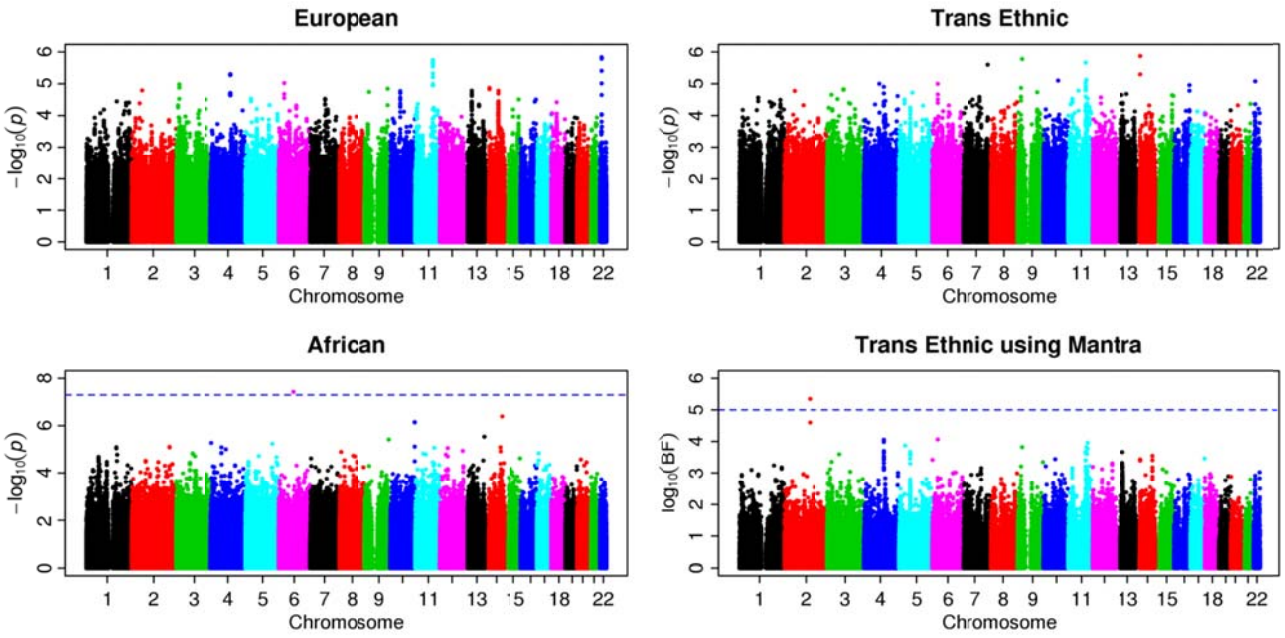


Supplementary Figure 6: QQ plots for cohort-specific GWAS results on LDL-thiazide diuretic analysis. Each panel shows *P*-values based on a standard normal distribution (black circles), *P*-values based on a t-distribution (red crosses), and their genomic inflation factor lambda.



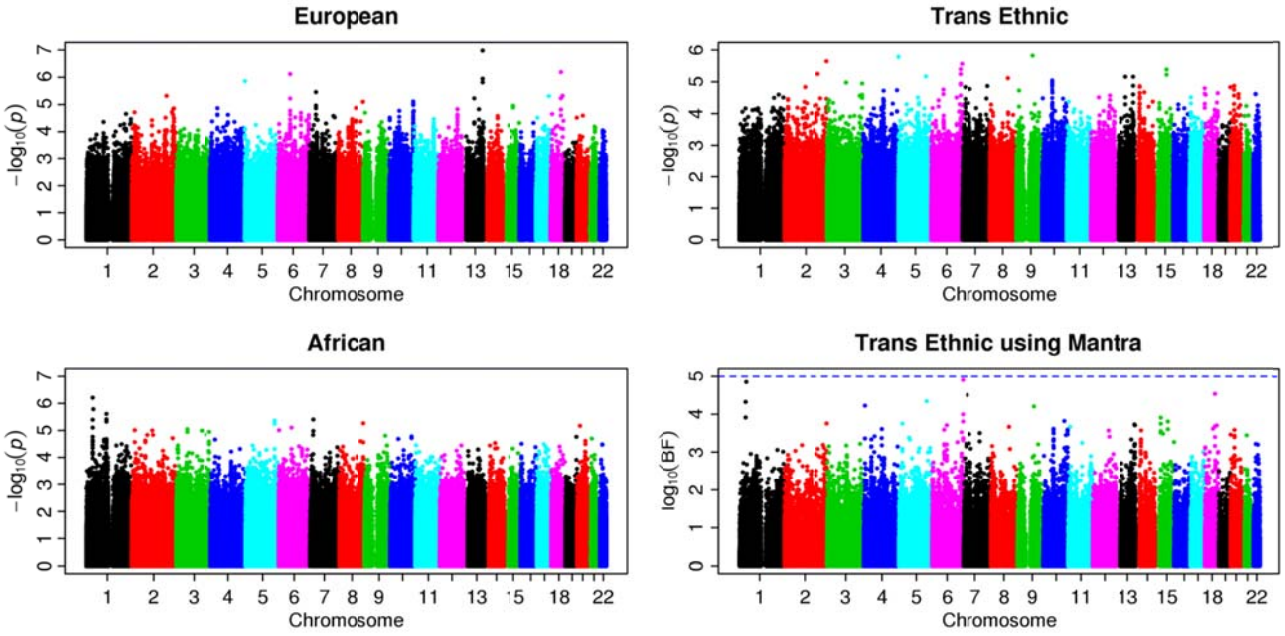
Supplementary Figure 7: Manhattan plots for HDL-loop diuretic interaction meta-analysis.

The ancestry-sepcific meta-analysis used 14 cohorts of European ancestry (upper-left panel) and 7 cohorts of African ancestry (lower-left panel). Trans-ancestry meta-analysis used fixed-effect weighted Z-statistics with METAL (upper-right panel) and a Bayesian framework with MANTRA (lower-right panel). The $-\log_{10}(P)$ from METAL or $\log_{10}(Bayes\ Factor)$ from MANTRA was plotted at the chromosomal location of each variant.



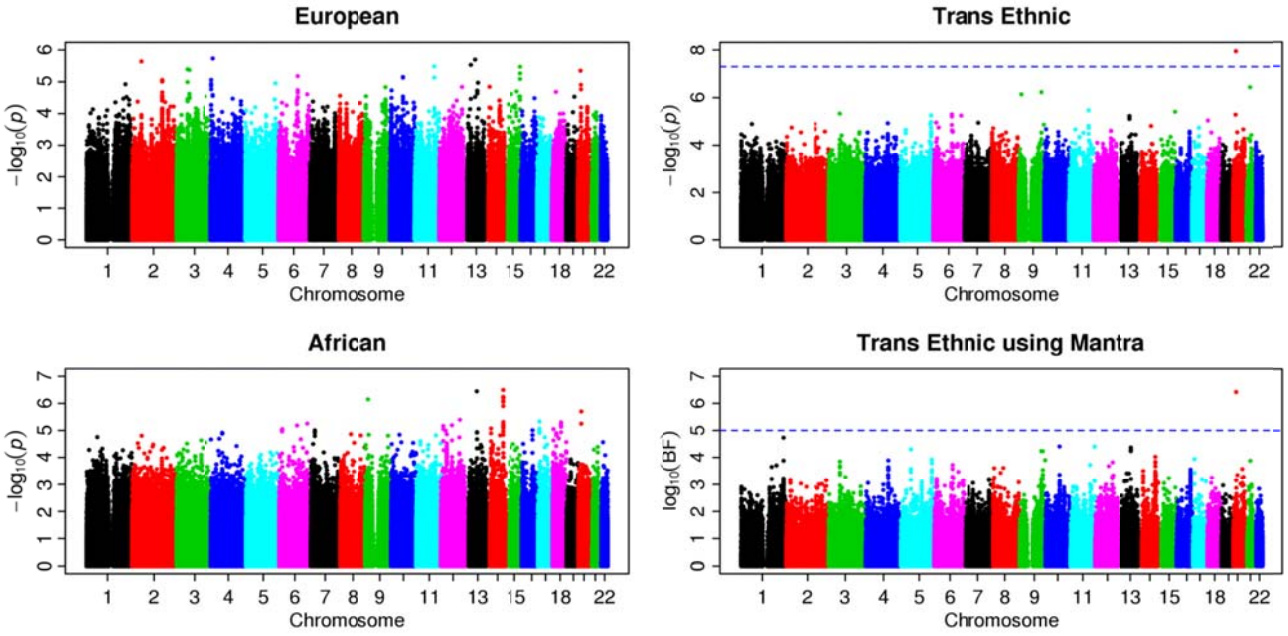
Supplementary Figure 8: Manhattan plots for LDL-loop diuretic interaction meta-analysis.

The ancestry-sepcific meta-analysis used 14 cohorts of European ancestry (upper-left panel) and 7 cohorts of African ancestry (lower-left panel). Trans-ancestry meta-analysis used fixed-effect weighted Z-statistics with METAL (upper-right panel) and a Bayesian framework with MANTRA (lower-right panel). The $-\log_{10}(P)$ from METAL or $\log_{10}(\textit{Bayes Factor})$ from MANTRA was plotted at the chromosomal location of each variant.



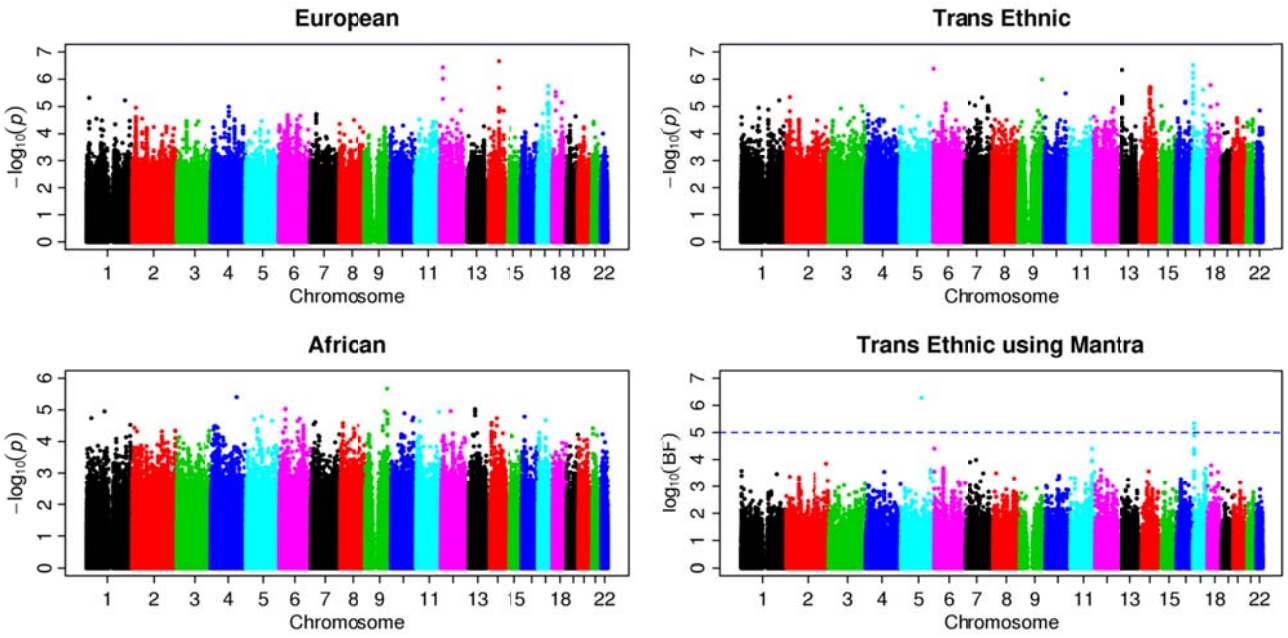
Supplementary Figure 9: Manhattan plots for TG-thiazide diuretic interaction meta-analysis.

The ancestry-sepcific meta-analysis used 14 cohorts of European ancestry (upper-left panel) and 7 cohorts of African ancestry (lower-left panel). Trans-ancestry meta-analysis used fixed-effect weighted Z-statistics with METAL (upper-right panel) and a Bayesian framework with MANTRA (lower-right panel). The $-\log_{10}(P)$ from METAL or $\log_{10}(\textit{Bayes Factor})$ from MANTRA was plotted at the chromosomal location of each variant.



Supplementary Figure 10: Manhattan plots for HDL-thiazide diuretic interaction meta-analysis.

The ancestry-sepcific meta-analysis used 14 cohorts of European ancestry (upper-left panel) and 7 cohorts of African ancestry (lower-left panel). Trans-ancestry meta-analysis used fixed-effect weighted Z-statistics with METAL (upper-right panel) and a Bayesian framework with MANTRA (lower-right panel). The $-\log_{10}(P)$ from METAL or $\log_{10}(\textit{Bayes Factor})$ from MANTRA was plotted at the chromosomal location of each variant.



Supplementary Figure 11: Manhattan plots for LDL-thiazide diuretic interaction meta-analysis.

The ancestry-sepcific meta-analysis used 14 cohorts of European ancestry (upper-left panel) and 7 cohorts of African ancestry (lower-left panel). Trans-ancestry meta-analysis used fixed-effect weighted Z-statistics with METAL (upper-right panel) and a Bayesian framework with MANTRA (lower-right panel). The $-\log_{10}(P)$ from METAL or $\log_{10}(\textit{Bayes Factor})$ from MANTRA was plotted at the chromosomal location of each variant.

Study Descriptions

AGES (Age Gene/Environment Susceptibility Reykjavik Study): The Reykjavik Study cohort originally was composed of a random sample of 30 795 men and women born in 1907-1935 and living in Reykjavik in 1967.[1] A total of 19 381 attended, resulting in 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow-up and was examined in all stages. Another group was designated a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the AGES-Reykjavik study re-examined 5 764 survivors of the original cohort who had participated before in the Reykjavik Study.

ARIC (Atherosclerosis Risk in Communities): The ARIC study is an ongoing population-based cohort of 15 792 predominantly Caucasian and African-American males and females aged 45-64 years at baseline and selected using probability sampling from four United States communities (Forsyth County NC, Jackson MS, suburban Minneapolis MN, and Washington County MD).[2] Participants were recruited in 1987-1989 to examine cardiovascular and pulmonary disease, patterns of medical care, and disease variation over time. Standardized physical examinations and interviewer-administered questionnaires were conducted at baseline (1987-1989), and at three triennial follow-up examinations (1990-1998).

CHS (Cardiovascular Health Study): CHS is a population-based cohort study of risk factors for CHD and stroke in adults ≥65 years conducted across four field centers.[3] The original predominantly Caucasian cohort of 5 201 persons was recruited in 1989-1990 from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African-American cohort of 687 persons was enrolled for a total sample of 5 888. DNA was extracted from blood samples drawn on all participants at their baseline examination in 1989-90. In 2007-2008, genotyping was performed at the General Clinical Research Center's Phenotyping/Genotyping Laboratory at Cedars-Sinai using the Illumina 370CNV BeadChip system on 3 980 CHS participants who were free of CVD at baseline, consented to genetic testing, and had DNA available for genotyping. Because the other cohorts were predominantly white, the African American participants were excluded from this analysis to reduce the possibility of confounding by population structure.

FHS (Framingham Heart Study): The FHS is a prospective, community based cohort study that was initiated in 1948 and now spans 3 generations, including the original cohort, their offspring and spouses of the offspring (Offspring Cohort, enrollment- beginning in 1971), and children from the largest offspring families (Generation 3 Cohort, enrollment beginning in 2000). Details regarding study recruitment and design have been reported previously.[4,5] All study protocols were approved by the Institutional Review Board for Boston University Medical Center. All study participants provided informed written consent.

Health ABC (Health, Aging, and Body Composition): The Health ABC Study is a NIA-sponsored cohort study of the factors that contribute to incident disability and the decline in function of healthier older persons, with a particular emphasis on changes in body composition in old age. Between 4/15/97 and 6/5/98 the Health ABC study has recruited 3 075 70-79 year old community-dwelling adults (41% African-American), who were initially free of mobility and activities of daily living disability. The key components of Health ABC include a baseline exam, annual follow-up clinical exams, and phone contacts every 6 months to identify major health events and document functional status between clinic visits. Provision has been made for banking of blood specimens and extracted DNA (Health ABC repository).

HVH (Heart and Vascular Health Study): The Heart and Vascular Health Study is a case-control study of risk factors for the development of cardiovascular events, including myocardial infarction (MI) and stroke. All study participants were Group Health (GH) members and aged 30-79 years.[6] MI and stroke cases were identified from hospital discharge diagnosis codes and were validated by medical record review. Controls were a random sample of GH members frequency matched to MI cases on age (within decade), sex, treated hypertension, and calendar year of identification. Only hypertensive control participants were included in this analysis. Lipid measures were obtained from the GH laboratory results database. Medication use was ascertained using computerized GH pharmacy records. The HVH data were analyzed in two phases because genotyping was

done with two different panels and at two different times. Across both phases, 676 persons of EA descent contributed data to this analysis.

HyperGEN (Hypertension Genetic Epidemiology Network): HyperGEN is a family-based study that looks at the genetic causes of hypertension and related conditions in EA and AA subjects.[7] HyperGEN recruited hypertensive sibships, along with their normotensive adult offspring, and an age-matched random sample. HyperGEN has collected data on 2 471 Caucasian-American subjects and 2 300 African-American subjects, from five field centers in Alabama, Massachusetts, Minnesota, North Carolina, and Utah.

JHS (Jackson Heart Study): The Jackson Heart Study is a longitudinal, community-based observational cohort study investigating the role of environmental and genetic factors in the development of cardiovascular disease in African Americans. Between 2000 and 2004, a total of 5 301 participants were recruited from a tri-county area (Hinds, Madison, and Rankin Counties) that encompasses Jackson, MS. Details of the design and recruitment for the Jackson Heart Study cohort has been previously published.[8-10] Briefly, approximately 30% of participants were former members of the Atherosclerosis Risk in Communities (ARIC) study. The remainder were recruited by either 1) random selection from the Accudata list, 2) commercial listing, 3) a constrained volunteer sample, in which recruitment was distributed among defined demographic cells in proportions designed to mirror those in the overall population, or through the Jackson Heart Study Family Study.

MESA (Multi-Ethnic Study of Atherosclerosis): MESA is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular disease or progression of the subclinical disease. MESA researchers study a diverse, population-based sample of 6 814 asymptomatic men and women aged 45-84. Thirty-eight percent of the recruited participants are white, 28% African-American, 22% Hispanic, and 12% Asian, predominantly of Chinese descent. [11] Participants were recruited from six field centers across the United States. Four physical examinations (at baseline and at 3 follow-up time points) were conducted and the electrocardiography was taken only at baseline. The tenets of the Declaration of Helsinki were followed and institutional review board approval was granted at all MESA sites. Written informed consent was obtained from each participant.

PROSPER (Prospective Study of Pravastatin in the Elderly at Risk): All data come from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the study has been published elsewhere.[12,13] PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5 804 subjects were randomly assigned to pravastatin or placebo. A large number of prospective tests were performed including Biobank tests and cognitive function measurements.

RS (Rotterdam Study): The Rotterdam study is a prospective population based cohort study comprising 7 983 participants aged 55 years or older (RS1), which started in 1990.[14] In 2000-2001, an additional 3 011 individuals aged 55 years or older were recruited (RS2). At baseline, participants were interviewed at home and were examined at the research center. Since then, participants are followed continuously and re-examined during several follow-up examination rounds. Medical information is available of all participants by collaboration with the general practitioners and with the pharmacies in the area of Ommoord. The medical ethics committee of Erasmus University, Rotterdam, approved the study, and all participants gave informed consent.

WHI (Women's Health Initiative): WHI is a long-term national health study that focuses on strategies for preventing common diseases such as heart disease, cancer and fracture in postmenopausal women. A total of 161 838 women aged 50–79 years old were recruited from 40 clinical centers in the US between 1993 and 1998. WHI consists of an observational study (OS) and clinical trials (CT) of postmenopausal hormone therapy, calcium / vitamin D supplementation, and dietary modification.[15] Study recruitment and exclusion criteria have been described previously.[15] Recruitment was done through mass mailing to age-eligible women obtained from voter registration, driver's license and Health Care Financing Administration or other insurance list, with emphasis on recruitment of minorities and older women.[16] Exclusions included participation in other

randomized trials, predicted survival < 3 years, alcoholism, drug dependency, mental illness and dementia. Study protocols and consent forms were approved by the IRB at all participating institutions. Medical history, medication use, anthropometrics (height; weight; body mass index), and fasting lipid concentrations were determined at screening and follow-up visits.[16] In this context, women of European ancestry were controls from a case-control study of incident coronary heart disease, stroke, venous thromboembolism, and diabetes (Genome-wide Association Research Network into Effects of Treatment (WHI CT GARNET),[17] while women of African ancestry were from the WHI Single Nucleotide Polymorphism Health Association Resource (WHI CT or OS SHARe).[18] Analyses of non-overlapping WHI CT and OS participant subsets were stratified by availability of lipid concentrations measured in serum at only one (Baseline) visit or in plasma at repeated (Core) visits.

Study Acknowledgments

AGES (Age Gene/Environment Susceptibility Reykjavik Study): This study has been funded by NIH contract N01-AG012100, the NIA Intramural Research Program, an Intramural Research Program Award (ZIAEY000401) from the National Eye Institute, an award from the National Institute on Deafness and Other Communication Disorders (NIDCD) Division of Scientific Programs (IAA Y2-DC_1004-02), Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament). The study is approved by the Icelandic National Bioethics Committee, VSN: 00-063. The researchers are indebted to the participants for their willingness to participate in the study.

ARIC (Atherosclerosis Risk in Communities) Study: The ARIC Study is carried out as a collaborative study supported by National Heart, Lung and Blood Institute Contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute Contract U01HG004402; and National Institutes of Health Contract HHSN268200625226C. We thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant No. UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

CHS (Cardiovascular Health Study): This CHS research was supported by National Heart, Lung and Blood Institute (NHLBI) Contracts N01-HC-85239, N01-HC-85079–N01-HC-85086; N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133, HHSN268201200036C and NHLBI Grants HL080295, HL085251, HL087652, HL103612, HL105756, HL130114, and HL120393 with additional contribution from NINDS. Additional support was provided through AG-023629, AG-15928, AG-20098 and AG-027058 from the NIA. See also http://www.chs-nhlbi.org/pi.htm. DNA handling and genotyping was supported in part by National Center for Research Resources CTSI Grant UL 1RR033176, National Institute of Diabetes and Digestive and Kidney Diseases Grant DK063491 to the Southern California Diabetes Endocrinology Research Center and the Cedars-Sinai Board of Governors' Chair in Medical Genetics (JIR).

FHS (Framingham Heart Study): FHS work was supported by the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine (Contract No. N01-HC-25195), its contract with Affymetrix for genotyping services (Contract No. N02-HL-6-4278), based on analyses by FHS investigators participating in the SNP Health Association Resource (SHARe) project. A portion of this research was conducted using the Linux Cluster for Genetic Analysis (LinGA-II), funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. Measurement of the Gen 3 ECGs was supported by grants from the Doris Duke Charitable Foundation and the Burroughs Wellcome Fund (Newton-Cheh) and the NIH (HL080025, Newton-Cheh).

Health ABC (Health, Aging, and Body Composition): This research was supported by NIA Contracts N01AG62101, N01AG62103 and N01AG62106. The genome-wide association study was funded by NIA Grant 1R01AG032098-01A1 to Wake Forest University Health Sciences and genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the

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HVH (Heart and Vascular Health): The research of the Heart and Vascular Health Studies has been funded in part by NHLBI grants R01HL085251, R01HL073410, and R01HL068986.

HyperGEN (Hypertension Genetic Epidemiology Network): The hypertension network is funded by cooperative agreements (U10) with NHLBI: HL54471, HL54472, HL54473, HL54495, HL54496, HL54497, HL54509, HL54515, and 2 R01 HL55673-12. The study involves: University of Utah: (Network Coordinating Center, Field Center, and Molecular Genetics Lab); Univ. of Alabama at Birmingham: (Field Center and Echo Coordinating and Analysis Center); Medical College of Wisconsin: (Echo Genotyping Lab); Boston University: (Field Center); University of Minnesota: (Field Center and Biochemistry Lab); University of North Carolina: (Field Center); Washington University: (Data Coordinating Center); Weil Cornell Medical College: (Echo Reading Center); National Heart, Lung, & Blood Institute. For a complete list of HyperGEN Investigators: http://www.biostat.wustl.edu/hypergen/Acknowledge.html.

Jackson Heart Study (JHS): We thank the Jackson Heart Study (JHS) participants and staff for their contributions to this work. The JHS 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.

MESA (Multi-Ethnic Study of Atherosclerosis): MESA is conducted and supported by the National Heart, Lung and Blood Institute (NHLBI) in collaboration with MESA investigators. Support is provided by Grants and Contracts N01 HC-95159–N01-HC-95169 and RR-024156. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-6-4278. Additional funding was supported in part by the Clinical Translational Science Institute Grant UL1RR033176 and the Cedars-Sinai General Clinical Research Center Grant RR00425. We also thank the other investigators, the staff and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

PROSPER (Prospective Study of Pravastatin in the Elderly at Risk): The PROSPER study was supported by an investigator initiated grant obtained from Bristol-Myers Squibb. Professor Dr J W Jukema is an Established Clinical Investigator of the Netherlands Heart Foundation (Grant No. 2001 D 032). Support for genotyping was provided by the seventh framework program of the European commission (Grant No. 223004) and by the Netherlands Genomics Initiative (Netherlands Consortium for Healthy Aging Grant 050-060-810).

RS (Rotterdam Study): The RS is supported by the Erasmus Medical Center and Erasmus University Rotterdam; The Netherlands Organization for Scientific Research; The Netherlands Organization for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly; The Netherlands Heart Foundation; the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission; and the Municipality of Rotterdam. Support for genotyping was provided by The Netherlands Organization for Scientific Research (NWO) (175.010.2005.011, 911.03.012) and Research Institute for Diseases in the Elderly (RIDE). This study was supported by The Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO) Project No. 050-060-810.

WHI (Women's Health Initiative): The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf. ELB was supported in part by a grant from the National Cancer Institute (5T32CA009001).

Supplementary References

1. Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G *et al*. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. *Am J Epidemiol* 2007; **165:** 1076-1087.
2. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol* 1989; **129:** 687-702.
3. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA *et al*. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991; **1:** 263-276.
4. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol* 1979; **110:** 281-290.
5. Dawber TR, Meadors GF, Moore FE, Jr. Epidemiological approaches to heart disease: the Framingham Study. *Am J Public Health Nations Health* 1951; **41:** 279-281.
6. Psaty BM, Heckbert SR, Koepsell TD, Siscovick DS, Raghunathan TE, Weiss NS *et al*. The risk of myocardial infarction associated with antihypertensive drug therapies. *JAMA* 1995; **274:** 620-625.
7. Williams RR, Rao DC, Ellison RC, Arnett DK, Heiss G, Oberman A *et al*. NHLBI family blood pressure program: methodology and recruitment in the HyperGEN network. Hypertension genetic epidemiology network. *Ann Epidemiol* 2000; **10:** 389-400.
8. Fuqua SR, Wyatt SB, Andrew ME, Sarpong DF, Henderson FR, Cunningham MF *et al*. Recruiting African-American research participation in the Jackson Heart Study: methods, response rates, and sample description. *Ethn Dis* 2005; **15:** S6-18-29.
9. Taylor HA, Jr., Wilson JG, Jones DW, Sarpong DF, Srinivasan A, Garrison RJ *et al*. Toward resolution of cardiovascular health disparities in African Americans: design and methods of the Jackson Heart Study. *Ethn Dis* 2005; **15:** S6-4-17.
10. Wyatt SB, Diekelmann N, Henderson F, Andrew ME, Billingsley G, Felder SH *et al*. A community-driven model of research participation: the Jackson Heart Study Participant Recruitment and Retention Study. *Ethn Dis* 2003; **13:** 438-455.
11. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR *et al*. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol* 2002; **156:** 871-881.
12. Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM *et al*. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 2002; **360:** 1623-1630.
13. Trompet S, de Craen AJ, Postmus I, Ford I, Sattar N, Caslake M *et al*. Replication of LDL GWAs hits in PROSPER/PHASE as validation for future (pharmaco)genetic analyses. *BMC Med Genet* 2011; **12:** 131.
14. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC *et al*. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol* 2011; **26:** 657-686.
15. Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C *et al*. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol* 2003; **13:** S18-77.
16. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* 1998; **19:** 61-109.
17. Genomics and Randomized Trials Network (GARNET). https://www.genome.gov/27541119/. National Institutes of Health, Date Accessed: 3/12/2017.
18. SHARe: SNP Health Association Resource. https://www.nhlbi.nih.gov/research/resources/genetics-genomics/share. National Institutes of Health, Date Accessed: 03/12/2017.