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# Genomic Association Analysis of Common Variants Influencing Antihypertensive Response to Hydrochlorothiazide

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***Abstract***—To identify novel genes influencing blood pressure response to thiazide diuretic therapy for hypertension, we conducted genome-wide association meta-analyses of ≈1.1 million single-nucleotide polymorphisms in a combined sample of 424 European Americans with primary hypertension treated with hydrochlorothiazide from the Pharmacogenomic Evaluation of Antihypertensive Responses study (n=228) and the Genetic Epidemiology of Responses to Antihypertensive study (n=196). Polymorphisms associated with blood pressure response at $P<10^{-5}$ were tested for replication of the associations in independent samples of hydrochlorothiazide-treated European hypertensives. The rs16960228 polymorphism in protein kinase C, α replicated for same-direction association with diastolic blood pressure response in the Nordic Diltiazem study (n=420) and the Genetics of Drug Responsiveness in Essential Hypertension study (n=206), and the combined 4-study meta-analysis *P* value achieved genome-wide significance ($P=3.3\times10^{-8}$). Systolic or diastolic blood pressure responses were consistently greater in carriers of the rs16960228 A allele than in GG homozygotes (>4/4 mm Hg) across study samples. The rs2273359 polymorphism in the *GNAS-EDN3* region also replicated for same-direction association with systolic blood pressure response in the Nordic Diltiazem study, and the combined 3-study meta-analysis *P* value approached genome-wide significance ($P=5.5\times10^{-8}$). The findings document clinically important effects of genetic variation at novel loci on blood pressure response to a thiazide diuretic, which may be a basis for individualization of antihypertensive drug therapy and identification of new drug targets. (***Hypertension.*** **2013;62:391-397.)** ● **Online Data Supplement**

**Key Words:** antihypertensive agents ■ genomics ■ hydrochlorothiazide ■ hypertension ■ pharmacogenomics ■ protein kinase C

The purpose of this study was to scan the human genome for single-nucleotide polymorphisms (SNPs) that predict blood pressure (BP) response to the most commonly prescribed thiazide diuretic, hydrochlorothiazide (HCT), in European Americans with primary hypertension. Since previous studies of genes hypothesized to regulate BP-reported polymorphisms associated with BP response to diuretic therapy,[1,2] none of the reported associations has been replicated across multiple independent studies.[3] In contrast to the approach of candidate gene studies, the genome-wide association (GWA) approach used in the present study requires no a priori selection of candidate genes and has the potential to identify genes not previously implicated to influence BP or drug response.[4] Recent GWA analyses in population of European descent

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document the success of this approach in identifying novel genetic variants influencing BP level, hypertension, and adverse cardiovascular disease outcomes.[5]

Our first objective was to conduct a GWA analysis for BP response to HCT in European American (ie, white) participants in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study,[6] in whom office, home, ambulatory daytime, and nighttime BP responses were measured and a weighted average BP response was calculated. Although all 4 methods measure the same BP response signal (but with different errors), calculation of the weighted average BP response minimizes measurement error and, thereby, maximizes the signal-to-noise ratio and power to identify genetic predictors of BP response.[7] Our second objective was to further increase statistical power to discover novel SNPs influencing BP response to HCT by conducting a meta-analysis of the combined GWA analysis results from the PEAR study participants and participants in our previous Genetic Epidemiology of Responses to Antihypertensives (GERA) study.[8] Our third objective was to validate the SNPs most strongly implicated in the meta-analysis by testing for replication of the associations with BP response to HCT in independent samples of European hypertensives from the Nordic Diltiazem (NORDIL) study,[9] the Genetics of Drug Responsiveness in Essential Hypertension study (GENRES),[10] and a study conducted in Milan, Italy.

## Methods

### Study Participants

The PEAR clinical study protocol (http://clinicaltrials.gov/ct2/show/NCT00246519) was approved by the Institutional Review Board at each site (the University of Florida, Gainesville, FL; Emory University, Atlanta, GA; and Mayo Clinic, Rochester, MN); all participants gave written informed consent; and all study procedures were in accordance with institutional guidelines and the Declaration of Helsinki and the US Code of the Federal Regulations for Protection of Human Subjects.[6] The methods and procedures for recruitment, the initial consent and screening visit, physical examination, BP measurement, and collection of blood and urine samples have been previously described (Methods in the online-only Data Supplement).[11] For the GWA analyses in PEAR participants (described below), a composite weighted average of the office, home, ambulatory daytime and nighttime BP responses was calculated on the basis of row sums of the inverse of the intermethod covariance matrices.[7] For the other study samples, the most precise measure of BP response available was analyzed (ie, the office BP response for the GERA and NORDIL study participants and the 24-hour ambulatory BP response for the GENRES and Milan (Italian) study participants).

### Statistical Analyses

In preliminary GWA analyses in HCT-treated European American PEAR study participants, each SNP was tested for association with the BP response phenotypes using an additive model that included pretreatment BP level, sex, and age as adjustment variables.[12] Although principal components analysis detected no population substructure, the first and second principal components were forced into all models. The SNP association results from both PEAR and GERA study samples were combined in a meta-analysis, assuming fixed effects and using inverse-variance weighting as implemented in the METAL software program.[13] SNPs with meta-analysis $P$ values $\leq 5\times10^{-8}$ were deemed genome-wide significant.[14] From SNPs with meta-analysis $P$ values $\leq 1\times10^{-5}$, we selected 1 to 2 SNPs with the smallest $P$ values at each locus to test for replication in the HCT-treated NORDIL study participants. Replication in NORDIL study participants was defined as a Bonferroni-corrected 1-sided $P<0.05$ because only SNPs with the same direction of effect as in PEAR and GERA study participants were of interest. SNPs that replicated in NORDIL study participants were further tested for replication in HCT-treated participants from the GENRES and the Milan (Italian) study.

Additional validation of the SNP associations that replicated among HCT-treated European hypertensives was pursued in 2 ways. First, we assessed whether variation across the entire region of the genes identified (in hypertensives of European descent) may be associated with BP response to HCT among African Americans (ie, black). Second, because known predictors of BP response to diuretics are inversely related to BP response to β blockers and other inhibitors of the renin–angiotensin system,[15] we assessed whether the SNPs associated with BP response to HCT had opposite direction associations with BP response to a β blocker in the PEAR study European Americans randomized to atenolol.[11] Finally, we tested the protein kinase C, α (*PRKCA*) SNP most strongly and consistently associated with BP response to HCT (ie, rs16960228) for association with lymphocyte mRNA expression (Methods in the online-only Data Supplement).

## Results

### Sample Descriptions

The HCT-treated European Americans from the PEAR and GERA studies did not differ significantly in the percentage of women or office systolic BP or diastolic BP responses (Table 1). Mean body mass index was significantly less and mean age and

**Table 1. Description of Hydrochlorothiazide-Treated European Americans From the PEAR and GERA Studies**

| Descriptive Characteristic | PEAR Study Participants (n=228) | GERA Study Participants (n=196) | *P* Value |
|---|---|---|---|
| Women, n (%) | 91 (40) | 84 (43) | 0.54 |
| Age, y | 50.4±9.4 | 48.5±7.3 | 0.02 |
| BMI, kg·m$^{-2}$ | 30.3±4.9 | 31.3±5.57 | 0.04 |
| Pretreatment office systolic BP, mm Hg | 151.8±12.4 | 142.7±12.6 | <0.0001 |
| Pretreatment office diastolic BP, mm Hg | 98.1±5.8 | 95.6±5.7 | <0.0001 |
| Office systolic BP response, mm Hg | −11.0±12.8 | −10.9±13.0 | 0.97 |
| Office diastolic BP response, mm Hg | −5.01±7.17 | −6.26±8.83 | 0.11 |
| Composite average systolic BP response, mm Hg | −8.5±7.02 | NA | NA |
| Composite average diastolic BP response, mm Hg | −4.68±4.79 | NA | NA |

BP response was defined as final minus baseline value (negative sign indicates BP decline in response to drug and was adjusted for pretreatment BP level, age, sex. In PEAR study participants, the composite average BP response is a weighted average of the office, home, ambulatory daytime, and nighttime BP responses. BMI indicates body mass index; BP, blood pressure; GERA, Genetic Epidemiology of Responses to Antihypertensives; NA, not available; and PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.

pretreatment office BP were significantly greater in the PEAR than GERA study participants. In the PEAR study participants, interindividual variation of the weighted average of office, home, ambulatory daytime, and nighttime BP response was less than for the office BP response, as expected.[7]

## Genome-Wide Association Analyses of BP Response to HCT

No SNP reached the genome-wide significance level (ie, $P<5\times10^{-8}$) for association with systolic BP or diastolic BP response in the GWA analysis of the composite weighted average BP responses in PEAR study HCT-treated European Americans (n=228), or in the separate GWA analysis of office BP responses in GERA study HCT-treated European Americans (n=196; Figure S1 and Table S1 in the online-only Data Supplement). However, in the meta-analysis of 1 092 841, SNP associations measured in both the PEAR and GERA study participants (n=424), 1 SNP association achieved genome-wide significance for diastolic BP response and 2 SNP associations achieved genome-wide significance for systolic BP response (Figure 1). The SNP that achieved genome-wide significance for association with diastolic BP response was on chromosome 14q31.3 (rs2776546; $P=4.9\times10^{-8}$), and the 2 SNPs that achieved genome-wide significance for association with systolic BP response were on chromosome 9q22.33 (rs238; $P=2.9\times10^{-8}$) and on chromosome 20p13 (rs4815273; $P=4.5\times10^{-8}$). Each of these loci included from 3 to as many as 15 SNPs associated with the BP response at the $P<10^{-5}$ level of significance (Table S2).

The meta-analysis *P* values for SNPs in genes previously reported to be associated with BP response to HCT[1,2] (eg, adducin 1 and WNK lysine-deficient protein kinase 1) did not achieve the $P<10^{-5}$ level of significance (Table S3) and were not considered in the replication analyses described below.

## Replication of SNP Associations With BP Responses to HCT in Independent Samples

We selected representative SNPs from each of the loci with meta-analysis *P* values $<1\times10^{-5}$ (Table 2). The selected SNPs were tested for replication of the associations with the office BP responses to HCT in an independent sample of HCT-treated hypertensive Europeans from the NORDIL study (n=420; Methods in the online-only Data Supplement and Table S4). Of the 10 SNPs tested for association with diastolic BP response, 2 SNPs in the chromosome 17q24.3 locus, rs4791040 and rs16960228, replicated for same-direction associations with office diastolic BP response (Table 2). The nominal 1-sided *P* values for rs4791040 ($P=6.3\times10^{-3}$) and for rs16960228 ($P=6.0\times10^{-3}$) remained statistically significant after Bonferroni correction for the 6 diastolic BP response loci tested ($P=0.04$, for both). When the SNP association results from the 3 independent samples were combined, the 3-study meta-analysis *P* values approached genome-wide significance (for rs4791040, $P=6.2\times10^{-8}$; and for rs16960228, $P=6.0\times10^{-8}$).

Although neither of the 2 chromosome 20p13 SNPs replicated for association with systolic BP response in the NORDIL study participants, a chromosome 20q13.32 SNP, rs2273359, was nominally associated ($P=2.5\times10^{-2}$) and the 3-study meta-analysis *P* value for its association with systolic BP response closely approached genome-wide significance ($P=5.5\times10^{-8}$; Table 2).

The chromosome 17q24.3 and chromosome 20p13 SNPs that replicated in NORDIL study participants were further tested for replication in the GENRES and Milan (Italian) study participants. The chromosome 17q24.3 SNP rs16960228 replicated for same-direction association with 24-hour ambulatory diastolic BP response to HCT in GENRES study participants (n=206; 1-sided $P=0.04$) but not with office diastolic BP response in the Milan (Italian) study participants (n=195; 1-sided $P=0.58$). The combined 4-study meta-analysis *P* value for rs16960228 achieved genome-wide significance ($P=3.3\times10^{-8}$). The variant A allele carriers from each of the 5 studies demonstrated consistently greater BP responses to HCT than the GG homozygotes (Figure 2). On the basis of weighted average BP response phenotypes measured in PEAR study participants, the estimated difference in systolic or diastolic BP response was 4/4 mm Hg greater among the rs16960228 variant A allele carriers.

Although the chromosome 20q13 SNP rs2273359 was not measured or imputed in the GENRES or Milan (Italian) study participants (and, therefore, could not be tested for replication in these additional independent samples), among the PEAR, GERA, and NORDIL study participants, the variant G allele carriers from each of the 3 studies demonstrated consistently

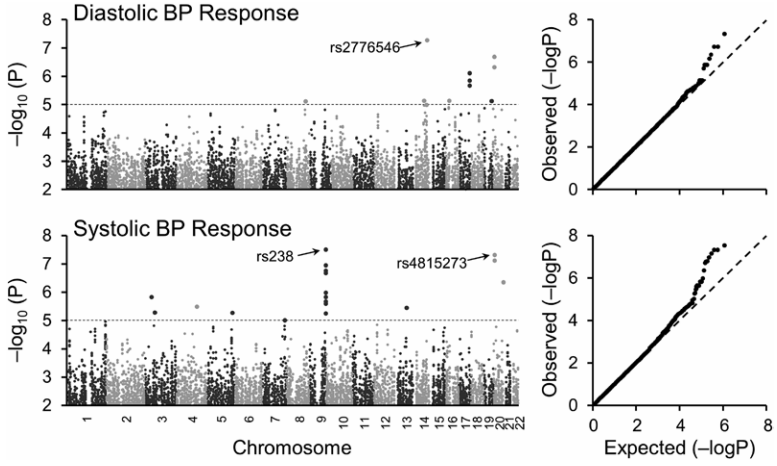


**Figure 1.** Manhattan plots and quantile–quantile plots from meta-analysis of genome-wide association analysis results for blood pressure (BP) response to hydrochlorothiazide in European American Pharmacogenomic Evaluation of Antihypertensive Responses and Genetic Epidemiology of Responses to Antihypertensives study participants.

**Table 2. Single-Nucleotide Polymorphisms Associated With BP Response to Hydrochlorothiazide in Meta-Analyses of GWA Analyses of European American Samples and Replication Analysis of European Sample**

| BP Response | SNP | Chr | Alleles | | Meta-Analysis of PEAR+GERA Study GWA Analyses | | | Replication Analysis in NORDIL Study Participants | | | PEAR+GERA+NORDIL Study Meta-Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Allele Freq | β | *P* Value | Allele Freq | β | *P* Value* | β | *P* Value |
| Diastolic | rs2432742 | 8 | A | G | 0.86 | −2.52 | 7.03E−06 | 0.82 | 0.07 | 6.16E−01 | −1.57 | 5.22E−04 |
| | rs221903 | 14 | T | C | 0.37 | 1.77 | 6.98E−06 | 0.37 | 0.08 | 4.33E−01 | 1.15 | 2.40E−04 |
| | rs2776546 | 14 | A | C | 0.87 | −3.24 | 4.9E−08 | 0.84 | −0.88 | 9.80E−02 | −2.22 | 7.07E−07 |
| | rs9933692 | 16 | A | G | 0.22 | −2.09 | 6.93E−06 | 0.19 | 0.05 | 6.79E−01 | −1.36 | 2.95E−04 |
| | rs4074471 | 16 | T | G | 0.78 | 2.09 | 6.92E−06 | 0.81 | −0.05 | 6.80E−01 | 1.36 | 2.94E−04 |
| | rs4791040 | 17 | T | C | 0.96 | 4.46 | 1.36E−06 | 0.96 | 3.30 | 6.25E−03 | 4.17 | 6.19E−08 |
| | rs16960228 | 17 | A | G | 0.04 | −4.46 | 1.37E−06 | 0.04 | −3.26 | 5.96E−03 | −4.16 | 6.03E−08 |
| | rs7247267 | 19 | T | G | 0.25 | 2.81 | 7.05E−06 | 0.21 | 0.07 | 4.79E−01 | 1.40 | 1.43E−03 |
| | rs4815273 | 20 | T | C | 0.46 | −1.93 | 1.96E−07 | 0.45 | 0.28 | 7.36E−01 | −1.20 | 7.36E−05 |
| | rs6083538 | 20 | T | C | 0.45 | −1.90 | 4.6E−07 | 0.45 | 0.27 | 7.35E−01 | −1.18 | 1.28E−04 |
| Systolic | rs2306667 | 3 | T | C | 0.83 | 3.64 | 4.88E−06 | 0.77 | 0.45 | 3.35E−01 | 2.63 | 6.05E−05 |
| | rs17010902 | 4 | A | G | 0.07 | −7.35 | 3.12E−06 | 0.06 | 1.28 | 8.11E−01 | −4.34 | 8.02E−04 |
| | rs4376293 | 5 | T | C | 0.49 | −2.50 | 5.01E−06 | 0.41 | 0.38 | 7.14E−01 | −1.77 | 2.08E−04 |
| | rs11763492 | 7 | A | G | 0.22 | −2.89 | 9.35E−06 | 0.24 | 1.14 | 9.69E−01 | −1.59 | 4.51E−03 |
| | rs13223171 | 7 | T | C | 0.22 | −2.89 | 9.44E−06 | 0.24 | 1.12 | 9.67E−01 | −1.59 | 4.29E−03 |
| | rs689979 | 9 | T | C | 0.45 | 2.68 | 2.02E−06 | 0.44 | −0.26 | 6.79E−01 | 1.84 | 1.36E−04 |
| | rs238 | 9 | A | G | 0.46 | 3.11 | 2.90E−08 | 0.45 | −0.45 | 1.10E−01 | 2.22 | 9.61E−6 |
| | rs4815273 | 20 | T | C | 0.46 | −2.91 | 4.50E−08 | 0.45 | −0.39 | 3.45E−01 | −2.34 | 5.65E−07 |
| | rs6083538 | 20 | T | C | 0.45 | −2.91 | 6.84E−08 | 0.44 | −0.41 | 3.38E−01 | −2.34 | 7.95E−07 |
| | rs2273359 | 20 | C | G | 0.04 | 8.21 | 4.15E−07 | 0.02 | 7.79 | 2.47E−02 | 8.15 | 5.54E−08 |

Alleles: coded allele shown to the left of the noncoded allele is the modeled allele as in the example of A/G SNP in which AA=0, AG=1, and GG=2, where G is the coded and A the noncoded allele; allele freq, frequency of the coded allele; β, model regression coefficient, mm Hg per coded allele. BP indicates blood pressure; GERA, Genetic Epidemiology of Responses to Antihypertensives; NORDIL, Nordic Diltiazem; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; and SNP, single nucleotide polymorphisms.

*One-sided *P* value for same-direction association as in meta-analysis of PEAR+GERA study participants.

greater BP responses to HCT than the CC homozygotes (no GG homozygotes were observed; Figure 3). On the basis of weighted average BP response phenotypes measured in PEAR study participants, the estimated difference in systolic or diastolic BP response was 7/5 mm Hg greater among the rs2273359 variant G allele carriers (Figure 3).

## Further Validation of SNP Associations With BP Responses to Antihypertensive Drug Therapy

### *Regional Associations in Blacks With HCT Response*

The chromosome 17q24.3 SNP rs16960228 are located in the gene encoding *PRKCA*, a plausible candidate to influence BP (see the Discussion section). SNPs in the nearby chromosome 17q24.2 region of *PRKCA* were significantly associated with diastolic BP response to HCT in PEAR blacks (n=148; eg, rs6504428; $P=8.8\times10^{-5}$; Figure S2). The chromosome 20q13.32 SNP rs2273359 is in the gene encoding TH1-like (*TH1L*) between G-protein α subunit (*GNAS*) and *EDN3*, a region associated with BP level and hypertension in GWA meta-analyses of large samples of European descent.[5] SNPs in the chromosome 20q13.32 region between *TH1L* and *GNAS1* were also associated with systolic BP response to HCT in PEAR blacks (eg, rs234613; *P*=0.02; Figure S4). There were differences between races in the linkage disequilibrium between these and other SNPs across the *PRKCA* gene and the *GNAS-TH1L* regions (Figures S4 and S5).

### *Opposite Direction Association With BP Response to Atenolol*

In a parallel, independent sample of PEAR study European Americans randomized to atenolol (n=233),[6] the chromosome *PRKCA* SNP rs16960228 was associated with diastolic BP response to the β blocker with the direction of association opposite to that observed with BP response to HCT (1-sided *P*=0.01). The chromosome 20q13 SNP rs2273359, however, was not significantly associated with systolic BP response to atenolol in the PEAR European American study participants (*P*=0.95).

### *Gene Expression Analysis of PRKCA*

Gene expression of *PRKCA* was measured using RNA isolated from whole blood collected before HCT treatment (baseline) from 36 European American PEAR study participants selected on the basis of rs16960228 genotype (Methods in the online-only Data Supplement). Carriers of the rs16960228 variant A allele (n=12) had significantly greater mean relative *PRKCA* expression level than the GG homozygotes (n=24; *P*=0.03; Figure 4).

## Discussion

We sought to identify common genetic variants in whites of European descent that are predictive of BP response to HCT, the most commonly prescribed diuretic for the treatment of

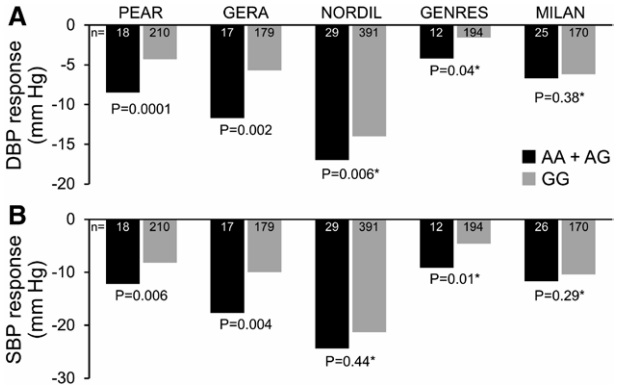


**Figure 2.** Plot of blood pressure response to hydrochlorothiazide by chromosome 17 rs16960228 genotype of participants from 5 independent studies. **A**, Diastolic blood pressure (DBP) response. **B**, Systolic blood pressure (SBP) response. The blood pressure responses are adjusted for pretreatment blood pressure levels, age, and sex and *P* values are for contrast of adjusted means between genotype groups. GENRES indicates the Genetics of Drug Responsiveness in Essential Hypertension Study; GERA, Genetic Epidemiology of Responses to Antihypertensives; NORDIL, the Nordic Diltiazem; and PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.

hypertension.[16] Our results provide substantial evidence that chromosome 17q24 variation within *PRKCA* influences interindividual variation in BP response to HCT. We found statistically significant and directionally consistent associations of rs16960228 with diastolic BP response to HCT in 4 independent samples of white hypertensives of European descent, with a directionally consistent albeit not statistically significant association in a fifth European sample. The association of rs16960228 with diastolic BP response to HCT achieved genome-wide significance in a meta-analysis combining results from the first 4 samples. The contribution of *PRKCA* variation to differences in BP response to HCT was further validated by finding that other SNPs within *PRKCA* were associated with diastolic BP response to HCT in hypertensive blacks. Among European American hypertensives, the association of rs16960228 with diastolic BP response to atenolol was directionally opposite to its association with diastolic BP response to HCT, as is the case for known predictors of BP response to these 2 drug classes.[15] Finally, the rs16960228 variant A allele that predicted greater BP response to HCT was also found to be associated with greater pretreatment *PRKCA* expression.

Our results also provide evidence that chromosome 20q13.32 variation in a region between *GNAS* and *EDN3*, which previous GWA meta-analyses of large samples of European descent have associated with BP level and hypertension,[5] may also influence BP response to HCT. We found statistically significant and directionally consistent associations of rs2273359 within *TH1L* with systolic BP response to HCT in the 3 independent samples of white hypertensives of European descent in which this SNP was available for analyses, and the 3-study meta-analysis *P* value closely approached genome-wide significance. The possible role for variation in this region in influencing BP response was bolstered by finding that other chromosome 20q13.32 SNPs between *TH1L* and *GNAS* were associated with systolic BP response to HCT in black hypertensives.

The target of HCT and other thiazide-like diuretics is the sodium-chloride cotransporter in the distal convoluted tubule.[17] Variants in the regulators of renal sodium transport, or in the vasoactive systems opposing BP decline in response to sodium and volume loss, are obvious candidates to influence BP response to HCT.[15] From this perspective, variations in both of the identified gene regions seem to be plausible candidates to influence BP response to HCT. *PRKCA* expression has been reported in brain, endothelium, heart and cardiac myocytes, smooth muscle, kidney, and adrenal cortex.[18–20] The PRKCA protein is involved in calcium signaling, vascular smooth muscle contraction, vascular endothelial growth factor signaling, and aldosterone-regulated sodium reabsorption pathways.[21] *TH1L* is downstream of *GNAS*, the stimulatory G-protein α subunit (Gs-α), a key component of the signal transduction pathway linking receptor–ligand interactions with the activation of adenylyl cyclase and a variety of cellular responses, including calcium signaling and vascular smooth muscle contraction.[21]

Replication across multiple, appropriately designed, well-powered, independent samples has become the gold standard

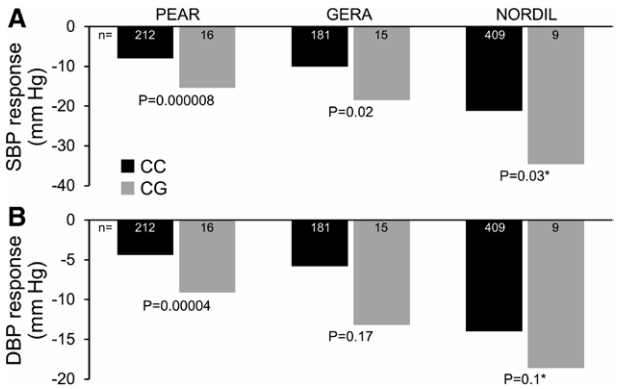


**Figure 3.** Plot of blood pressure response to hydrochlorothiazide by chromosome 20 rs2273359 genotype of participants from 3 independent studies. **A**, Systolic blood pressure (SBP) response. **B**, Diastolic blood pressure (DBP) response. The blood pressure responses are adjusted for pretreatment blood pressure levels, age, and sex and *P* values are for contrast of adjusted means between genotype groups. GERA indicates Genetic Epidemiology of Responses to Antihypertensives; NORDIL, the Nordic Diltiazem; and PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses.

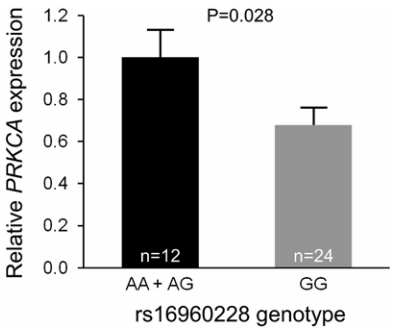


**Figure 4.** Plot of relative gene expression of *PRKCA* by rs16960228 genotype in whole blood collected from European American Pharmacogenomic Evaluation of Antihypertensive Responses study participants at baseline before HCT treatment. Expression data were normalized to β-2 microglobulin; error bars indicate SE of mean.

for reliability of pharmacogenetic associations.[4] By this standard, the present GWA meta-analysis of BP response to HCT is unique among genetic studies of antihypertensive drug responses. The only 2 previously reported GWA analyses for BP response to antihypertensive drugs did not have available samples to test for replication across independent studies.[22,23] None of the several prior report associations of polymorphisms in hypothesized candidate genes[1,24] has been consistently replicated across independent studies,[3] and none of the hypothesized candidate genes is within the regions identified in subsequent GWA analyses.[22,23]

The present study has several limitations. First, even though the sample size for the combined PEAR and GERA study GWA meta-analysis was 2-fold greater than the previous GWA analysis of BP response to HCT,[22] power was not adequate to detect variants with small effects on BP response comparable with those found for BP level and hypertension.[5,7] Second, even though the identified chromosome 17q24 and 20q13.32 regions harbor genes that are biologically plausible candidates, the SNPs we analyzed are intronic in *PRKCA* and *TH1L* and unlikely to be functional. Presumably, they are in linkage disequilibrium with functional variants that influence gene expression or protein structure but have not been identified.

### Perspective

Large interindividual differences in BP response reported since the earliest trials involving thiazide diuretics[25] have been attributed to variation in activity of the BP regulatory systems targeted by antihypertensive drugs.[26] Measurements of genetic variation hold the promise of individualization of antihypertensive drug therapy on the basis of matching the pathophysiologic disturbance elevating BP to the pharmacological action of the drug prescribed. Results of the present study support GWA analysis as an effective method to identify common genetic variants that may be a basis for individualization of antihypertensive drug therapy and identification of new drug targets.

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## Disclosures

None.

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## Novelty and Significance

### What Is New?

- Meta-analysis of 2 genome-wide association analyses of blood pressure response to the most commonly prescribed antihypertensive drug hydrochlorothiazide in European American hypertensives with replication of single-nucleotide polymorphism associations in independent samples of European hypertensives.

### What Is Relevant?

- Common variants in protein kinase C, α (*PRKCA*) and in the stimulatory G-protein α subunit (*GNAS*) region have clinically relevant effects on blood pressure response to hydrochlorothiazide in hypertensives of European descent that may be a basis for individualization of antihypertensive drug therapy and identification of new drug targets.

### Summary

Meta-analysis of 2 genome-wide association analyses of blood pressure response to hydrochlorothiazide in European American hypertensives succeeded in identifying common genetic variants that have clinically relevant effects on blood pressure response that replicate in European hypertensives treated with a thiazide diuretic.

**ONLINE SUPPLEMENT**

# Genomic Association Analysis of Common Variants Influencing Antihypertensive Response to Hydrochlorothiazide

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## SUPPLEMENTARY METHODS

### *PEAR Study*

At an initial consent and screening visit, trained study personnel administered standardized questionnaires, performed a limited physical examination, and obtained blood and urine samples for testing to establish eligibility.[1] Participants were provided an automated BP monitor (MicroLife 3AC1-PC, Minneapolis MN), instructed to take a set of three readings twice daily (upon arising in the morning and just before retiring in the evening), and withdrawn from previous antihypertensive drug therapy for a washout period averaging 31 days (range: 13-125 days). At subsequent visits prior to beginning and at the end of therapy, an additional set of three readings was obtained with the MicroLife monitor as a measure of office BP. At each of these visits, 24-hour ambulatory BP recordings were obtained using Spacelabs (Redmond WA) model 90207 monitors. Office, home, ambulatory daytime and nighttime BP responses were each calculated as the difference between pre- and post-treatment BP averages.

To qualify for randomization to HCT (or atenolol), the average home diastolic BP in the previous week had to be ≥85 mmHg (consisting of at least five morning and five evening sets of readings) *and* the average office diastolic BP ≥90 mmHg. HCT (or atenolol) therapy began at a dose of 12.5 mg (or 50 mg) daily for two weeks, after which, if BP remained >120/70 mmHg, the dose was increased to 25 mg (or 100 mg) daily for six additional weeks in 98% of the participants randomized to HCT (83% of those randomized to atenolol).

While both the PEAR and GERA Studies included African Americans, because of differences in allele frequencies and linkage disequilibrium between races,[2] and racial differences in antihypertensive drug responses,[3-5] all within-study analyses were conducted in each race

separately. Because the datasets available for replication included only Europeans, the PEAR and GERA Study analyses focused on the European American PEAR and GERA Study participants.

Genotyping: The PEAR DNA samples were genotyped for >1 million SNP markers using the Illumina Human Omni1-Quad BeadChip (Illumina, San Diego CA). Genotypes were called using BeadStudio software and the GenTrain2 calling algorithm (Illumina, San Diego CA). Using SNPs that passed quality-control filtering, we employed the MaCH software program12 (version 1.0.16) to impute genotypes at >1 million SNPs based on HapMap III phased haplotypes. SNPs were filtered and excluded from analysis if the minor allele frequency (MAF) was <3% or the imputation $r^2$ was <0.3.

Gene expression analysis: Expression of PRKCA was measured in whole blood collected from 36 European American PEAR Study participants at the pretreatment (baseline) study visit, i.e., at the end of the drug-free period just prior to HCT administration. RNA was isolated from whole blood using the PAXgene Blood RNA Kit IVD (Qiagen, Valenica, CA, USA) and converted to cDNA. Gene expression was measured by quantitative real-time RT-PCR using Taqman Gene Expression Assays and the Taqman 7900HT Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Expression levels were normalized to the reference gene β-2-microglobulin. Relative gene expression was calculated using the $2^{-\Delta Ct}$ method.6 Expression levels between genotype groups at baseline was compared using the Wilcoxon Two Sample test, with a p-value <0.05 considered significant.

***GERA Study***

The hypertensive European American participants from the GERA Study whose phenotypic data and genetic measurements were analyzed consisted of 98 "good" and 98 "poor" responders to hydrochlorothiazide.[7] Between 1997 and 2002, 300 hypertensive European Americans from

Rochester MN were treated with HCT at a dose of 25 mg daily for four weeks following a drug-free washout period of at least four weeks; BP was measured at the end of the drug-free and drug-treatment periods using a random zero sphygmomanometer (Hawksley and Sons, Ltd.; West Sussex, England).[5] After adjusting the race-and-gender specific distributions of diastolic BP response to remove variation attributable to differences in age and pretreatment level of BP, the "good" and "poor" (i.e., most extreme) responders to each drug were selected from opposite extremes of the sex-specific BP response distributions.[7]

The GERA Study DNA samples were genotyped for ≈500,000 SNP markers genome-wide using Affymetrix GeneChip® Human Mapping 500K Array Sets. The manufacturer recommended protocols were followed, and genotyping calls were made using the Dynamic Modeling and Birdseed algorithms.[8, 9] For participants included in the analyses, genotype call rates exceeded 95% over all SNPs; SNPs with call rates <80% over all GERA Study participants were excluded from the analyses. Using SNPs that passed the quality-control filtering, we employed the MACH software program (version 1.0.16) to impute genotypes at >1 million SNPs based on HapMap III phased haplotypes. Imputed SNP genotype results were filtered at an $r^2$ threshold of 0.3 and a minor allele frequency threshold of 0.03.

***NORDIL Study***

The NORDIL Study is a prospective,randomized, open, blinded endpoint study conducted at 1032 healthcare centers in Sweden and Norway between 1992 and 1999.[10] Ten thousand eight hundred eighty-one middle-aged Swedish and Norwegian participants who had diastolic BP of 100 mmHg or more on two occasions were included. Participants were previously untreated or if previously treated, had diastolic blood pressure of 100 mmHg or greater on two consecutive visits, at least one week apart, during a run in period when no antihypertensive treatment was given. Participants were randomized to treatment with either the nonselective calcium channel

blocker diltiazem or to therapy with beta-blockers, diuretics, or both. The thiazide diuretic was either hydrochlorothiazide or bendroflumethiazide at the discretion of the treating physician, as was the dose administered. Office BP was measured every six months with participants in the recumbent position, with the usual method of measurement for each participating center. Participants with diastolic BP still over 90 mmHg on follow-up visits received additional therapy in steps. In the beta-blocker/diuretic group, participants were initially treated with a beta-blocker or thiazide diuretic. In step 2, the two were combined if needed for adequate BP reduction. In step 3, an ACE inhibitor or an alpha-blocker was added. If participants were still hypertensive, any other antihypertensive compound except a calcium antagonist could be added. Participants were followed for a mean time of 4.5 years, with no differences of the primary endpoint (fatal and nonfatal stroke and myocardial infarction, death from cardiovascular causes) between the diltiazem and the beta-blocker/diuretic groups.

DNA was extracted from 5152 Swedish participants, constituting 72.4% of the Swedish NORDIL Study cohort. From these, 420 participants on monotherapy with a thiazide diuretic during the first 6 months of the study were selected for the current study. Office BP response to HCT was calculated as the difference between BP measured prior to and after six-months of HCT monotherapy. The study protocol was approved by the ethics committee at Lund University and Gothenburg University. All participants had formerly given their informed consent. The procedures followed were in accordance with institutional guidelines.

The NORDIL Study genome-wide genotyping was performed using the Illumina 610 Quad V1 BeadChip (Illumina, Inc., San Diego, CA, USA). SNPs with a minor allele frequency (MAF) <1% or in significant Hardy-Weinberg disequilibrium ($p<1\times10^{-7}$) in pooled samples were removed leaving 521,220 SNPs for analysis. Population structure was assessed using principal

components analysis as implemented in EIGENSTRAT. Imputation was performed using IMPUTE v.2 using HapMap release 22 (build 35).[11]

***GENRES Study***

The GENRES Study is a prospective, randomized, double-blind, cross-over, placebo-controlled antihypertensive drug trial in 313 moderately hypertensive Finnish men, aged 35-60 years, previously described in detail.[12] Inclusion criteria were diastolic BP ≥95 mm Hg in repeated measurements or use of antihypertensive medication. Any previously prescribed antihypertensive medication were withdrawn at least four-weeks prior to initiating study medication. Exclusion criteria were use of three or more antihypertensive drugs, secondary hypertension, or significant comorbidity. Each study participant received losartan 50 mg, bisoprolol 5 mg, hydrochlorothiazide 25 mg, and amlodipine 5 mg daily, each as a monotherapy in randomized order for four weeks. The study started with a four-week run-in placebo period, and all four drug treatment periods were separated by four-week placebo periods. Twenty-four-hour ambulatory BP readings were recorded at the end of each treatment period with a device equipped with a QRS complex detector and a position sensor (Diasys Integra; Novacor, Rueil-Malmaison, France). Recordings were available for 207 subjects during hydrochlorothiazide therapy. A total of 236 were successfully genotyped using the Illumina HumanOmniExpress-12 BeadChip (Illumina, Inc., San Diego, CA, USA). Imputation was performed using IMPUTE2 (version 2.2.2)[11] and the 1000Genomes panel. Because of low imputation quality scores, only measured SNPs were used for the replication analysis.

***Milan Italian Study***

The design of the study protocol was similar to one described previously.[13] Two-hundred-twenty-seven newly-discovered and never-treated participants with primary hypertension (defined by mean of three consecutive measurements of office BP >140/95 but <160/110 mm Hg

at an initial office visit or mean daytime ambulatory BP >135/85 mm) were enrolled after exclusion of secondary hypertension. The protocol was approved by the Ethics Committee of San Raffale Hospital, and all subjects provided informed, written consent before being screened for enrollment. After a 1-month run-in period in which participants were advised to ingest a diet containing <150 mmol sodium daily, the participants were treated for eight weeks with HCT, beginning at a dose of 12.5 mg daily for four weeks followed 25 mg daily for four more weeks. At each study visit, office BP was recorded by the same investigator using an Omron 750IT monitor (Omron Healthcare; Kyoto, Japan) between 8:00 and 10:00 AM, about 24 hours after the last HCT dose. The last 3 stable measures taken after clinical examination were averaged and used in the analysis. The response to therapy was computed as the difference between the average of the last 3 BP values at the last pretreatment visit and the average of the last 3 BP values after 2 months of HCT therapy described above.

The DNA samples were genotyped using The Illumina 1M-Duo array.[14] Imputation was performed with MACH using as reference the 1000Genomes haplotypes (release June 2010). Imputation quality judged by $r^2$ values exceeded 0.86 for the imputed SNPs used in the analysis. However, only the measured SNPs were used in the replication analysis.

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**SUPPLEMENTARY TABLES**

**Table S1. Single nucleotide polymorphisms associated with blood pressure response in separate genome-wide association analyses of hydrochlorothiazide-treated European Americans from the PEAR study (N=228) and the GERA study (N=196)**

| Study | BP response | SNP | Chr | Alleles | | Allele Freq | β | SE | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| PEAR | Diastolic | rs17048656 | 2 | T | G | 0.85 | -2.86 | 0.62 | 4.3E-06 |
| PEAR | Diastolic | rs6439260 | 3 | C | T | 0.64 | -1.98 | 0.44 | 8.69E-06 |
| PEAR | Diastolic | rs221903 | 14 | T | C | 0.38 | 1.96 | 0.44 | 9.33E-06 |
| PEAR | Diastolic | rs1876529 | 14 | A | G | 0.13 | 3.02 | 0.63 | 1.76E-06 |
| PEAR | Diastolic | rs12894586 | 14 | C | G | 0.26 | 2.20 | 0.49 | 6.65E-06 |
| PEAR | Diastolic | rs2776546 | 14 | C | A | 0.13 | 3.14 | 0.63 | 7.14E-07 |
| PEAR | Diastolic | rs12385949 | 15 | G | A | 0.95 | 4.12 | 0.93 | 9.79E-06 |
| PEAR | Diastolic | rs16977265 | 15 | T | C | 0.93 | 3.81 | 0.82 | 3.95E-06 |
| PEAR | Diastolic | rs8031593 | 15 | T | A | 0.93 | 3.81 | 0.83 | 4E-06 |
| PEAR | Diastolic | rs9319902 | 18 | T | C | 0.96 | 4.96 | 1.05 | 2.5E-06 |
| PEAR | Diastolic | rs4815273 | 20 | T | C | 0.46 | -2.13 | 0.42 | 3.53E-07 |
| PEAR | Diastolic | rs6083536 | 20 | C | T | 0.46 | -2.13 | 0.42 | 3.53E-07 |
| PEAR | Diastolic | rs6083538 | 20 | T | C | 0.44 | -2.12 | 0.42 | 5.44E-07 |

| Study | BP response | SNP | Chr | Alleles | | Allele Freq | β | SE | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| PEAR | Systolic | rs11556868 | 1 | C | T | 0.90 | 4.75 | 1.02 | 3.09E-06 |
| PEAR | Systolic | rs1323123 | 1 | C | T | 0.07 | 5.83 | 1.27 | 4.74E-06 |
| PEAR | Systolic | rs16848979 | 1 | T | C | 0.07 | 5.77 | 1.27 | 5.59E-06 |
| PEAR | Systolic | rs10913063 | 1 | A | G | 0.07 | 5.68 | 1.26 | 6.95E-06 |
| PEAR | Systolic | rs6750487 | 2 | G | A | 0.87 | 4.35 | 0.95 | 5.07E-06 |
| PEAR | Systolic | rs4385227 | 5 | G | T | 0.79 | 3.73 | 0.78 | 1.51E-06 |
| PEAR | Systolic | rs12004422 | 9 | T | C | 0.56 | -3.10 | 0.64 | 1.41E-06 |
| PEAR | Systolic | rs689871 | 9 | G | A | 0.56 | -3.14 | 0.64 | 8.88E-07 |
| PEAR | Systolic | rs643815 | 9 | C | T | 0.56 | -3.14 | 0.64 | 8.83E-07 |
| PEAR | Systolic | rs680754 | 9 | C | T | 0.56 | -3.13 | 0.64 | 8.74E-07 |
| PEAR | Systolic | rs583716 | 9 | C | T | 0.56 | -3.13 | 0.64 | 8.75E-07 |
| PEAR | Systolic | rs6479036 | 9 | G | A | 0.56 | -3.13 | 0.64 | 8.75E-07 |
| PEAR | Systolic | rs601916 | 9 | G | A | 0.56 | -3.13 | 0.64 | 8.75E-07 |
| PEAR | Systolic | rs689979 | 9 | C | T | 0.56 | -3.14 | 0.64 | 8.56E-07 |
| PEAR | Systolic | rs10760692 | 9 | C | T | 0.56 | -3.22 | 0.63 | 3.87E-07 |

| Study | BP response | SNP | Chr | Alleles | | Allele Freq | β | SE | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| PEAR | Systolic | rs690484 | 9 | A | G | 0.56 | -3.25 | 0.63 | 2.55E-07 |
| PEAR | Systolic | rs690455 | 9 | T | C | 0.56 | -3.27 | 0.63 | 2.25E-07 |
| PEAR | Systolic | rs7024710 | 9 | C | T | 0.56 | -3.28 | 0.63 | 2.06E-07 |
| PEAR | Systolic | rs913408 | 9 | C | T | 0.56 | -3.34 | 0.63 | 1.31E-07 |
| PEAR | Systolic | rs238 | 9 | G | A | 0.55 | -3.36 | 0.63 | 8.89E-08 |
| PEAR | Systolic | rs11606101 | 11 | T | C | 0.77 | 3.28 | 0.72 | 5.13E-06 |
| PEAR | Systolic | rs4753176 | 11 | T | G | 0.40 | -2.82 | 0.63 | 8.07E-06 |
| PEAR | Systolic | rs12148640 | 15 | A | G | 0.92 | 5.07 | 1.13 | 7.78E-06 |
| PEAR | Systolic | rs16977265 | 15 | T | C | 0.93 | 5.38 | 1.21 | 8.21E-06 |
| PEAR | Systolic | rs7180274 | 15 | G | C | 0.92 | 5.07 | 1.13 | 7.76E-06 |
| PEAR | Systolic | rs8031593 | 15 | T | A | 0.93 | 5.38 | 1.21 | 8.2E-06 |
| PEAR | Systolic | rs9319902 | 18 | T | C | 0.96 | 7.03 | 1.54 | 4.95E-06 |
| PEAR | Systolic | rs4815273 | 20 | T | C | 0.46 | -3.24 | 0.61 | 8.76E-08 |
| PEAR | Systolic | rs6083536 | 20 | C | T | 0.46 | -3.24 | 0.60 | 8.81E-08 |
| PEAR | Systolic | rs6083538 | 20 | T | C | 0.44 | -3.28 | 0.61 | 8.29E-08 |

| Study | BP response | SNP | Chr | Alleles | | Allele Freq | β | SE | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| PEAR | Systolic | rs2273359 | 20 | C | G | 0.97 | 7.99 | 1.79 | 7.82E-06 |
| GERA | Diastolic | rs41505547 | 3 | C | T | 0.91 | -7.21 | 1.55 | 3.30E-06 |
| GERA | Diastolic | rs2936970 | 5 | G | A | 0.78 | -4.98 | 1.06 | 2.50E-06 |
| GERA | Diastolic | rs6933781 | 6 | T | C | 0.66 | -3.99 | 0.88 | 6.40E-06 |
| GERA | Diastolic | rs10957895 | 8 | G | A | 0.06 | 13.54 | 2.87 | 2.38E-06 |
| GERA | Diastolic | rs11779540 | 8 | G | T | 0.66 | -4.45 | 0.98 | 5.46E-06 |
| GERA | Diastolic | rs17245685 | 11 | T | C | 0.96 | -10.42 | 2.29 | 5.58E-06 |
| GERA | Diastolic | rs1958552 | 14 | A | C | 0.84 | -5.17 | 1.14 | 6.07E-06 |
| GERA | Diastolic | rs4981200 | 14 | G | A | 0.84 | -5.15 | 1.14 | 6.33E-06 |
| GERA | Diastolic | rs17708453 | 17 | T | C | 0.95 | -8.25 | 1.79 | 4.25E-06 |
| GERA | Diastolic | rs17638474 | 19 | A | G | 0.93 | -7.24 | 1.62 | 8.10E-06 |
| GERA | Systolic | rs7641321 | 3 | G | T | 0.96 | -13.47 | 2.78 | 1.31E-06 |
| GERA | Systolic | rs41505547 | 3 | C | T | 0.91 | -11.55 | 2.11 | 4.07E-08 |
| GERA | Systolic | rs300550 | 4 | A | C | 0.88 | -11.01 | 2.38 | 3.89E-06 |
| GERA | Systolic | rs300556 | 4 | C | T | 0.87 | -9.43 | 2.00 | 2.49E-06 |

| Study | BP response | SNP | Chr | Alleles | | Allele Freq | β | SE | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| GERA | Systolic | rs300570 | 4 | G | A | 0.86 | -9.33 | 2.01 | 3.32E-06 |
| GERA | Systolic | rs17010902 | 4 | A | G | 0.93 | -11.29 | 2.30 | 9.42E-07 |
| GERA | Systolic | rs1381339 | 8 | A | G | 0.85 | -7.74 | 1.68 | 4.30E-06 |
| GERA | Systolic | rs1983124 | 11 | G | A | 0.79 | -6.95 | 1.51 | 4.10E-06 |
| GERA | Systolic | rs17090322 | 13 | T | C | 0.97 | -15.96 | 3.42 | 3.09E-06 |
| GERA | Systolic | rs9543429 | 13 | C | T | 0.95 | -17.06 | 3.51 | 1.21E-06 |
| GERA | Systolic | rs9939391 | 16 | T | C | 0.93 | -10.12 | 2.29 | 9.92E-06 |
| GERA | Systolic | rs4148413 | 17 | C | G | 0.80 | 7.58 | 1.58 | 1.61E-06 |

PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; GERA, Genetic Epidemiology of Responses to Antihypertensives; BP, blood pressure; alleles: coded allele shown to the left of the non-coded allele is the modeled allele as in the example of A/G SNP in which AA=0, AG=1 and GG=2, where G is the coded and A the non-coded allele; allele freq, frequency of the coded allele; β, model regression coefficient, mmHg per coded allele; SE, standard error of the regression coefficient.

**Table S2. Single nucleotide polymorphisms associated with blood pressure response in meta-analysis of the genome-wide association analyses of hydrochlorothiazide-treated European Americans from the PEAR study (N=228) and the GERA study (N=196)**

| BP Response | SNP | Chr | Alleles | | Meta-analysis of PEAR+GERA study GWA analyses Allele Freq | β | *p*-value | PEAR study GWA analysis *p*-value | GERA study GWA analysis *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| Diastolic | rs2432742 | 8 | A | G | 0.86 | -2.52 | 7.03E-06 | 9.10E-05 | 2.69E-02 |
| Diastolic | rs221903 | 14 | T | C | 0.37 | 1.77 | 6.98E-06 | 9.33E-06 | 2.30E-01 |
| Diastolic | rs12894586 | 14 | C | G | 0.26 | 2.00 | 9.28E-06 | 6.65E-06 | 4.84E-01 |
| Diastolic | rs2776546 | 14 | A | C | 0.87 | -3.24 | 4.9E-08 | 7.14E-07 | 1.98E-02 |
| Diastolic | rs9933692 | 16 | A | G | 0.22 | -2.09 | 6.93E-06 | 2.44E-05 | 1.15E-01 |
| Diastolic | rs4074471 | 16 | T | G | 0.78 | 2.09 | 6.92E-06 | 2.44E-05 | 1.15E-01 |
| Diastolic | rs4791040 | 17 | T | C | 0.96 | 4.46 | 1.36E-06 | 1.31E-04 | 2.06E-03 |
| Diastolic | rs4791037 | 17 | A | G | 0.96 | 4.46 | 1.37E-06 | 1.31E-04 | 2.08E-03 |
| Diastolic | rs16960228 | 17 | A | G | 0.04 | -4.46 | 1.37E-06 | 1.31E-04 | 2.08E-03 |
| Diastolic | rs7216764 | 17 | A | G | 0.04 | -6.20 | 6.89E-07 | 1.23E-04 | 1.37E-03 |
| Diastolic | rs7247267 | 19 | T | G | 0.25 | 2.81 | 7.05E-06 | 4.49E-05 | 5.92E-02 |
| Diastolic | rs4815273 | 20 | T | C | 0.46 | -1.93 | 1.96E-07 | 3.53E-07 | 1.38E-01 |
| Diastolic | rs6083538 | 20 | T | C | 0.45 | -1.90 | 4.6E-07 | 5.44E-07 | 2.03E-01 |

| BP Response | SNP | Chr | Alleles | | Meta-analysis of PEAR+GERA study GWA analyses | | | PEAR study GWA analysis | GERA study GWA analysis |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Allele Freq | β | *p*-value | *p*-value | *p*-value |
| Systolic | rs2306667 | 3 | T | C | 0.83 | 3.64 | 4.88E-06 | 3.373E-03 | 1.723E-05 |
| Systolic | rs17010902 | 4 | A | G | 0.93 | -7.35 | 3.12E-06 | 6.893E-02 | 9.424E-07 |
| Systolic | rs4376293 | 5 | T | C | 0.49 | -2.50 | 5.01E-06 | 3.170E-05 | 5.831E-02 |
| Systolic | rs11763492 | 7 | A | G | 0.22 | -2.89 | 9.35E-06 | 1.873E-04 | 1.495E-02 |
| Systolic | rs13223171 | 7 | T | C | 0.22 | -2.89 | 9.44E-06 | 1.876E-04 | 1.510E-02 |
| Systolic | rs12004422 | 9 | T | C | 0.55 | -2.68 | 2.36E-06 | 1.413E-06 | 3.198E-01 |
| Systolic | rs689871 | 9 | A | G | 0.45 | 2.72 | 1.43E-06 | 8.884E-07 | 3.060E-01 |
| Systolic | rs643815 | 9 | T | C | 0.45 | 2.72 | 1.44E-06 | 8.830E-07 | 3.079E-01 |
| Systolic | rs680754 | 9 | T | C | 0.45 | 2.68 | 2.06E-06 | 8.744E-07 | 3.938E-01 |
| Systolic | rs583716 | 9 | T | C | 0.45 | 2.68 | 2.05E-06 | 8.748E-07 | 3.937E-01 |
| Systolic | rs6479036 | 9 | A | G | 0.45 | 2.68 | 2.06E-06 | 8.748E-07 | 3.938E-01 |
| Systolic | rs601916 | 9 | A | G | 0.45 | 2.68 | 2.06E-06 | 8.748E-07 | 3.938E-01 |
| Systolic | rs689979 | 9 | T | C | 0.45 | 2.68 | 2.02E-06 | 8.562E-07 | 3.939E-01 |
| Systolic | rs10760692 | 9 | T | C | 0.45 | 2.75 | 1.01E-06 | 3.865E-07 | 3.939E-01 |

| BP Response | SNP | Chr | Alleles | | Meta-analysis of PEAR+GERA study GWA analyses | | | PEAR study GWA analysis | GERA study GWA analysis |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Allele Freq | β | *p*-value | *p*-value | *p*-value |
| Systolic | rs690484 | 9 | A | G | 0.55 | -2.90 | 1.95E-07 | 2.555E-07 | 1.632E-01 |
| Systolic | rs690455 | 9 | T | C | 0.55 | -2.92 | 1.69E-07 | 2.250E-07 | 1.589E-01 |
| Systolic | rs7024710 | 9 | T | C | 0.45 | 2.92 | 1.56E-07 | 2.058E-07 | 1.589E-01 |
| Systolic | rs913408 | 9 | T | C | 0.45 | 2.97 | 1.05E-07 | 1.314E-07 | 1.589E-01 |
| Systolic | rs238 | 9 | A | G | 0.46 | 3.11 | 2.9E-08 | 8.890E-08 | 8.532E-02 |
| Systolic | rs1556025 | 9 | T | C | 0.38 | -2.62 | 5.23E-06 | 3.470E-05 | 5.309E-02 |
| Systolic | rs4815273 | 20 | T | C | 0.46 | -2.91 | 4.5E-08 | 8.764E-08 | 1.064E-01 |
| Systolic | rs6083536 | 20 | T | C | 0.54 | 2.91 | 4.54E-08 | 8.808E-08 | 1.073E-01 |
| Systolic | rs6083538 | 20 | T | C | 0.45 | -2.91 | 6.84E-08 | 8.292E-08 | 1.575E-01 |
| Systolic | rs2273359 | 20 | C | G | 0.96 | 8.21 | 4.15E-07 | 7.820E-06 | 1.626E-02 |

PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; GERA, Genetic Epidemiology of Responses to Antihypertensives; BP, blood pressure; alleles: coded allele shown to the left of the non-coded allele is the modeled allele as in the example of A/G SNP in which AA=0, AG=1 and GG=2, where G is the coded and A the non-coded allele; allele freq, frequency of the coded allele; β, model regression coefficient, mmHg per coded allele.

**Table S3. Associations of single nucleotide polymorphisms in *WNK1* and *ADD1* with blood pressure response in meta-analysis of the hydrochlorothiazide-treated European Americans from the PEAR study (N=228) and the GERA study (N=196)**

| | | | | | Diastolic blood pressure response | | | Systolic blood pressure response | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles | | Allele Freq | β | SE | *p*-value | β | SE | *p*-value |
| *WNK1* | rs3858703 | A | G | 0.69 | -0.65 | 0.43 | 0.13 | -0.91 | 0.62 | 0.14 |
| *WNK1* | rs7972667 | A | G | 0.30 | 0.65 | 0.43 | 0.13 | 0.87 | 0.61 | 0.16 |
| *WNK1* | rs6489746 | C | G | 0.23 | 0.50 | 0.49 | 0.31 | 1.21 | 0.70 | 0.09 |
| *WNK1* | rs4980968 | A | T | 0.22 | 0.32 | 0.48 | 0.50 | 1.02 | 0.69 | 0.14 |
| *WNK1* | rs7295704 | A | T | 0.80 | -0.52 | 0.49 | 0.29 | -1.07 | 0.71 | 0.13 |
| *WNK1* | rs7976964 | A | G | 0.83 | 0.35 | 0.51 | 0.49 | 0.20 | 0.74 | 0.78 |
| *WNK1* | rs2107612 | A | G | 0.73 | -0.65 | 0.44 | 0.13 | -0.82 | 0.63 | 0.19 |
| *WNK1* | rs2107613 | T | C | 0.78 | -0.33 | 0.48 | 0.50 | -1.00 | 0.69 | 0.15 |
| *WNK1* | rs11064524 | T | G | 0.74 | 0.58 | 0.44 | 0.18 | 0.59 | 0.63 | 0.35 |
| *WNK1* | rs10774461 | A | C | 0.52 | -0.21 | 0.38 | 0.58 | -0.54 | 0.55 | 0.32 |
| *WNK1* | rs724709 | A | C | 0.75 | -0.66 | 0.45 | 0.14 | -1.29 | 0.65 | 0.05 |
| *WNK1* | rs765250 | T | C | 0.67 | -0.79 | 0.41 | 0.06 | -1.10 | 0.59 | 0.06 |
| *WNK1* | rs12314329 | A | G | 0.91 | -0.26 | 0.68 | 0.70 | 0.04 | 0.97 | 0.97 |

| | | | | | Diastolic blood pressure response | | | Systolic blood pressure response | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles | | Allele Freq | β | SE | *p*-value | β | SE | *p*-value |
| *WNK1* | rs10849559 | A | G | 0.48 | -0.45 | 0.39 | 0.24 | -0.91 | 0.56 | 0.10 |
| *WNK1* | rs10774464 | T | C | 0.50 | -0.55 | 0.38 | 0.15 | -0.92 | 0.55 | 0.09 |
| *WNK1* | rs7963376 | T | C | 0.50 | 0.55 | 0.38 | 0.15 | 0.92 | 0.55 | 0.09 |
| *WNK1* | rs6489750 | A | C | 0.19 | 0.67 | 0.50 | 0.18 | 1.54 | 0.72 | 0.03 |
| *WNK1* | rs2158502 | C | G | 0.25 | 0.68 | 0.45 | 0.13 | 1.33 | 0.64 | 0.04 |
| *WNK1* | rs10774466 | A | G | 0.25 | 0.68 | 0.45 | 0.13 | 1.33 | 0.64 | 0.04 |
| *WNK1* | rs11611246 | T | G | 0.21 | -1.03 | 0.47 | 0.03 | -1.23 | 0.67 | 0.07 |
| *WNK1* | rs2158501 | A | G | 0.50 | 0.56 | 0.38 | 0.15 | 0.93 | 0.55 | 0.09 |
| *WNK1* | rs10849568 | A | G | 0.66 | -0.88 | 0.41 | 0.03 | -1.19 | 0.59 | 0.04 |
| *WNK1* | rs7980163 | A | C | 0.75 | -0.67 | 0.45 | 0.13 | -1.33 | 0.64 | 0.04 |
| *WNK1* | rs6489755 | T | C | 0.81 | -0.67 | 0.50 | 0.18 | -1.54 | 0.72 | 0.03 |
| *WNK1* | rs6489756 | A | G | 0.50 | -0.55 | 0.38 | 0.15 | -0.92 | 0.55 | 0.09 |
| *WNK1* | rs7311423 | T | C | 0.34 | 0.88 | 0.41 | 0.03 | 1.19 | 0.59 | 0.04 |
| *WNK1* | rs2240283 | T | C | 0.25 | 0.67 | 0.45 | 0.13 | 1.34 | 0.64 | 0.04 |

| | | | | | Diastolic blood pressure response | | | Systolic blood pressure response | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles | | Allele Freq | β | SE | *p*-value | β | SE | *p*-value |
| *WNK1* | rs765891 | T | C | 0.84 | 0.38 | 0.52 | 0.46 | 0.20 | 0.75 | 0.79 |
| *WNK1* | rs2286006 | T | C | 0.84 | 0.38 | 0.52 | 0.46 | 0.20 | 0.75 | 0.79 |
| *WNK1* | rs12816718 | T | G | 0.16 | -0.38 | 0.52 | 0.46 | -0.20 | 0.75 | 0.79 |
| *WNK1* | rs7305099 | T | G | 0.40 | 0.74 | 0.40 | 0.07 | 0.79 | 0.58 | 0.17 |
| *WNK1* | rs12309274 | T | G | 0.83 | -0.01 | 0.52 | 0.99 | 0.67 | 0.75 | 0.37 |
| *WNK1* | rs16931965 | T | C | 0.16 | -0.27 | 0.54 | 0.61 | 0.06 | 0.77 | 0.94 |
| *WNK1* | rs10849573 | A | G | 0.16 | -0.21 | 0.53 | 0.70 | 0.12 | 0.77 | 0.88 |
| *WNK1* | rs11064580 | A | G | 0.58 | 0.61 | 0.39 | 0.12 | 0.90 | 0.56 | 0.11 |
| *WNK1* | rs4980973 | A | G | 0.10 | 0.19 | 0.66 | 0.78 | 0.18 | 0.95 | 0.85 |
| *WNK1* | rs1012729 | A | G | 0.74 | -0.77 | 0.44 | 0.08 | -1.36 | 0.63 | 0.03 |
| *WNK1* | rs12312603 | A | G | 0.18 | 0.01 | 0.50 | 0.98 | -0.40 | 0.72 | 0.58 |
| *WNK1* | rs880054 | T | C | 0.56 | -0.63 | 0.40 | 0.11 | -0.85 | 0.57 | 0.14 |
| *WNK1* | rs9804992 | A | G | 0.18 | -0.01 | 0.50 | 0.99 | -0.42 | 0.72 | 0.56 |
| *WNK1* | rs16928108 | A | G | 0.84 | 0.21 | 0.53 | 0.70 | -0.12 | 0.77 | 0.88 |

| | | | | | Diastolic blood pressure response | | | Systolic blood pressure response | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles | | Allele Freq | β | SE | *p*-value | β | SE | *p*-value |
| *WNK1* | rs7953912 | T | C | 0.25 | 0.66 | 0.45 | 0.14 | 1.28 | 0.64 | 0.05 |
| *WNK1* | rs7300444 | T | C | 0.41 | -0.48 | 0.39 | 0.21 | -0.85 | 0.56 | 0.13 |
| *WNK1* | rs10744727 | A | G | 0.56 | -0.63 | 0.40 | 0.11 | -0.85 | 0.57 | 0.14 |
| *WNK1* | rs12828016 | T | G | 0.41 | 0.75 | 0.40 | 0.06 | 0.84 | 0.58 | 0.14 |
| *WNK1* | rs2255390 | A | G | 0.41 | -0.53 | 0.39 | 0.18 | -0.96 | 0.57 | 0.09 |
| *WNK1* | rs2301880 | T | C | 0.25 | 0.68 | 0.45 | 0.13 | 1.30 | 0.64 | 0.04 |
| *WNK1* | rs7972490 | A | G | 0.25 | 0.68 | 0.45 | 0.13 | 1.30 | 0.64 | 0.04 |
| *WNK1* | rs10849582 | A | G | 0.56 | -0.63 | 0.40 | 0.11 | -0.85 | 0.57 | 0.14 |
| *WNK1* | rs2286028 | C | G | 0.19 | -1.10 | 0.48 | 0.02 | -1.24 | 0.70 | 0.07 |
| *WNK1* | rs2286029 | T | G | 0.59 | 0.43 | 0.39 | 0.28 | 0.83 | 0.57 | 0.14 |
| *WNK1* | rs1060499 | T | C | 0.84 | 0.42 | 0.54 | 0.43 | 0.09 | 0.77 | 0.90 |
| | | | | | | | | | | |
| *ADD1* | rs1877723 | T | C | 0.33 | -0.43 | 0.43 | 0.31 | -0.13 | 0.62 | 0.84 |
| *ADD1* | rs16843452 | T | C | 0.19 | 0.60 | 0.50 | 0.23 | 0.48 | 0.72 | 0.51 |

| | | | | | Diastolic blood pressure response | | | Systolic blood pressure response | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles | | Allele Freq | β | SE | *p*-value | β | SE | *p*-value |
| *ADD1* | rs12503220 | A | G | 0.17 | -0.40 | 0.54 | 0.46 | -0.27 | 0.79 | 0.73 |
| *ADD1* | rs6600769 | A | T | 0.30 | -0.47 | 0.43 | 0.28 | -0.16 | 0.62 | 0.79 |
| *ADD1* | rs12509447 | A | G | 0.16 | -0.39 | 0.56 | 0.49 | -0.19 | 0.81 | 0.82 |
| *ADD1* | rs10026792 | A | G | 0.32 | -0.47 | 0.43 | 0.27 | -0.19 | 0.62 | 0.75 |
| *ADD1* | rs17833250 | C | G | 0.30 | -0.52 | 0.43 | 0.23 | -0.23 | 0.62 | 0.71 |
| *ADD1* | rs16843511 | T | C | 0.03 | 2.33 | 2.77 | 0.40 | 3.55 | 3.84 | 0.36 |
| *ADD1* | rs4690001 | T | C | 0.21 | 0.54 | 0.48 | 0.26 | 0.46 | 0.69 | 0.51 |
| *ADD1* | rs16843523 | T | C | 0.20 | 0.59 | 0.48 | 0.22 | 0.51 | 0.69 | 0.46 |
| *ADD1* | rs2097081 | A | G | 0.19 | 0.58 | 0.49 | 0.24 | 0.46 | 0.71 | 0.52 |
| *ADD1* | rs624833 | T | G | 0.68 | 0.39 | 0.42 | 0.36 | 0.11 | 0.61 | 0.86 |
| *ADD1* | rs1242228 | T | C | 0.49 | -0.05 | 0.39 | 0.90 | 0.08 | 0.57 | 0.88 |
| *ADD1* | rs6824567 | T | C | 0.30 | -0.47 | 0.43 | 0.27 | -0.20 | 0.62 | 0.75 |
| *ADD1* | rs3775068 | A | G | 0.41 | -0.23 | 0.41 | 0.57 | -0.48 | 0.59 | 0.41 |
| *ADD1* | rs3775067 | A | G | 0.36 | -0.19 | 0.42 | 0.65 | -0.51 | 0.61 | 0.40 |

| | | | | | Diastolic blood pressure response | | | Systolic blood pressure response | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Gene | SNP | Alleles | | Allele Freq | β | SE | *p*-value | β | SE | *p*-value |
| *ADD1* | rs2071695 | A | G | 0.80 | -0.53 | 0.49 | 0.28 | -0.40 | 0.71 | 0.57 |

*WNK1*, WNK lysine deficient protein kinase 1; *ADD1*, adducin 1; PEAR, Pharmacogenomic Evaluation of Antihypertensive Responses; GERA, Genetic Epidemiology of Responses to Antihypertensives; BP, blood pressure; alleles: coded allele shown to the left of the non-coded allele is the modeled allele as in the example of A/G SNP in which AA=0, AG=1 and GG=2, where G is the coded and A the non-coded allele; allele freq, frequency of the coded allele; β, model regression coefficient, mmHg per coded allele; SE, standard error of the regression coefficient.

**Table S4. Description of hydrochlorothiazide-treated European samples for replication analyses**

| Descriptive characteristic | NORDIL study N=420 | GENRES Study N=206 | Milan Study N=215 |
|---|---|---|---|
| Women, N (%) | 265 (63) | 0 (0) | 33 (15) |
| Age, years | 61.2 ±6.7 | 50.5 ±6.4 | 45.9 ±8.2 |
| BMI, $kg \cdot m^{-2}$ | 28.4 ±4.7 | 26.7 ±2.8 | 26.1 ±3.0 |
| Pretreatment systolic BP, mmHg | 170.8 ±18.2 | 151.3 ±12.7 | 148.4 ±13.2 |
| Pretreatment diastolic BP, mmHg | 102.3 ±6.6 | 99.4 ±6.7 | 98.0 ±8.4 |
| Systolic BP response, mmHg | -22.8 ±21.8 | -4.5 ±11.1 | -10.6 ±12.4 |
| Diastolic BP response, mmHg | -15.2 ± 11.6 | -2.5 ±6.5 | -6.3 ±8.7 |

NORDIL, Nordic Diltiazem; GENRES, Genetics of Drug Responsiveness in Essential Hypertension study; BMI, body mass index; BP, blood pressure; NA, not available. BP response was defined as final minus baseline value (negative sign indicates BP decline in response to drug and was adjusted for pretreatment BP level, age, gender. In the GENRES Study pretreatment BP is based on the mean of four placebo treatment periods.

**SUPPLEMENTARY FIGURES**

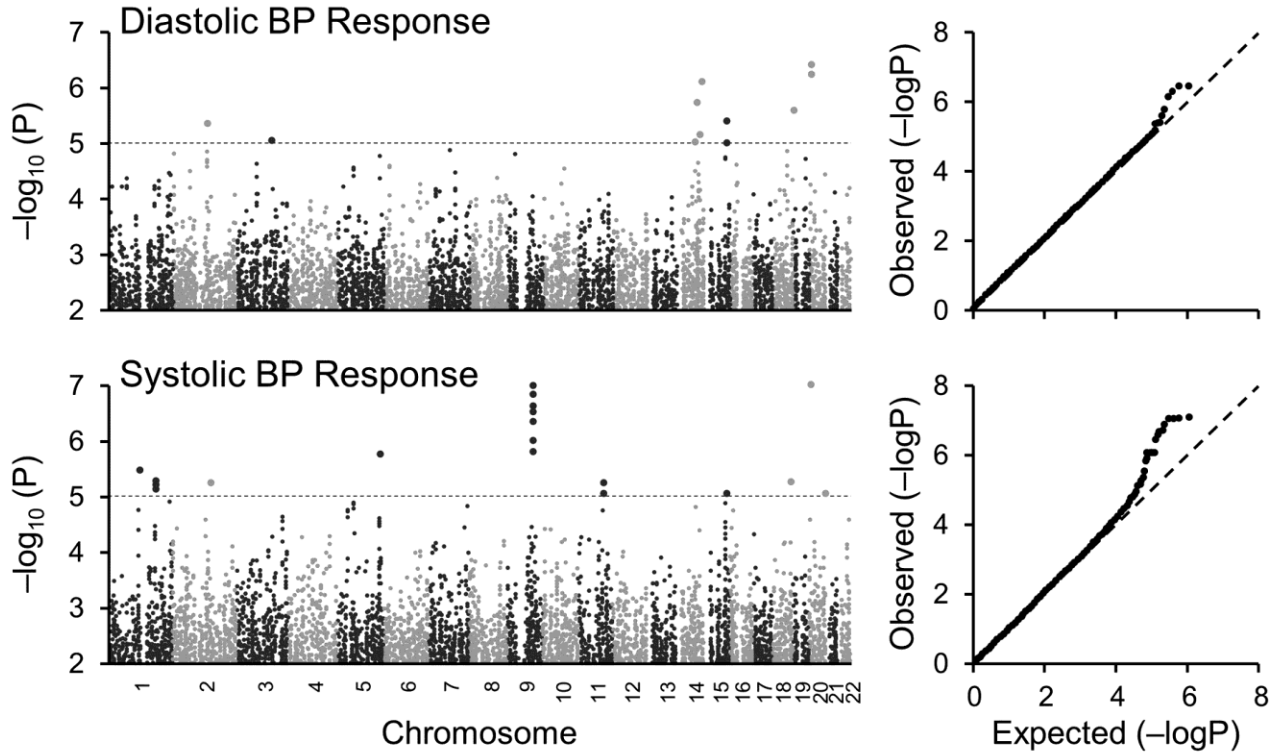


**Figure S1**. Manhattan plots and quantile-quantile plots from genome-wide association analysis for blood pressure response to hydrochlorothiazide in European American PEAR study participants.

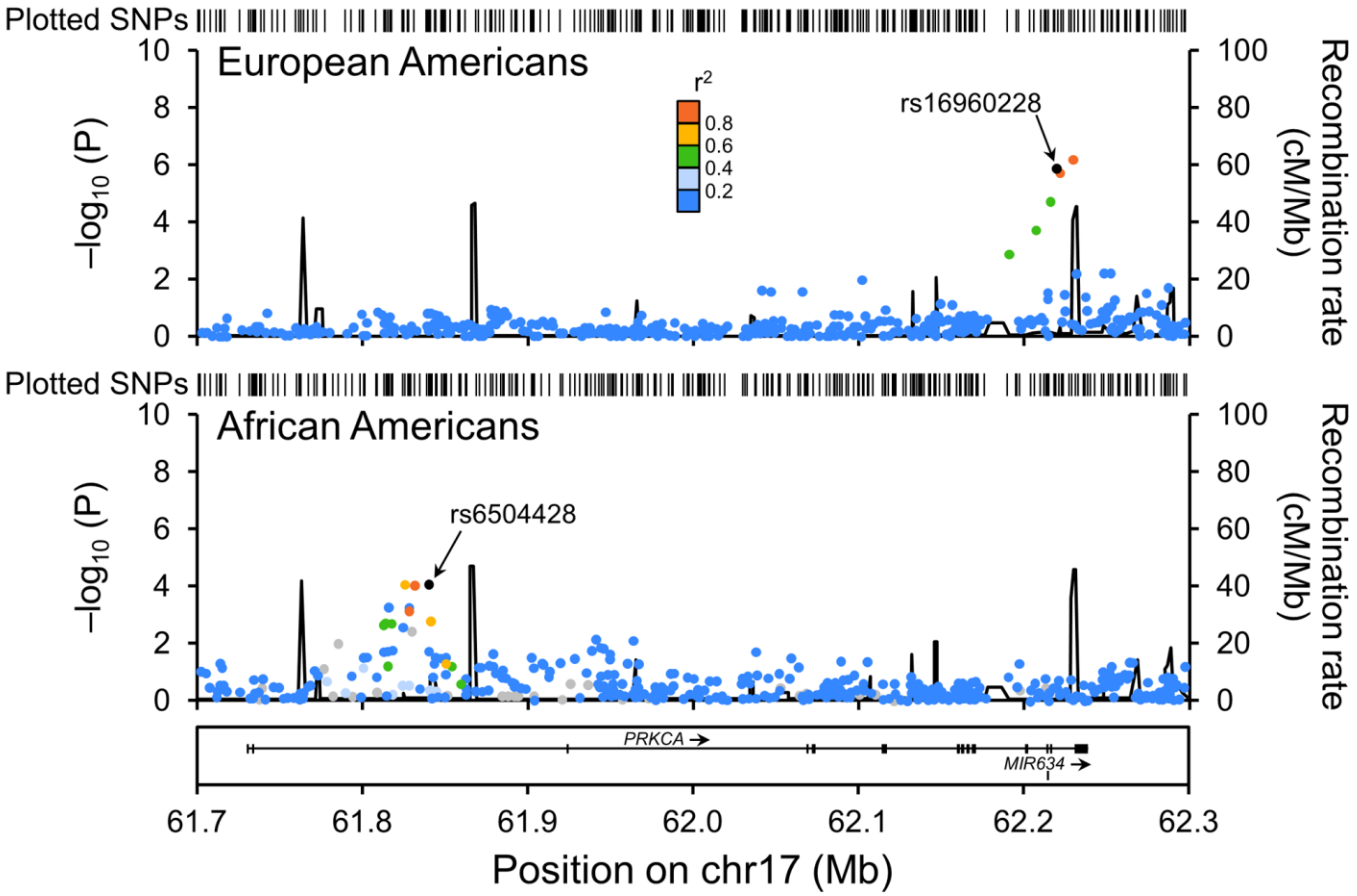


**Figure S2**. Regional plot of chromosome 17q24.3 region of protein kinase C, alpha (*PRKCA*) showing significance of the associations of single nucleotide polymorphisms with diastolic blood pressure response to hydrochlorothiazide in the PEAR Study European Americans (upper panel) and African Americans (lower panel). In both races, the most strongly associated single nucleotide polymorphisms are within *PRKCA*.

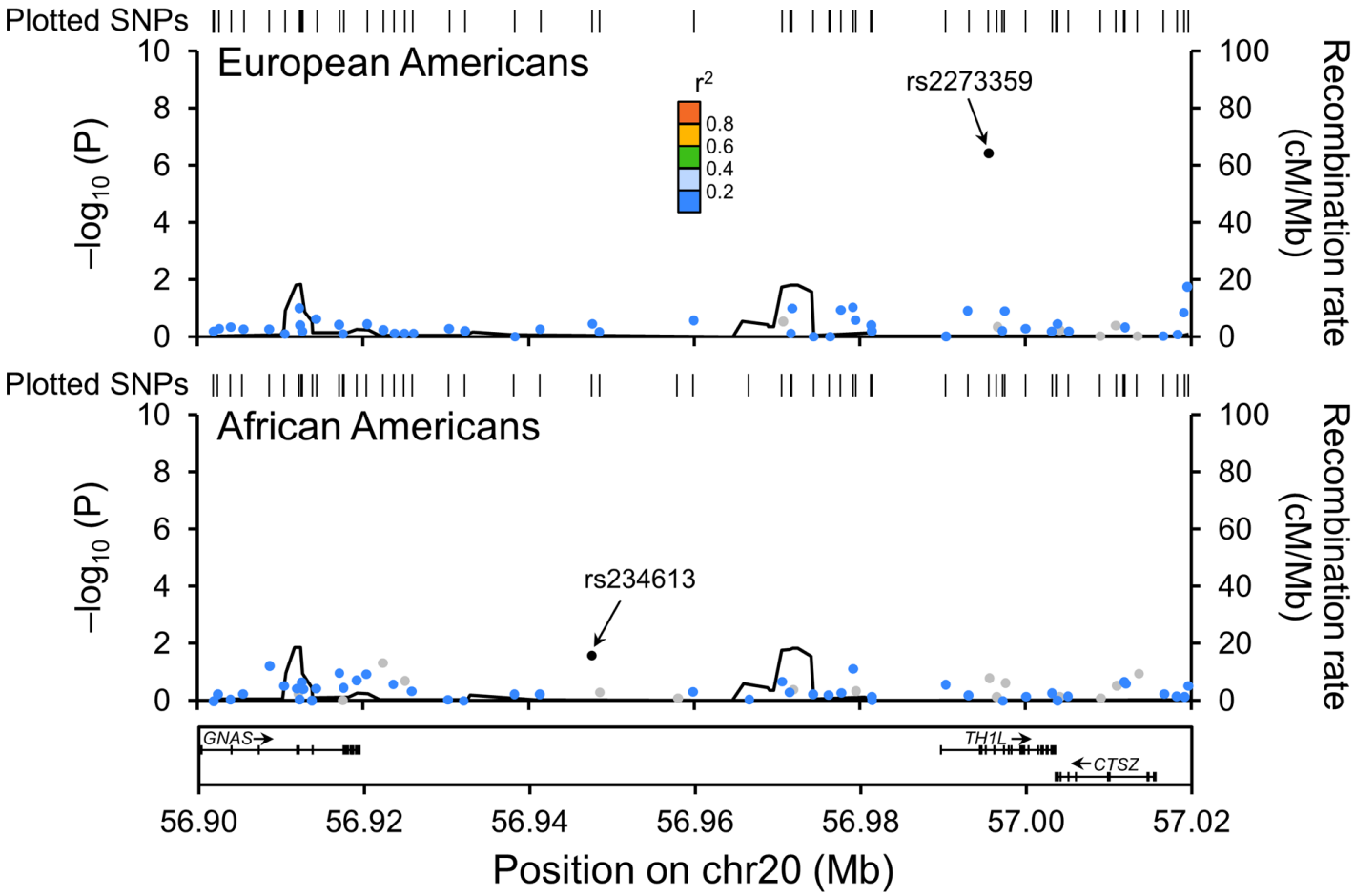


**Figure S3**. Regional plot of chromosome 20q13.32 region of *TH1L* and *GNAS* showing significance of the associations of single nucleotide polymorphisms with systolic blood pressure response to hydrochlorothiazide in the PEAR Study European Americans (upper panel) and African Americans (lower panel).

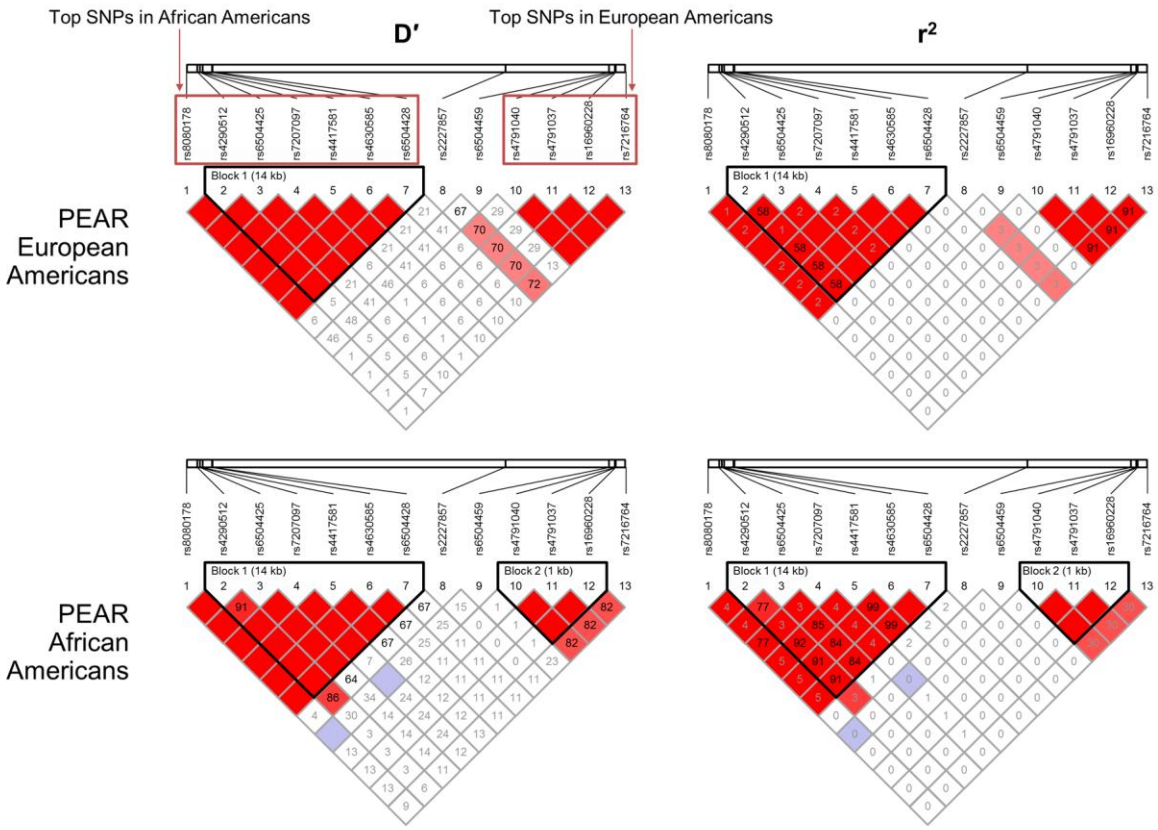


**Figure S4**. Linkage disequilibrium as measured by D' and $r^2$ in PEAR European and African American study participants between SNPs at the chromosome17q22-q23.2 locus associated with diastolic BP response to HCT in European Americans and the 17q24 locus associated with diastolic BP response to HCT in African Americans. Shown also are two coding SNPs, rs2227857 (synonymous) and rs6504459 (missense, ILE568VAL).

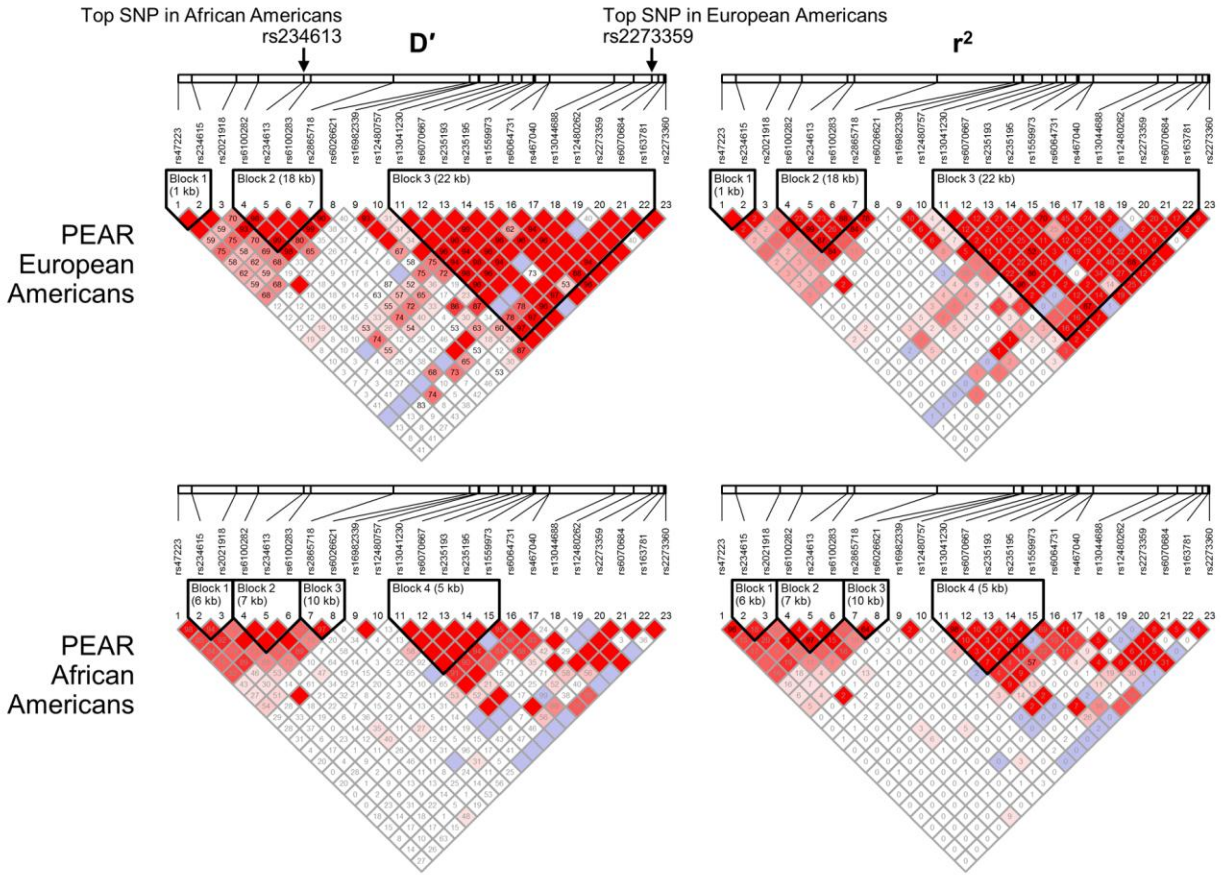


**Figure S5**. Linkage disequilibrium as measured by D' and r2 in PEAR European and African American study participants between SNPs at the chromosome 20q13.32 loci associated with systolic BP response to HCT in European and African Americans. The chromosome 20q13.32 SNP rs2273359 that is associated with systolic BP response in European Americans is in the gene encoding TH1-like (TH1L) between GNAS and EDN3. A SNP in the chromosome 20q13.32 region between TH1L and GNAS1, rs234613, was most significantly associated with systolic BP response to HCT in African Americans.