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Association of *KCNJ1* variation with change in fasting glucose and new onset diabetes during HCTZ treatment

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Abstract

Thiazide-induced potassium loss may contribute to new onset diabetes (NOD). *KCNJ1* encodes a potassium channel and one study observed that a *KCNJ1* single-nucleotide polymorphism (SNP) was associated with changes in fasting glucose (FG) during hydrochlorothiazide (HCTZ) treatment. We used linear regression to test association of *KCNJ1* SNPs and haplotypes with FG changes during HCTZ treatment in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. We used logistic regression to test association of *KCNJ1* variation with NOD in HCTZ-treated patients from the International Verapamil SR Trandolapril Study (INVEST). Multivariate regression analyses were performed by race/ethnicity with false discovery rate (FDR) correction. In PEAR blacks, a *KCNJ1* SNP was associated with increased FG during HCTZ treatment (beta = 8.47, P_{FDR} = 0.009). KCNJ1 SNPs and haplotypes were associated with NOD risk in all INVEST race/ethnic groups (strongest association: odds ratio 2.14 (1.31–3.53), P_{FDR} = 0.03). Our findings support that *KCNJ1* variation is associated with HCTZ-induced dysglycemia and NOD.

Keywords

pharmacogenetics; *KCNJ1*; ROMK1; hypertension; diabetes mellitus; hydrochlorothiazide

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CONFLICT OF INTEREST

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INTRODUCTION

Hypertension and type 2 diabetes are major causes of morbidity and mortality.[1] Hypertension is an independent risk factor for type 2 diabetes and a patient with both diabetes and hypertension is at an increased risk of an adverse cardiovascular (CV) outcome versus a patient with hypertension alone.[2,3] Thiazide diuretics are considered first-line agents for the treatment of hypertension, but thiazides are also associated with glucose elevation and new onset diabetes (NOD).[4–6] Because thiazides contribute to NOD development, their benefit in a hypertensive patient, including reduction in CV risk, may be offset by this adverse metabolic effect.

The importance of identifying predictors of thiazide-induced dysglycemia was emphasized by a working group from the National Heart Lung and Blood Institute.[5] *A priori* identification of patients who will develop dysglycemia during thiazide treatment could guide thiazide prescribing to reduce the risk of NOD. Inter-individual variability in thiazide-induced dysglycemia and strong genetic associations with type 2 diabetes suggest that drug–gene interactions influence thiazide-induced dysglycemia.[5,7] Despite the potential utility of personalized medicine in avoiding adverse metabolic effects of thiazides, few studies have investigated pharmacogenetics of thiazide-induced dysglycemia.[8–10]

Although thiazides raise fasting glucose (FG) levels, the mechanisms of thiazide-induced dysglycemia are not fully understood. Thiazide diuretics such as hydrochlorothiazide (HCTZ) may increase FG through increased potassium excretion, which can impair insulin release and decrease glucose uptake into skeletal muscle.[11,12] Supporting the role of potassium depletion in thiazide-induced dysglycemia, the non-synonymous single-nucleotide polymorphism (SNP) rs59172778 in the potassium inwardly rectifying channel, subfamily J, member 1 gene (*KCNJ1*) has been associated with decreased FG during HCTZ treatment.[9]

We investigated the association of *KCNJ1* SNPs and haplotypes with change in FG during short-term HCTZ treatment in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. PEAR compared adverse metabolic effects in hypertensive patients treated with atenolol and HCTZ. We also investigated the association of *KCNJ1* SNPs and haplotypes with NOD during long-term HCTZ treatment in the International Verapamil SR Trandolapril Study (INVEST). INVEST compared CV outcomes and NOD in hypertensive coronary artery disease patients treated with two antihypertensive treatment strategies.

MATERIALS AND METHODS

All patients enrolled in PEAR and INVEST provided written informed consent, and the institutional review boards of participating study centers approved the study protocols.

PEAR study design and population

PEAR is a prospective, randomized, parallel group study to evaluate the pharmacogenomic effects of HCTZ, atenolol and their combination on blood pressure (BP) response and adverse metabolic effects. Details of the PEAR study design have been previously published.[13] PEAR patients, aged 17–65 years, had mild-to-moderate essential hypertension without a history of heart disease or diabetes. After a 3- to 8-week washout period, patients were randomized to receive HCTZ 12.5 mg or atenolol 50 mg daily followed by dose titration to HCTZ 25 mg or atenolol 100 mg daily for 6–9 weeks (Figure 1). The other agent was then added, with similar dose titration for 6–9 weeks of combination treatment. BP, FG, serum potassium, urinary potassium and plasma insulin were acquired at baseline, after

monotherapy, and after subsequent combination therapy. We investigated the impact of 25 *KCNJ1* SNPs on change in FG during HCTZ treatment, combining data for FG changes during both HCTZ monotherapy (HCTZ arm) and HCTZ add-on therapy (atenolol arm) (Figure 1). Change in FG during HCTZ monotherapy was defined as the difference in FG from the start of HCTZ monotherapy to the end of HCTZ monotherapy. Change in FG during HCTZ add-on therapy was defined as the difference in FG from the start of HCTZ to the end of the trial (Figure 1). In addition, similar analyses were performed in PEAR atenolol-treated patients to assess specificity of *KCNJ1* associations with HCTZ.

INVEST study design and population

INVEST evaluated adverse CV outcomes and NOD occurring during randomized treatment with either an atenolol-based or a verapamil sustained release (SR)-based antihypertensive strategy in patients with hypertension and coronary artery disease who were ≥50 years of age. The design, primary outcome and NOD results have been previously published in detail.[14–17] Briefly, the verapamil SR strategy consisted of stepped therapy with verapamil SR, trandolapril add-on, dose titration, then HCTZ add-on treatment for BP control and end organ protection as necessary. The atenolol-based strategy consisted of atenolol, HCTZ add-on, dose titration, then trandolapril add-on treatment as necessary.

INVEST-GENES collected DNA samples from 5979 INVEST patients at 187 sites in the United States and Puerto Rico, who provided additional written informed consent for genetic studies. We conducted a nested case–control study including those who developed NOD during follow-up (cases) and age, race and gender-matched participants who remained diabetes-free over a mean 2.8 years follow-up (controls). NOD was determined by site investigators from a review of all available patient data, as well as use of diabetic medication.[16] Patients taking anti-diabetic medication or with diabetes history at baseline were excluded from this analysis.

Genotyping and quality control

In both cohorts, genotyping for *KCNJ1* tag SNPs was accomplished using the HumanCVD BeadChip (Illumina, San Diego, CA, USA). The HumanCVD BeadChip contains approximately 50 000 cosmopolitan tag SNPs for 2100 CV and metabolic-related genes.[18] Twenty-four *KCNJ1* tag SNPs were present on the HumanCVD BeadChip, selected for r^2>0.5 and minor allele frequency (MAF) >0.05. Patients were excluded if sample genotype call rates were below 95% and SNPs were excluded if genotype call rates were below 90%. Genotype data quality was further ensured in PLINK using concordance rates for blind duplicates, gender confirmation using X-chromosome genotype data, cryptic relatedness using pairwise identity-by-descent and estimation of heterozygosity using the inbreeding coefficient F.[19] Genotyping for rs59172778 (hcv632615) was accomplished using the TaqMan 7900HT real-time PCR system (Applied Biosystem, Carlsbad, CA, USA).

Definition and treatment of race/ethnicity

PEAR race/ethnicity was self-described by study patient and confirmed by principal components analysis (PCA) performed using linkage disequilibrium (LD)-pruned data from the HumanOmni1-Quad BeadChip (Illumina). In PEAR, any patient that did not report black ancestry during screening was considered non-black in statistical analyses. INVEST race/ethnic groups were determined by patient report with interaction by the study investigator and confirmed through PCA with LD-pruned HumanCVD BeadChip data.[20] To minimize confounding by population stratification, pharmacogenetic analyses were performed by race/ethnicity and adjusted for principal components (PCs) one, two and three. PCs were generated separately for each race/ethnic group. PCs one, two and three were used in both PEAR and INVEST, as three PCs provided adequate separation of race/ethnic groups, fewer

PCs did not provide adequate separation of race/ethnic groups and additional PCs provided no further precision. PCA was performed using JMP Genomics version 5.0 (SAS, Cary, NC, USA) in which the EIGENSTRAT method was implemented.[21]

Statistical analysis

All statistical analyses were performed using SAS version 9.2 and JMP Genomics version 5.0 (SAS). Significance was determined using an false discovery rate (FDR) correction for all SNPs and haplotypes within each race/ethnic group. Deviations from Hardy–Weinberg Equilibrium were assessed using Fisher's Exact Test by race/ethnicity with alpha = 0.05.

PEAR—Differences in change in FG and serum potassium during HCTZ monotherapy versus HCTZ add-on therapy were tested using Wilcoxon Rank Sum. Laboratory values were tested for normality using Kolmogorov–Smirnov and variables were log-transformed if non-normal. Linear regression was used to model *KCNJ1* SNP and haplotype effects on change in log(FG) during HCTZ using allelic trend tests in an additive model. *P*-values were determined using log-transformed data whereas betas were calculated using non-transformed data to provide clinically interpretable genotype effects on change in FG. Variables for adjustment were selected for potential impact on FG and included log(FG) at start of HCTZ, age, gender, waist circumference, potassium supplementation during the study, drug arm, average home systolic and diastolic BP, HCTZ dose, duration of HCTZ treatment and PCs one, two and three. In secondary analyses, linear regressions for change in FG were also performed for significant SNPs with additional adjustment for baseline and change in serum potassium, urinary potassium and plasma insulin. *KCNJ1* SNP effects on change in serum potassium during HCTZ treatment were also tested.

INVEST—Baseline differences in patient characteristics between cases and controls were determined using *t*-tests, Wilcoxon Rank Sum and chi-square tests as appropriate. Multivariable logistic regression models were used to assess *KCNJ1* SNP effects on NOD risk in patients treated with HCTZ. Odds ratios (ORs) per allele copy and 95% confidence intervals were calculated using allelic trend tests. A patient was considered to be HCTZ-treated if HCTZ was prescribed prior to NOD diagnosis. Similar treatment definitions were used for atenolol and trandolapril, which belong to drug classes known to affect NOD incidence.[6] For significant SNPs and haplotypes, additional regressions were performed in patients not treated with HCTZ, in patients treated with ≥25 mg per day HCTZ and in patients treated with continuous HCTZ for ≥6 months. SNP*HCTZ treatment interaction *P*-values were also determined for significant SNPs. Variables for adjustment were chosen for potential impact on NOD and included age, gender, body mass index, average on-treatment systolic BP, hypercholesterolemia, smoking history, potassium supplementation during the study, PCs one, two and three and trandolapril or atenolol treatment.

LD and haplotype design

LD analysis and pairwise LD (r^2) were performed within race/ethnic groups using Haploview.[22] We used SAS to create phased haplotypes within race/ethnic groups from SNPs reaching nominal significance ($P<0.05$). Common haplotypes (frequency>0.05) were tested for association with change in FG during HCTZ treatment and NOD risk in previously described statistical models. If two nominally associated SNPs were in very high LD ($r^2>0.9$), one SNP was included in haplotypes.

RESULTS

Association of *KCNJ1* variation with increased FG during HCTZ Treatment in PEAR

A total of 768 patients were included in PEAR analysis, including 382 who received HCTZ monotherapy and 386 who received HCTZ add-on therapy. Differences in baseline characteristics for patients randomized to HCTZ and atenolol drug arms are presented in Table 1. Median increase in FG was 2.50 mg dl $^{-1}$ (interquartile range (IQR) −3.5 to 7.5) during 9 weeks of HCTZ monotherapy and 2.00 mg dl $^{-1}$ (IQR −4.0 to 8.0) during 9 weeks of HCTZ add-on therapy (P = 0.69). Serum potassium decreased a median 0.27 mEq l $^{-1}$ (IQR 0.04–0.57) during HCTZ monotherapy and 0.31 mEq l $^{-1}$ (IQR 0.03–0.59) during HCTZ add-on therapy (P = 0.60). These results support the similarity of distributions in FG data from HCTZ monotherapy and HCTZ add-on therapy and support pooling change in log(FG) data.

Three SNPs (rs581472, rs583352 and rs7933427) deviated from Hardy–Weinberg Equilibrium in at least one race/ethnic group at P<0.05 and were included in the analysis. (Supplementary Table S1) In PEAR blacks (n = 304), one SNP (rs17137967, (MAF 0.05)) was associated with an increased FG during HCTZ treatment after FDR correction (beta = 8.47 (s.e. 2.45), P = 0.0008, P_{FDR} = 0.009). FG increased a median 2.0 mg dl $^{-1}$ (IQR −4.0 to 7.5) among 271 T/T homozygotes, 6.8 mg dl $^{-1}$ (IQR 2.0–10.0) in 25 T/C heterozygotes and 15.8 mg dl $^{-1}$ (IQR −2.5 to 34.0) in two C/C homozygotes (Figure 2). This association remained significant after adjustment for baseline and change in serum potassium (beta = 7.94 (s.e 2.46), P = 0.002, P_{FDR} = 0.01), urinary potassium (beta = 8.17 (s.e. 2.47), P = 0.001, P_{FDR} = 0.009), and plasma insulin (beta = 4.96 (s.e. 1.79), P = 0.002, P_{FDR} = 0.01). Nominal (P<0.05) associations with increased FG were also observed for the rs2846680 A allele (beta = 3.55 (s.e. 1.44), P = 0.03, P_{FDR} = 0.13) in blacks. These two SNPs (r^2 = 0.01) were used to create three common haplotypes for black PEAR patients. Haplotype findings in blacks were driven by rs17137967 and therefore were not considered further.

In non-blacks (n = 464), one SNP (rs7933427, MAF = 0.04) was nominally associated with increased FG (beta = 3.46 (s.e. 1.55), P = 0.02, P_{FDR} = 0.29). The significant SNP rs17137967 in PEAR blacks was monomorphic in non-blacks. The previously reported missense SNP rs59172778, which was previously associated with a decrease in FG,[9] occurred only in non-blacks (MAF = 0.01), was not in LD with any other SNP tested, and was not associated with change in FG during HCTZ treatment (P = 0.58). However, we did observe a significant association between this SNP's G allele and increased serum potassium during HCTZ treatment in non-blacks, increasing a median 0.38 mEq l $^{-1}$ (IQR −0.07 to 0.48) in eight rs59172778 A/G heterozygotes and decreasing 0.29 mEq l $^{-1}$ (IQR 0.04–0.58) in 222 A/A homozygotes (P = 0.001, P_{FDR} = 0.02). Associations between model covariates and change in FG are presented in Supplementary Table S2. No significant associations were observed in any race/ethnic groups in atenolol-treated PEAR patients.

Association of *KCNJ1* variation with NOD risk after HCTZ treatment in INVEST

Over a mean of 2.8 years, we identified 446 NOD cases in the INVEST GENEtic Substudy (INVEST-GENES), 410 of which were successfully genotyped on the HumanCVD BeadChip. NOD cases had higher baseline body mass index and diastolic BP, and a higher percentage of left ventricular hypertrophy than age, race/ethnicity and gender-matched controls. (Table 2) During INVEST, the proportion of patients who were treated with HCTZ was higher in cases (74%) than in controls (62%, P<0.0001). Mean atenolol dose was higher in cases (72 mg) than in controls (67 mg, P = 0.02) in those who were treated with atenolol.

No *KCNJ1* SNP deviated from Hardy–Weinberg Equilibrium in control patients from any race/ethnic group in INVEST. (Supplementary Table S3) In HCTZ-treated whites (n = 371),

two SNPs (rs12795437 and rs11600347) were significantly associated with a greater than two-fold increase in NOD risk per variant allele ($P = 0.006$ ($P_{FDR} = 0.04$) and $P = 0.008$ ($P_{FDR} = 0.04$), respectively). (Table 3) These two SNPs were also associated in HCTZ-treated Hispanics ($n = 464$) at nominal ($P<0.05$) significance. (Supplementary Figure S1) One SNP (rs658903) was associated with a more than 60% reduced risk of NOD ($P = 0.002$ ($P_{FDR} = 0.04$)) in Hispanics. (Table 3) In HCTZ-treated black patients ($n = 131$), one SNP (rs675388) was associated with a 3.13-fold increased NOD risk with each allele copy ($P = 0.004$ ($P_{FDR} = 0.03$)). We found 10 other SNPs associated with NOD in HCTZ-treated patients at nominal ($P<0.05$) significance in at least one race/ethnic group. (Supplementary Figure S1).

After LD analysis in whites, three SNPs (rs2238009, rs12795437 and rs11600347) were used to construct three haplotypes with frequency>0.05. The haplotype HapW1 (GCA) was associated with significantly increased risk for NOD during HCTZ treatment ($P = 0.006$ ($P_{FDR} = 0.04$)). (Table 3) In Hispanics, haplotypes were inferred from five nominally associated SNPs (rs675388, rs1148058, rs658903, rs12795437 and rs3016774), resulting in five haplotypes with frequency>0.05. HapH1 (CATCT) was associated with an approximately two-fold increased risk for NOD per haplotype copy ($P = 0.003$ ($P_{FDR} = 0.03$)) and the opposite haplotype HapH2 (TGAGC) was associated with a 57% reduction in NOD risk per haplotype copy ($P = 0.007$ ($P_{FDR} = 0.04$)). In blacks, two nominally associated SNPs (rs675388 and rs1148059) were used to infer three haplotypes with frequency>0.05. HapB1 (GC) was associated with a 72% decreased NOD risk during HCTZ treatment ($P = 0.003$ ($P_{FDR} = 0.02$)). The SNP rs59172778 previously reported to be associated with changes in FG had a MAF of 0.01 in whites and Hispanics and was not associated with NOD in either race/ethnic group.

Consistent associations were observed for SNPs and haplotypes in all three ethnic groups when HCTZ treatment was defined as ≥6 months or ≥25 mg per day. (Table 3) Importantly, no *KCNJ1* SNPs or haplotypes were associated with NOD in patients not treated with HCTZ in any race/ethnic group. This resulted in significant SNP*HCTZ treatment interactions for NOD. (Table 3) Associations between model covariates and NOD are presented in Supplementary Table S4.

DISCUSSION

In the present study, we observed a significant association between *KCNJ1* variation and increased FG in blacks treated with short-term HCTZ. We also observed significant increases in serum potassium with a *KCNJ1* SNP previously associated with decreased glucose during HCTZ treatment. Our study also observed that *KCNJ1* variation was associated with NOD in patients treated with long-term HCTZ in all race/ethnic groups. These associations remained significant in patients treated with HCTZ for an extended duration and in patients taking higher daily doses of HCTZ. We observed no associations achieving even nominal significance in non-HCTZ-treated patients, suggesting effects of *KCNJ1* variation on dysglycemia are specific to HCTZ treatment.

KCNJ1 variation was associated with change in FG in PEAR and NOD in INVEST, during both short- and long-term HCTZ treatment, and in most race/ethnic groups studied, suggesting that variability in *KCNJ1* affects HCTZ-induced dysglycemia. Disparate results in PEAR and INVEST suggest either different pharmacogenetic markers for FG versus NOD during HCTZ treatment or that the SNPs studied here are all tagging a functional SNP. Disparate associations between race/ethnic groups suggest differences in LD, which are observable in the PEAR and INVEST populations (Supplementary Figure S2 through Supplementary Figure S6), and the need to identify functional variants in *KCNJ1*. PEAR

and INVEST enrolled different study populations, used varied durations of HCTZ treatment and employed contrasting antihypertensive treatment, which might have contributed to disparate genetic associations with the dysglycemia phenotype of focus in each study.

Our study is strengthened by the inclusion of two different metabolic phenotypes across the diabetes continuum and two different study populations. While different associations were observed in PEAR and INVEST, significant associations of *KCNJ1* variation in two independent populations and a previous association support that *KCNJ1* variation influences thiazide-induced dysglycemia.[9] Our results also suggest that *KCNJ1* variation affects FG during both short- and long-term HCTZ treatment.

The relationship between potassium and glucose is well described, and decreased serum potassium has been implicated in thiazide-induced dysglycemia.[5] *KCNJ1* encodes the renal outer medullary potassium channel (ROMK1, Kir1.1) that is responsible for potassium excretion in exchange for sodium absorption through the epithelial sodium channel (ENaC).[23] This action occurs in the collecting duct distal to the thiazide-sensitive sodium/chloride co-transporter, the direct target of thiazide diuretics. SNPs may influence HCTZ's effect on ROMK1 function, disrupting potassium homeostasis and affecting glucose-dependent insulin secretion from pancreatic beta cells or glucose uptake into skeletal muscle.[23,24] *KCNJ1*'s direct role in potassium excretion and the previous association of a *KCNJ1* SNP with thiazide-induced dysglycemia make *KCNJ1* a particularly compelling candidate gene in the pharmacogenetics of thiazide-induced dysglycemia. Many other compelling candidate genes exist for pharmacogenomic study of thiazide-induced dysglycemia, including the ENaC genes, *SCNN1A* and *SCNN1G*, and the thiazide-sensitive sodium-chloride co-transporter *SLC12A3*.

Our study adds to existing literature by attempting to replicate a previous SNP association and investigating the effect of *KCNJ1* variation on both FG changes and NOD. An analysis from the Genetic Epidemiology of Responses to Antihypertensives (GERA) study observed decreased FG in rs59172778 (M338T) G allele carriers ($n = 8$, mean -4.6 mg dl^{-1}) and increased FG in A/A homozygotes ($n = 532$, mean 3.8 mg dl^{-1}) after 4 weeks of HCTZ treatment.[9] In the present study, we did not observe an association between this SNP and change in FG or NOD during HCTZ, but we did observe a significant increase in serum potassium in PEAR with the G allele, which is consistent with a lower FG. However, the GERA study reported a lack of association between the SNP and change in serum potassium. Additional studies are necessary to refine the role of potassium depletion and *KCNJ1* variation in thiazide-induced dysglycemia.

While secondary analyses of antihypertensive trial data have provided evidence that potassium depletion is associated with increased FG during thiazide treatment,[11,25] a recent analysis from the PEAR study observed no correlation between change in serum potassium and change in FG levels during thiazide treatment, using individual patient data rather than aggregate pooled data.[26] Our *KCNJ1* SNP findings in PEAR blacks remained significant after adjustment for serum and urinary potassium, and plasma insulin, suggesting that effects of *KCNJ1* variation are independent of potassium and insulin levels. In addition, potassium supplementation in INVEST was associated with increased NOD risk (Supplementary Table S4). The increased NOD risk may suggest that patients with lower potassium levels are at greater risk for NOD or may indicate a higher rate of potassium supplementation in HCTZ-treated individuals, which is insufficient to offset NOD risk after HCTZ treatment. While further research is required, the pathophysiology of thiazide-induced dysglycemia appears to involve potassium, but is likely more complex than a simple inverse relationship between potassium and glucose.

Our study has several limitations worthy of mention. We recognize the potential for false positive results in our analyses and our observations need to be independently replicated. We attempted to reduce false positives by using an FDR correction for all *P*-values acquired in SNP and haplotype analyses within each race/ethnic group. In INVEST, NOD diagnosis was based on investigator reports, but this phenotype is well described in a previous publication,[16] the accuracy of such reporting has been verified by others,[27] and has been used in other trials.[28–30] Change in FG in PEAR and NOD in INVEST may be confounded by the use of other antihypertensive agents that affect FG levels, ongoing environmental factors and potassium supplementation. We attempted to reduce confounding by controlling for metabolically important variables, antihypertensive treatment and potassium supplementation.

In summary, our results add to available evidence suggesting that genetic variation in *KCNJ1* influences the dysglycemic effect of HCTZ. Our observation of different SNP and haplotype effects between race/ethnic groups suggests differences in LD and highlights the need to identify functional SNPs. Functional studies and replication of these associations are needed to better define the potential role of *KCNJ1* SNPs in predicting adverse metabolic effects of HCTZ. After further study and replication, *KCNJ1* SNPs might be used to provide genotype-guided thiazide prescribing to avoid NOD in patients with risk genotypes or haplotypes. Whether these findings are specific to HCTZ or can be generalized to all thiazide diuretics is also unclear, but *KCNJ1* remains a compelling candidate gene in the pharmacogenetic study of thiazide-induced dysglycemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, et al. Executive summary: Heart Disease and Stroke Statistics--2012 Update: a report from the American Heart Association. Circulation. 2012; 125:188–197. [PubMed: 22215894]
2. Verdecchia P, Reboldi G, Angeli F, Borgioni C, Gattobigio R, Filippucci L, et al. Adverse prognostic significance of new diabetes in treated hypertensive subjects. Hypertension. 2004; 43:963–969. [PubMed: 15037557]
3. Alderman MH, Cohen H, Madhavan S. Diabetes and cardiovascular events in hypertensive patients. Hypertension. 1999; 33:1130–1134. [PubMed: 10334799]
4. 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. [PubMed: 12748199]
5. Carter BL, Einhorn PT, Brands M, He J, Cutler JA, Whelton PK, et al. Thiazide-induced dysglycemia: call for research from a working group from the national heart, lung, and blood institute. Hypertension. 2008; 52:30–36. [PubMed: 18504319]

6. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. Lancet. 2007; 369:201–207. [PubMed: 17240286]
7. Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes. 2000; 49:2201–2207. [PubMed: 11118026]
8. Bozkurt O, de Boer A, Grobbee DE, de Leeuw PW, Kroon AA, Schiffers P, et al. Variation in Renin-Angiotensin system and salt-sensitivity genes and the risk of diabetes mellitus associated with the use of thiazide diuretics. Am J Hypertens. 2009; 22:545–551. [PubMed: 19247266]
9. 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. [PubMed: 16109321]
10. Irvin MR, Lynch AI, Kabagambe EK, Tiwari HK, Barzilay JI, Eckfeldt JH, et al. Pharmacogenetic association of hypertension candidate genes with fasting glucose in the GenHAT Study. J Hypertens. 2010; 28:2076–2083. [PubMed: 20577119]
11. Zillich AJ, Garg J, Basu S, Bakris GL, Carter BL. Thiazide diuretics, potassium, and the development of diabetes: a quantitative review. Hypertension. 2006; 48:219–224. [PubMed: 16801488]
12. Shafi T, Appel LJ, Miller ER III, Klag MJ, Parekh RS. Changes in serum potassium mediate thiazide-induced diabetes. Hypertension. 2008; 52:1022–1029. [PubMed: 18981326]
13. Johnson JA, Boerwinkle E, Zineh I, Chapman AB, Bailey K, Cooper-DeHoff RM, et al. Pharmacogenomics of antihypertensive drugs: rationale and design of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. Am Heart J. 2009; 157:442–449. [PubMed: 19249413]
14. Pepine CJ, Handberg-Thurmond E, Marks RG, Conlon M, Cooper-DeHoff R, Volkers P, et al. Rationale and design of the International Verapamil SR/Trandolapril Study (INVEST): an Internet-based randomized trial in coronary artery disease patients with hypertension. J Am Coll Cardiol. 1998; 32:1228–1237. [PubMed: 9809930]
15. Pepine CJ, Handberg EM, Cooper-DeHoff RM, Marks RG, Kowey P, Messerli FH, et al. A calcium antagonist vs a non-calcium antagonist hypertension treatment strategy for patients with coronary artery disease. The International Verapamil-Trandolapril Study (INVEST): a randomized controlled trial. JAMA. 2003; 290:2805–2816. [PubMed: 14657064]
16. Cooper-Dehoff R, Cohen JD, Bakris GL, Messerli FH, Erdine S, Hewkin AC, et al. Predictors of development of diabetes mellitus in patients with coronary artery disease taking antihypertensive medications (findings from the INternational VErapamil SR-Trandolapril STudy [INVEST]). Am J Cardiol. 2006; 98:890–894. [PubMed: 16996868]
17. Cooper-DeHoff RM, Handberg EM, Mancia G, Zhou Q, Champion A, Legler UF, et al. INVEST revisited: review of findings from the International Verapamil SR-Trandolapril Study. Expert Rev Cardiovasc Ther. 2009; 7:1329–1340. [PubMed: 19900016]
18. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, et al. Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One. 2008; 3:e3583. [PubMed: 18974833]
19. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]
20. Cooper-DeHoff RM, Aranda JM Jr, Gaxiola E, Cangiano JL, Garcia-Barreto D, Conti CR, et al. Blood pressure control and cardiovascular outcomes in high-risk Hispanic patients--findings from the International Verapamil SR/Trandolapril Study (INVEST). Am Heart J. 2006; 151:1072–1079. [PubMed: 16644338]
21. Conway J, Lauwers P. Hemodynamic and hypotensive effects of long-term therapy with chlorothiazide. Circulation. 1960; 21:21–27. [PubMed: 13811661]
22. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]
23. Welling PA, Ho K. A comprehensive guide to the ROMK potassium channel: form and function in health and disease. Am J Physiol Renal Physiol. 2009; 297:F849–F863. [PubMed: 19458126]

24. Braun M, Ramracheya R, Bengtsson M, Zhang Q, Karanauskaite J, Partridge C, et al. Voltage-gated ion channels in human pancreatic beta-cells: electro-physiological characterization and role in insulin secretion. Diabetes. 2008; 57:1618–1628. [PubMed: 18390794]

25. Chatterjee R, Yeh HC, Shafi T, Selvin E, Anderson C, Pankow JS, et al. Serum and dietary potassium and risk of incident type 2 diabetes mellitus: The Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med. 2010; 170:1745–1751. [PubMed: 20975023]

26. Smith SM, Anderson SD, Wen S, Gong Y, Turner ST, Cooper-Dehoff RM, et al. Lack of correlation between thiazide-induced hyperglycemia and hypokalemia: subgroup analysis of results from the pharmacogenomic evaluation of antihypertensive responses (PEAR) study. Pharmacotherapy. 2009; 29:1157–1165. [PubMed: 19792989]

27. Goldman N, Lin IF, Weinstein M, Lin YH. Evaluating the quality of self-reports of hypertension and diabetes. J Clin Epidemiol. 2003; 56:148–154. [PubMed: 12654409]

28. Aguilar D, Solomon SD, Kober L, Rouleau JL, Skali H, McMurray JJ, et al. Newly diagnosed and previously known diabetes mellitus and 1-year outcomes of acute myocardial infarction: the VALsartan In Acute myocardial iNfarcTion (VALIANT) trial. Circulation. 2004; 110:1572–1578. [PubMed: 15364810]

29. Bosch J, Lonn E, Pogue J, Arnold JM, Dagenais GR, Yusuf S. Long-term effects of ramipril on cardiovascular events and on diabetes: results of the HOPE study extension. Circulation. 2005; 112:1339–1346. [PubMed: 16129815]

30. Yusuf S, Gerstein H, Hoogwerf B, Pogue J, Bosch J, Wolffenbuttel BH, et al. Ramipril and the development of diabetes. JAMA. 2001; 286:1882–1885. [PubMed: 11597291]

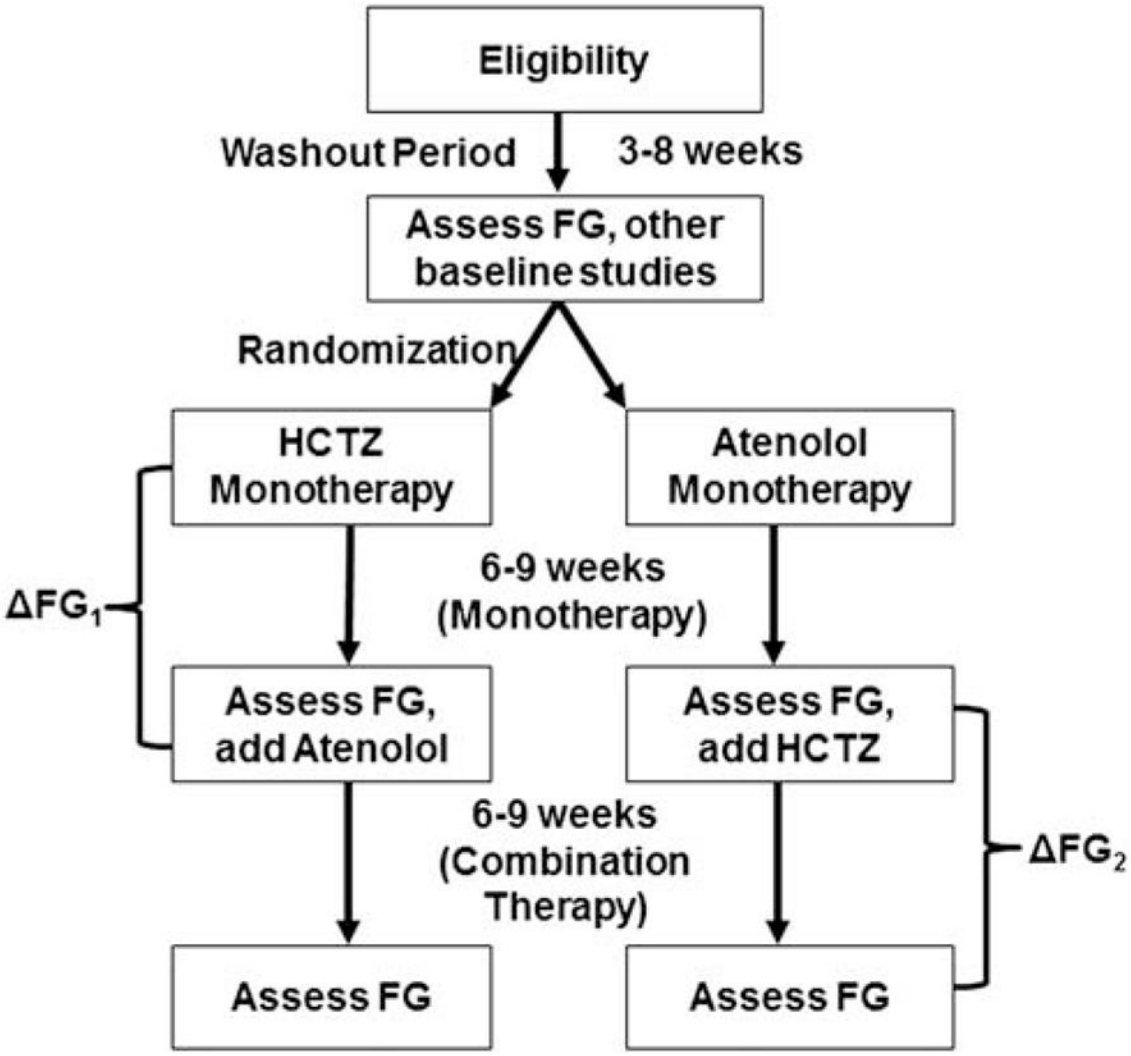

Figure 1.
Assessment of fasting glucose (FG) in the Pharmacogenomic Evaluation of Antihypertensive Responses study design. ΔFG_1, change in FG during HCTZ monotherapy; ΔFG_2, change in FG during HCTZ add-on therapy to atenolol; HCTZ, hydrochlorothiazide.

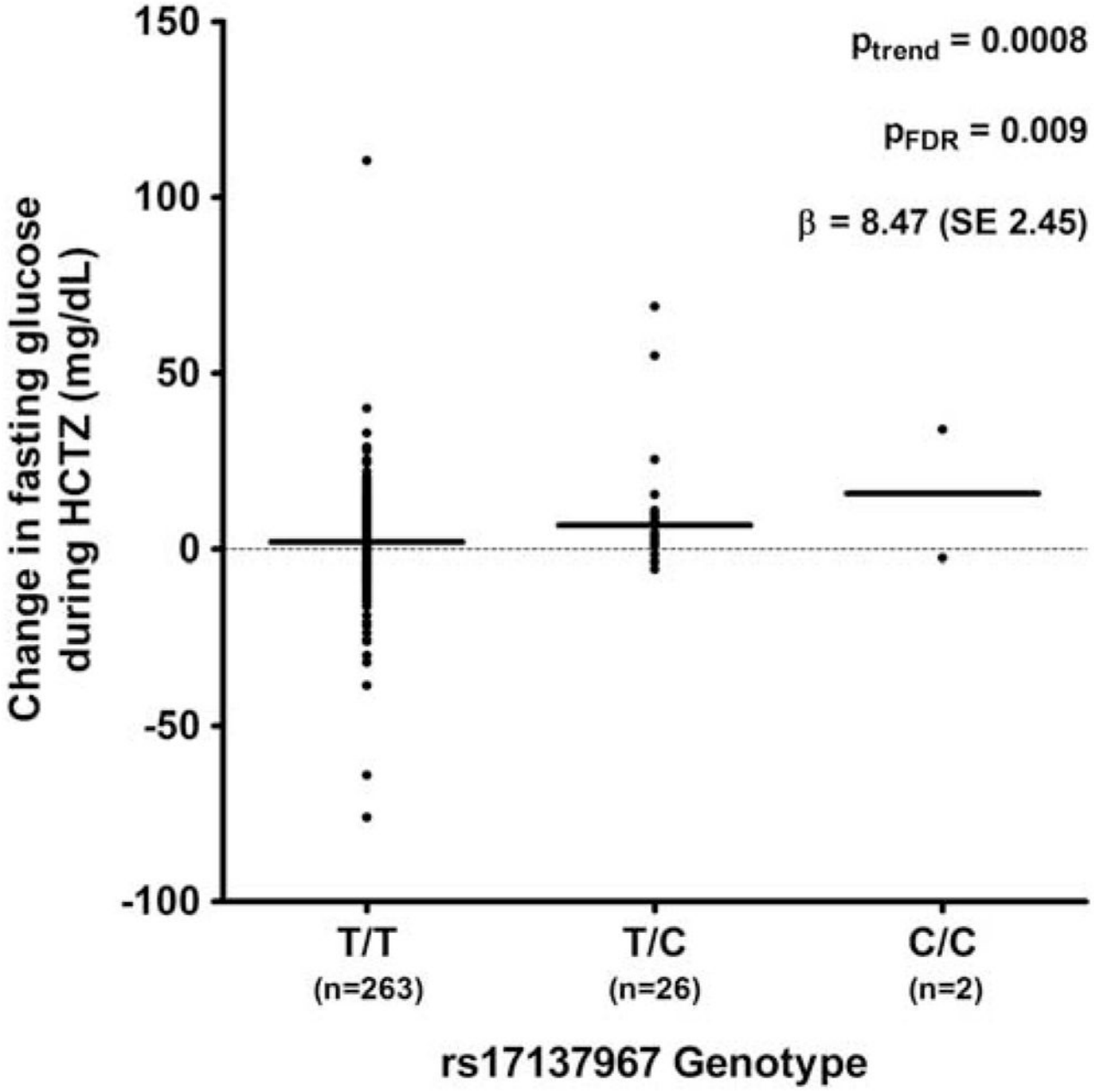

Figure 2.

Change in fasting glucose during hydrochlorothiazide (HCTZ) treatment by rs17137967 genotype in black Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) patients. Bars represent medians and P_{trend} indicates *P*-value for change in log(fasting glucose) during HCTZ using an allelic trend test adjusted for log fasting glucose at start of HCTZ, age, gender, waist circumference, potassium supplementation, drug arm, average home systolic and diastolic blood pressure, duration of HCTZ treatment and principal components one, two and three. P_{FDR} indicates P_{trend} false discovery rate (FDR)-corrected for all SNP and haplotypes in PEAR blacks.

Table 1

Baseline characteristics of PEAR patients by randomized drug arm

Characteristic[a]	HCTZ (*n* = 382)	Atenolol (*n* = 386)	*P*-value[b]
Age (years)	49 (9)	49 (9)	0.57
Female, *n* (%)	188 (49)	217 (56)	0.05
BMI (kg m^{-2})	31 (5)	31 (6)	0.96
Waist circumference (cm)	98 (13)	98 (13)	0.55
Race/ethnicity, *n* (%)			0.88
Black	152 (40)	152 (39)	
Non-black	230 (60)	234 (61)	
Current smoker	61 (16)	50 (13)	0.23
Home blood pressure (mm Hg)[c]			
Systolic	147 (11)	145 (10)	0.13
Diastolic	94 (6)	93 (6)	0.03
Fasting glucose (mg dl^{-1}), median (IQR)	91 (85–98)	89 (84–96)	0.53
Serum potassium (mEq l^{-1}), median (IQR)	4.2 (4.0–4.5)	4.2 (4.0–4.5)	0.47

Abbreviations: BMI, body mass index; HCTZ, hydrochlorothiazide; HDL, high-density lipoprotein; IQR, interquartile range.

[a] Values are mean±s.d. unless otherwise noted.

[b] *P*-values are for *t*-tests and chi-square tests as appropriate. Wilcoxon rank sum was used for continuous, non-normal variables.

[c] Represents average of home blood pressure values.

Table 2

Characteristics of NOD cases and controls at baseline and during INVEST

Characteristic[a]	NOD cases (n = 446)	Controls (n = 1025)	P-value[b]
Baseline			
Age	65 (10)	65 (9)	0.73
Female, n (%)	250 (56)	573 (56)	0.96
BMI (kg m $^{-2}$)	31 (6)	29 (5)	<0.0001
Race/ethnicity, n (%)			0.66
White	176 (40)	409 (40)	
African American	51 (11)	121 (12)	
Hispanic	217 (49)	492 (48)	
Blood pressure (mm Hg)			
Systolic	149 (19)	148 (18)	0.26
Diastolic	87 (10)	86 (10)	0.04
Verapamil SR strategy, n (%)	208 (47)	519 (51)	0.16
Hypercholesterolemia, n (%)[c]	242 (54)	548 (54)	0.78
History of LVH, n (%)	77 (17)	128 (13)	0.02
History of smoking, n (%)	184 (41)	405 (40)	0.53
During INVEST			
Blood pressure (mm Hg)[d]			
Systolic	135 (11)	134 (11)	0.07
Diastolic	79 (6)	79 (6)	0.49
HCTZ treatment, n (%)	328 (74)	640 (62)	<0.0001
HCTZ (≥6 months), n (%)	320 (72)	627 (61)	<0.0001
HCTZ dose (mg)	28 (12)	26 (12)	0.17
Atenolol treatment, n (%)	229 (51)	479 (47)	0.10
Atenolol dose (mg)	72 (32)	67 (31)	0.02
Trandolapril treatment, n (%)	267 (60)	664 (65)	0.07
Trandolapril dose (mg)	3.3 (2.6)	3.4 (2.6)	0.49
Verapamil SR treatment, n (%)	208 (47)	519 (51)	0.16
Verapamil SR dose (mg)	234 (75)	238 (75)	0.71
Potassium supplementation, n (%)	75 (17)	100 (10)	0.0001

Abbreviations: BMI indicates body mass index; HCTZ, hydrochlorothiazide; INVEST, International Verapamil SR and Trandolapril Study; LVH, left ventricular hypertrophy; NOD, new onset diabetes; SR, sustained release.

[a] Values are mean±s.d. unless otherwise noted.

[b] P-values represent t-tests and chi-square tests where appropriate.

[c] History of or currently taking lipid-lowering medications.

[d] Average of clinic blood pressure measurements during study.

Table 3

Odds ratios per copy of *KCNJ1* SNPs and haplotypes and 95% CIs for new onset diabetes during hydrochlorothiazide treatment by race/ethnicity in INVEST[a]

SNP or haplotype	Allele(s) (frequency)	OR (95% CI) HCTZ-treated	OR (95% CI), HCTZ-treated (≥25 mg per day)	OR (95% CI), HCTZ-treated (≥6 months)	OR (95% CI), not HCTZ-treated	Interaction *P*-value[b]
Whites						
rs12795437	C (0.07)	2.36 (1.28–4.37), $P = 0.006^*$	2.43 (1.11–5.34), $P = 0.03$	2.41 (1.30–4.47), $P = 0.005^*$	0.40 (0.13–1.27), $P = 0.12$	$P = 0.002^*$
rs11600347	A (0.07)	2.29 (1.24–4.21), $P = 0.008^*$	2.31 (1.06–5.02), $P = 0.03$	2.33 (1.26–4.30), $P = 0.007^*$	0.54 (0.19–1.50), $P = 0.23$	$P = 0.003^*$
HapW1[c]	GCA (0.08)	2.35 (1.27–4.34), $P = 0.006^*$	3.09 (1.49–6.41), $P = 0.002^*$	2.39 (1.29–4.44), $P = 0.006^*$	0.42 (0.13–1.32), $P = 0.14$	$P = 0.002^*$
Hispanics						
rs658903	A (0.12)	0.38 (0.21–0.69), $P = 0.002^*$	0.31 (0.15–0.62), $P = 0.0009^*$	0.41 (0.22–0.74), $P = 0.003^*$	1.17 (0.55–2.50), $P = 0.69$	$P = 0.03$
HapH1[d]	CATCT (0.09)	2.14 (1.31–3.53), $P = 0.003^*$	2.06 (1.23–3.45), $P = 0.006^*$	2.06 (1.24–3.41), $P = 0.005^*$	0.86 (0.35–2.11), $P = 0.74$	$P = 0.07$
HapH2[d]	TGAGC (0.10)	0.43 (0.24–0.79), $P = 0.007^*$	0.39 (0.20–0.76), $P = 0.005^*$	0.48 (0.26–0.86), $P = 0.01$	0.94 (0.42–2.10), $P = 0.69$	$P = 0.18$
Blacks						
rs675388	T (0.18)	3.13 (1.45–6.75), $P = 0.004^*$	4.16 (1.72–10.03), $P = 0.002^*$	3.06 (1.42–6.60), $P = 0.004^*$	NE $P = 0.63$	$P = 0.06$
HapB1[e]	GG (0.70)	0.28 (0.12–0.66), $P = 0.003^*$	0.25 (0.11–0.60), $P = 0.002^*$	0.29 (0.13–0.67), $P = 0.004^*$	NE $P = 0.73$	$P = 0.10$

Abbreviations: CI, confidence interval; HCTZ, hydrochlorothiazide; INVEST, International Verapamil SR and Trandolapril Study; NE, odds ratio not estimable due to sample size; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] All point estimates represent increased risk of NOD per copy of allele and are adjusted for age, gender, body mass index, average on-treatment systolic blood pressure, history of smoking and hypercholesterolemia, principal components one, two and three, and treatment with trandolapril, atenolol or potassium supplementation.

[b] Interaction *P*-values for allele by HCTZ treatment.

[c] Haplotype inferred from SNPs rs2238009, rs12795437 and rs11600347.

[d] Haplotypes inferred from SNPs rs675388, rs1148058, rs658903, rs12795437 and rs3016774.

[e] Haplotypes inferred from SNPs rs675388 and rs1148059.

[*] *P*-value significant after false discovery rate correction for all SNP and haplotype associations within race/ethnic group.