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Pharmacogenetic Association of Hypertension Candidate Genes with Fasting Glucose in the GenHAT Study

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Abstract

Several clinical studies report increased risk of diabetes mellitus (DM) with pharmacologic treatment for hypertension (HTN). HTN genes may modify glycemic response to antihypertensive treatment. The current study examined the association of 24 single nucleotide polymorphisms (SNPs) in 11 HTN candidate genes with fasting glucose measured at 2, 4, and 6 years after treatment initiation. The study sample included participants free of diabetes at baseline in the Genetics of Hypertension Associated Treatment (GenHAT) study (N=9,309). GenHAT participants were randomized to receive treatment with a diuretic (chlorthalidone), calcium channel blocker (amlodipine), or ACE inhibitor (lisinopril). Mixed models for repeated measures were employed to test for gene and pharmacogenetic associations with fasting glucose during follow-up. Fasting glucose at year 2 increased on average 6.8 mg/dL, 4.8 mg/dL and 3.0 mg/dL from baseline in the chlorthalidone, amlodipine and lisinopril groups, respectively. Carrying the I allele (rs1799752) of the angiotensin-converting enzyme (ACE) I/D polymorphism was associated with lower fasting glucose levels (P=0.02). Additionally, an ACE promoter polymorphism (−262, rs4291) was associated with lower fasting glucose for the model AA/AT vs. TT which remained significant after correction for multiple testing (P=0.001). Finally, a SNP in the α-subunit of the amiloride-sensitive epithelial sodium channel (SCNN1A, rs2228576) modified the association of amlodipine versus chlorthalidone treatment with fasting glucose (P<0.001). Further examination of these genes and their relationships with cardiometabolic disease could foster development of pharmacogenetic guidelines aimed to prevent increases in fasting glucose during antihypertensive treatment.

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Keywords

ACE; SCNN1A; RAAS; Lisinopril; Chlorthalidone; Amlodipine; Thiazide Diuretics; Hypertension Treatment

Introduction

Current clinical practice guidelines, including the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) and results from the Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial (ALLHAT), support the use of thiazide diuretics as the first-line of therapy for most patients with hypertension (HTN) [1;2]. However, diuretics are associated with a number of metabolic disturbances, including raised glucose levels. In ALLHAT at year 2, fasting glucose increased 8.5 mg/dL, 5.5 mg/dL, and 3.5 mg/dL for the chlorthalidone group (thiazide-like diuretic), amlodipine (calcium channel blocker) group, and lisinopril (ACE inhibitor) group, respectively [3]. This result is concerning as HTN is a risk factor for cardiovascular disease (CVD) and glucose dysregulation is an additional comorbidity that significantly compounds that risk [4–6]. Similarly, a recent meta-analysis of over 143,000 eligible participants from 22 clinical trials reported incident diabetes risk reduction with angiotensin receptor blockers, ACE inhibitors, and calcium-channel blockers versus diuretic [7].

Data suggest that there is crosstalk at multiple levels between pathways that mediate glucose regulation and HTN [8]. However, the mechanisms through which antihypertensive treatments may improve or impair glucose tolerance among hypertensives are not completely understood. Pharmacologic interruption of the ACE enzyme with ACE inhibitors might improve glucose metabolism by interfering with angiotensin II (Ang II) generation or Ang II receptor activation. Recent studies have suggested that Ang II may promote altered glucose metabolism through effects on insulin signaling pathways, tissue blood flow, oxidative stress, sympathetic activity, and adipogenesis [8;9]. In contrast, thiazide diuretics increase glucose levels. Several studies have suggested that the mechanism for this effect is through hypokalemia resulting in decreased insulin secretion by beta cells in the pancreas [10;11]. Thiazide diuretics can cause increased renal potassium excretion due to increased flow to the distal secretory site as a result of inhibition of NaCl and water reabsorption in the distal tubule as well as to increased secretion of aldosterone because of diuretic-induced volume contraction [12;13]. Supporting the hypothesis that diuretic induced hypokalemia has an effect on hyperglycemia, it has been reported that potassium supplementation helps curb thiazide-induced glucose intolerance [14].

The Genetics of Hypertension Associated Treatment (GenHAT) study, an ancillary study of ALLHAT, was designed to determine whether variants in HTN susceptibility genes interact with antihypertensive medication to modify coronary heart disease (CHD) risk in hypertensives [15]. In the current study we selected hypertension candidate genes belonging to pathways that may have effects on glucose metabolism during hypertension treatment including the renin-angiotensin-aldosterone system (RAAS), sodium metabolism, nitrous oxide (NO) synthesis, and oxidative stress reduction. We hypothesize that variation in genes belonging to these pathways may modify the relationship of antihypertensive treatment with subsequent increase in fasting glucose, a diabetes-related trait. Understanding gene by treatment interactive risk for diabetes could lead to selection of optimum antihypertensive treatment based on specific genotypes. Specifically, we examined SNPs in the RAAS including angiotensin-converting enzyme (ACE), angiotensinogen (AGT), angiotensin II type 1 receptor (AGTR1), and renin (REN). The genes involved in sodium sensitivity and transport that we examined included guanine nucleotide binding protein β-polypeptide 3 (GNB3), G protein-coupled receptor kinase

4 (GRK4), natriuretic peptide precursor A (NPPA), the alpha subunit of the amiloride-sensitive epithelial sodium channel (SCNN1A), and α-adducin (ADD1). We also included SNPs in NO-synthase (NOS3) and HDL associated paraoxonase 1 (PON1) which are involved in NO synthesis and oxidative stress response, respectively.

Methods

Study Population

ALLHAT (N=42,418) was a randomized, double-blind, multicenter clinical trial of persons ≥ 55 years of age with hypertension and at least one other CVD risk factor recruited from 623 centers which examined differences by antihypertensive treatment in fatal CHD and non-fatal myocardial infarction incidence [16]. In GenHAT 39,114 of the 42,418 ALLHAT participants with available DNA were genotyped for variants in several genes implicated in hypertension and stroke etiology. HTN was defined as systolic blood pressure of 140 mmHg or higher, and/or a diastolic blood pressure of 90 mmHg or higher, and/or taking antihypertensive medication (<3 drugs) with a blood pressure of 160/100 mmHg or lower. Randomization began in February, 1994 and concluded January 31, 1998. Active follow-up concluded in March 2002. Detailed inclusion and exclusion criteria are published elsewhere [17;18]. Informed consent was obtained for each patient, and the protocol was approved by the institutional review board at each participating center.

Intervention

Participants were randomized to treatment with one of 4 primary antihypertensive drugs belonging to different classes: (1) a calcium channel blocker (amlodipine), (2) an ACE inhibitor (lisinopril), and (3) a α-adrenergic blocker (doxazosin) as compared to (4) chlorthalidone, a thiazide-like diuretic. In February, 2000, the doxazosin arm was discontinued because of a significant increase (25%) in incidence of CVD compared with the chlorthalidone arm [19]. Participants randomized to the doxazosin treatment arm were not included in the current study. The remaining participants were randomized to chlorthalidone, lisinopril, and amlodipine—in a ratio of 1.7:1:1, respectively, at each center. The treatment was given once daily to achieve blood pressure control (≤140/90 mmHg) on the lowest possible dosage: chlorthalidone 12.5 mg for the first and second titration and 25 mg for the third; lisinopril 10, 20, or 40 mg; and amlodipine 2.5, 5, or 10 mg. If blood pressure control was not achieved on the maximum study medication dose, a second-step, open-label agent (reserpine, clonidine, or atenolol) and/or a third-step open-label agent (hydralazine) was added.

Sample for Current Analysis

Our study hypothesis centers on genes that may be associated with adverse metabolic outcomes during antihypertensive treatment among persons not diabetic at treatment initiation. To this end, we adopted the same inclusion criteria as a previous analysis of incident DM in the ALLHAT study [3]. Known DM was defined by physician diagnosis (N=11,002). Newly diagnosed DM cases (N=972) were defined as those entering the trial without a previously known DM diagnosis, but with a fasting glucose level > 125 mg/dL (6.9 mmol/L). Participants classified as having known or newly diagnosed DM were excluded from the current study. Those with a missing baseline fasting glucose measurement and a non-fasting baseline glucose level of 110 mg/dL (6.1 mmol/L) or higher were also excluded (N=1,333) to conservatively ensure only participants free of DM at baseline were examined. This left 17,473 participants eligible for the current analysis. Of these 17,473 participants, 9,309 had at least one follow-up fasting glucose measure (and often more than one) at 2 (N=7,284), 4 (N=6,153) and/or 6 (N=1,540) years of follow-up and were included in the present analysis.

We compared baseline characteristics of those with (N=9,309) and those without (N=8,164) a follow-up fasting glucose measure who were considered free of DM at baseline. The groups had similar mean age (66.4 for those included and 67.7 for those excluded). The distribution of treatment was similar among those included and excluded with 46% and 45% randomized to chlorthalidone, 27% and 26% randomized to amlodipine, and 26% and 28% randomized to lisinopril, respectively. However, the gender and race distribution between the groups was different. Specifically, 58% versus 51% of men did and did not have a follow-up fasting glucose and 42% versus 48% of women did and did not have a follow-up fasting glucose measure ($P<0.0001$). Finally, the distribution of race was similar between the two groups except 24% of non-Hispanic Blacks versus 33% did and did not have a follow-up fasting glucose measure, respectively ($P<0.0001$).

Laboratory Measurements

Baseline and follow-up serum glucose concentrations were obtained after an overnight fast (at least 8 hours) at each site through a certified laboratory [3]. Glucose was quantified with the Ortho-Clinical Diagnostics VITROS Analyzer (models 950 and 250), which used a glucose oxidase colorimetric method (Ortho-Clinical Diagnostics, Inc., Raritan, NJ).

SNP Selection

Our study was ancillary to GenHAT and utilized data on previously genotyped variants from a multilocus CVD SNP panel (Roche Molecular Systems, Pleasanton, California, USA). From this panel we focused on genes in pathways where we could find evidence for effects on both blood pressure and glucose metabolism. To this end we did not investigate all 43 cardiovascular candidate genes genotyped in GenHAT and only examined SNPs in genes involved in the renin-angiotensin-aldosterone system (RAAS), sodium metabolism, NO synthesis, and/or oxidative stress reduction.

Genotyping

DNA isolation techniques and genotyping methods within GenHAT have been described [15]. Briefly, DNA samples were anonymized as set forth in the Report of the Special Emphasis Panel on Opportunities and Obstacles to Genetic Research in NHLBI Clinical Studies [20]. DNA was isolated from blood clots using FTA paper assays (Fitzco Inc, Maple Plain, MN, USA). With the exception of the ACE I/D polymorphism, the current study used a multilocus CVD SNP panel described above where genotyping was performed by colorimetrically detecting the hybridization of biotinylated products from a multiplex PCR to sequence-specific oligonucleotide probes arrayed on a nylon membrane [21]. Genotype calls were made with the assistance of image processing software provided by Roche Molecular Systems. ACE I/D genotyping was performed using a modification of the procedure described by Kim *et al*. to enhance detection of heterozygotes [22].

Statistical methods

Twenty four SNPs in 11 candidate genes were tested for Hardy-Weinberg Equilibrium (HWE) stratified by self-reported ethnic group (non-Hispanic White, non-Hispanic Black, Hispanic White, Hispanic Black, and Other). One way ANOVA and chi square tests were used to examine differences in baseline characteristics of the study population for continuous and categorical traits by treatment group, respectively. Randomization to hypertension treatment resulted in equal distribution of confounders between treatment groups, thus, models including treatment group were not adjusted for covariates in our study. Outliers from the distribution of fasting glucose at each time-point (± 4 SD from the mean) were excluded from the analysis.

To examine the average change in fasting glucose by treatment group, change in fasting glucose from baseline to the 2 year visit was calculated for each participant and treatment group category was regressed on this outcome. To examine SNP association with fasting glucose at equally spaced follow-up visits (years 2, 4, and 6) we employed a mixed model approach in SAS v. 9.2 that allowed for repeated fasting glucose measures using a compound symmetry correlation structure which bases the estimates on the available measurements. For these main effect SNP models (i.e., the effect of the genotype on fasting glucose), we adjusted for visit number as an additive variable and ethnic group category since allele frequencies for some of the genetic variants differed by ethnicity. Though treatment group was not included in these models, we found the addition of other covariates such as gender and BMI did not change results. An additive model was tested for each SNP except when there were 10 or fewer participants with the minor allele homozygote genotype for an ethnic group category. In such a case heterozygotes were grouped with minor allele homozygotes. The ACE I/D polymorphism was modeled II/ID vs. DD (and similarly for the linked SNPs rs4343 and rs4363) as several previous studies have suggested the DD genotype is a risk factor for DM [23–26]. A similar mixed model was employed to estimate mean fasting glucose at each follow-up visit for each treatment group (Figure 1).

Pharmacogenetic models (examining gene by antihypertensive treatment interactions) used similar mixed model methods and were adjusted for visit number plus gene and treatment main effects, but not for other covariates as these were balanced in the randomization for treatment. Antihypertensive treatment was modeled as a categorical variable with chlorthalidone as the reference. Visit number and the genotypes were modeled as described above. Interactions with visit number were not significant in any model suggesting the time points measured in this study do not change the relationship of the treatments or SNPs with the outcome, fasting glucose.

We corrected for multiple testing using a method based on the Bonferroni criterion that accounts for correlations among markers in a multi-marker study [27]. Based on this method, we calculated the number of independent SNPs to be 23.35 yielding a significance level *P* value of 0.05/23.35=0.002 for tests of main effect and 0.05/46.7=0.001 for tests of gene by treatment interactions.

Results

Baseline characteristics of the study population were not different by treatment randomization group (Table 1). The description of SNP variation and genotype frequencies are presented in Table 2. Genotyping rates were greater than or equal to 97% for each SNP. SNPs deviating from HWE with *P*-value <0.01 include AGTR1 −535 G/A ($P=1.12*10^{-5}$) among non-Hispanic Blacks, SCNN1A T663A A/G ($P=2.82*10^{-8}$) among non-Hispanic Whites, and ADD1 G460T G/T (*P*=0.007) and NPPA V664M G/A (*P*=0.0004) in the “Other” racial group category. At year 2, mean fasting glucose increased on average 6.8 mg/dL, 4.8 mg/dL and 3.0 mg/dL from baseline in the chlorthalidone, amlodipine, and lisinopril groups, respectively. These estimates for change in fasting glucose after 2 years of follow-up in our GenHAT subgroup closely agree with those reported in a study of fasting glucose and incident DM in the parent ALLHAT study [3]. Figure 1 demonstrates that fasting glucose continues to increase over time within each treatment category.

No SNP was associated with baseline fasting glucose with $P<0.05$ among GenHAT participants without DM at baseline. The association of each SNP with fasting glucose during follow-up is presented in Table 3. Two SNPs in the ACE gene were associated with fasting glucose in our study with $P\leq0.05$. Carriers of the insertion (I) allele for the ACE I/D polymorphism had close to 1 mg/dL lower fasting glucose on average during antihypertensive treatment compared

to DD homozygotes. Carrying an A for the ACE −262 A/T (rs4291) polymorphism was associated with a 1.72 mg/dL lower fasting glucose level on average, which met significance criteria after adjustment for multiple testing. For all the other SNPs examined, we did not find any main effect associations with follow-up fasting glucose in GenHAT.

We report a gene by treatment interaction for a SNP in SCNN1A (T663A, rs2228576). Figure 2 shows estimated average fasting glucose levels at year 6/visit 3 by treatment group and genotype for this SNP. The pharmacogenetic association is for the amlodipine versus chlorthalidone comparison and is significant after correction for multiple testing (P=0.001). Specifically, the GG genotype was associated with lower fasting glucose levels for amlodipine versus chlorthalidone (100 vs. 106 mg/dL, respectively), while carrying the A allele for that SNP was not associated with as large a difference in fasting glucose levels between the two treatments (Figure 2: 103 vs. 106 mg/dL, respectively).

Discussion

This report describes the association of HTN genes with fasting glucose during antihypertensive treatment along with how genes modify the relationship between treatment and fasting glucose. The SNPs were carefully chosen from an available list of HTN and other cardiovascular candidate genes belonging to pathways for which we could explain dual roles in glucose metabolism and blood pressure. The extensively studied ACE I/D polymorphism was associated with fasting glucose during antihypertensive treatment (P=0.02), though the association was not significant after correction for multiple testing. The association of the DD genotype with diabetes has been replicated in some populations [23–26;28] but not all populations [29–31]. Consistent with the D allele being associated with metabolic disturbance, our results show a trend toward the I allele being associated with lower fasting glucose levels in treated hypertensives. Other studies of the association of the ACE I/D genotype with fasting and non-fasting glucose are inconsistent [29;31–35]. The D allele has been associated with higher ACE levels [36;37] leading to increased Ang II levels [9] followed by downstream adverse effects on metabolism that remain incompletely understood [32].

We extend results on fasting glucose from previous studies of the ACE gene by reporting an association for another polymorphism in the ACE gene. The ACE −262 A/T (rs4291) polymorphism was the only SNP that met significance criteria after adjustment for multiple testing. The SNP is located in the promoter region of the gene, and we report the A allele to be associated with lower fasting glucose levels during antihypertensive treatment. Evidence of the relationship of this allele with ACE concentration has not been consistent. One study reports the A allele is associated with increased ACE concentration in plasma [38], but two other studies report the opposite effect [39;40]. Another study reported increased expression of ACE gene products with the A allele and reported the presence of a transcription factor binding site at that location [41]. To our knowledge, no other study has considered this SNP's association with DM or related traits. However, the effect of the SNP on fasting glucose during antihypertensive treatment was small in our study (<2mg/dL for the risk genotype).

One recent nested case-control study based on pharmacy records in the Netherlands examined modification of the relationship between thiazide diuretics and DM by 5 SNPs in 5 genes examined by our study [12]. The SNPs included ACE G4656C (as a proxy for the ACE I/D polymorphism), AGT M235T, AGTR1 A1166C, ADD1 G460T, and GNB3 C825T. Results showed that among 497 incident cases of DM and 2633 controls the risk of DM due to thiazide diuretic treatment compared to other antihypertensives was different by ACE G4656C, GNB3 C825T, and AGTR1 A1166C genotypes. Our results considering the related phenotype, fasting glucose, did not agree with these findings even when we restricted our analysis to non-Hispanic Whites only. There could be several reasons our study was not able to replicate the results of

Bozkurt *et al*. [12]. They compared thiazide diurectics to all other hypertensive medications grouped together as the reference while our study compared amlodipine and lisonipril, individually, to chlorthalidone. Also, the outcome in that study was dichotomous diabetes status and our study examined fasting glucose as the outcome. Finally, chance cannot be excluded as the reason for the majority of their findings. Bozkurt *et al.* mentioned they did not adjust for multiple testing after examining 5 SNPs and acknowledged "when a more stringent significance level of 0.01 would have been applied, only the interaction between AGTR1 and thiazides remained significant."

We report a pharmacogenetic association involving a SCNN1A missense polymorphism (rs2228576) for amlodipine versus chlorthalidone treatment. The SCNN1A gene encodes one of three subunits of the amiloride-sensitive epithelial sodium channel (ENaC) that regulates sodium absorption in epithelial cells that line the distal renal tubule [42]. This interaction is biologically plausible as blockade of renal sodium transport has been hypothesized to be important for thiazide diuretic-induced glucose dysregulation [12;13]. It is also intriguing that the SNP is a missense SNP resulting in a threonine to alanine amino acid change and that could affect the function of the protein. However, we have been unable to determine the putative effect of the SNP on protein function based on the literature or using PupaSuite (http://www.pupasnp.org), which includes an estimator of effect of polymorphisms based on evolutionary information [43]. The SCNN1A interaction is the only interaction term that met statistical significance criteria after adjustment for multiple testing. However, we present this result with caution as the SNP was out of HWE in non-Hispanic Whites in the chlorthalidone treatment group only, an effect that could bias our result. The departure from HWE among non-Hispanic Whites appears to be due to a loss of heterozygosity which could result from structural variation and, in fact, according to the Database of Genomic Variants, the SNP lies in a region of copy number variation [44]. The interaction was still present when non-Hispanic Whites were removed from the analysis, although no longer statistically significant after correction for multiple testing (P=0.03).

Limitations of this analysis should be mentioned. Information concerning DM diagnosis and/or DM treatment was not collected during follow-up of ALLHAT. Other studies have used criteria of no DM at baseline and fasting glucose >125 mg/dL during ALLHAT follow-up to define DM onset [3;45]. However, given limitations of this definition with no information on DM treatment collected during follow-up and the potential for increased power to reveal pharmacogenetic associations using a continuous outcome, we chose to focus our analysis on fasting glucose. We realize that lack of appropriate correction for diabetes treatment in our analysis of fasting glucose could lead to substantial shrinkage in the estimated effect of genetic determinants (or shrinkage bias) and reduction in statistical power [46]. We could have missed some important associations, or our results could underestimate the effects we report. Additionally, in our tests of gene main effects we adjusted for self-reported race as a proxy for ancestry. We note allele frequency differences between the races could have biased our results for gene main effects. Optimally, if available, we would have included adjustment for ancestry informative markers in those models to exclude the possibility for residual confounding due to population substructure. A total of 47% of the 17,473 participants in GenHAT free of DM at baseline did not have a follow-up fasting glucose measure and were excluded from the current study. More non-Hispanic Blacks were excluded than included due to missing fasting glucose measures during follow-up. If the distribution of genes was different between the groups this could limit the generalizability of our study to the non-Hispanic Black population. The differences by gender should not limit the generalizability of this study since our genetic effects of interest should not be associated with gender. Our study is strengthened by its sample size and availability of genotype information, often including more than one functional marker, for a wide variety of important HTN candidate genes.

There is ample evidence of overlap in pathways and proteins affecting both HTN and glucose metabolism. We studied 24 SNPs in 11 cardiovascular candidate genes we hypothesized could have an effect on glucose metabolism during hypertension treatment in a biologically plausible way. The majority of these SNPs did not have an effect on fasting glucose. However, we report that the ACE gene has a small effect on fasting glucose during hypertension treatment. Future studies might examine whether the association of ACE with diabetes or related traits during hypertension treatment is modified by other genes or environmental factors. The magnitude of effect of the pharmacogenetic association reported for the SCNN1A polymorphism could have clinical implications. Despite the noted limitations of this result, it would be interesting to replicate this interaction in another study since inhibition of sodium transport is theorized to be important in diuretic-associated glucose disturbance. More recent studies have identified new loci associated with blood pressure that could be important to our hypothesis and interesting to study in the future. For instance phospholipase C, delta 3 (PLCD3) was highlighted by a recent genome wide association study [47] and belongs to a family of enzymes important to the insulin signaling pathway and lipid metabolism [48;49]. Understanding risk factors for developing glucose dysregulation during antihypertensive treatment continues to be an important research question to pursue to help prevent excess CVD risk in a vulnerable hypertensive population.

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Reference List

1. Jackson JH, Sobolski J, Krienke R, Wong KS, Frech-Tamas F, Nightengale B. Blood pressure control and pharmacotherapy patterns in the United States before and after the release of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) guidelines. J Am Board Fam Med 2008;21:512–521. [PubMed: 18988718]
2. Wright JT Jr. Probstfield JL, Cushman WC, Pressel SL, Cutler JA, Davis BR, et al. ALLHAT findings revisited in the context of subsequent analyses, other trials, and meta-analyses. Arch Intern Med 2009;169:832–842. [PubMed: 19433694]
3. Barzilay JI, Davis BR, Cutler JA, Pressel SL, Whelton PK, Basile J, et al. Fasting glucose levels and incident diabetes mellitus in older nondiabetic adults randomized to receive 3 different classes of antihypertensive treatment: a report from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Arch Intern Med 2006;166:2191–2201. [PubMed: 17101936]
4. Messerli FH, Grossman E, Goldbourt U. Antihypertensive therapy in diabetic hypertensive patients. Am J Hypertens 2001;14:12S–16S. [PubMed: 11370955]
5. Whaley-Connell A, Sowers JR. Hypertension management in type 2 diabetes mellitus: recommendations of the Joint National Committee VII. Endocrinol Metab Clin North Am 2005;34:63–75. [PubMed: 15752922]
6. UKPDS Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. BMJ 1998;317:703–713. [PubMed: 9732337]
7. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. Lancet 2007;369:201–207. [PubMed: 17240286]
8. Muscogiuri G, Chavez AO, Gastaldelli A, Perego L, Tripathy D, Saad MJ, et al. The crosstalk between insulin and renin-angiotensin-aldosterone signaling systems and its effect on glucose metabolism and diabetes prevention. Curr Vasc Pharmacol 2008;6:301–312. [PubMed: 18855718]
9. Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. J Hypertens 2004;22:2253–2261. [PubMed: 15614015]

10. Gorden P. Glucose intolerance with hypokalemia. Failure of short-term potassium depletion in normal subjects to reproduce the glucose and insulin abnormalities of clinical hypokalemia. Diabetes 1973;22:544–551. [PubMed: 4719191]
11. Rowe JW, Tobin JD, Rosa RM, Andres R. Effect of experimental potassium deficiency on glucose and insulin metabolism. Metabolism 1980;29:498–502. [PubMed: 6991855]
12. 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]
13. Liamis G, Milionis H, Elisaf M. Blood pressure drug therapy and electrolyte disturbances. Int J Clin Pract 2008;62:1572–1580. [PubMed: 18822027]
14. 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]
15. Arnett DK, Boerwinkle E, Davis BR, Eckfeldt J, Ford CE, Black H. Pharmacogenetic approaches to hypertension therapy: design and rationale for the Genetics of Hypertension Associated Treatment (GenHAT) study. Pharmacogenomics J 2002;2:309–317. [PubMed: 12439737]
16. ACR Group. Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). ALLHAT Collaborative Research Group. JAMA 2000;283:1967–1975. [PubMed: 10789664]
17. Davis BR, Cutler JA, Gordon DJ, Furberg CD, Wright JT Jr. Cushman WC, et al. Rationale and design for the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Research Group. Am J Hypertens 1996;9:342–360. [PubMed: 8722437]
18. Grimm RH Jr. Margolis KL, Papademetriou V, V, Cushman WC, Ford CE, Bettencourt J, et al. Baseline Characteristics of Participants in the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Hypertension 2001;37:19–27. [PubMed: 11208751]
19. ACR Group. Diuretic versus alpha-blocker as first-step antihypertensive therapy: final results from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Collaborative Research Group. Hypertension 2003;42:239–246. [PubMed: 12925554]
20. Report of the Special Emphasis Panel on Opportunities and Obstacles to Genetic Research in NHLBI Clinical Studies. National Heart, Lung, and Blood Institute, NIH; Bethesda, Md: 1997. 2009
21. Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, et al. A multilocus genotyping assay for candidate markers of cardiovascular disease risk. Genome Res 1999;9:936–949. [PubMed: 10523522]
22. Kim DS, Choi SI, Lee HS, Park JK, Yi HK. Determination of human angiotensin converting enzyme (ACE) gene polymorphisms in erectile dysfunction: frequency differences of ACE gene polymorphisms according to the method of analysis. Clin Chem Lab Med 2001;39:11–14. [PubMed: 11256792]
23. Baroudi T, Bouhaha R, Moran-Moguel C, Sanchez-Corona J, Ben Maiz H, Kammoun AH, et al. Association of the insertion/deletion polymorphism of the angiotensin-converting enzyme gene with type 2 diabetes in two ethnic groups of Jerba Island in Tunisia. J Renin Angiotensin Aldosterone Syst 2009;10:35–40. [PubMed: 19286757]
24. Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, et al. The D allele of the angiotensin-converting enzyme insertion/ deletion (I/D) polymorphism is a risk factor for type 2 diabetes in a population-based Japanese sample. Endocr J 2003;50:393–398. [PubMed: 14599112]
25. Feng Y, Niu T, Xu X, Chen C, Li Q, Qian R, et al. Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. Diabetes 2002;51:1986–1988. [PubMed: 12031990]
26. Yang M, Qiu CC, Xu Q, Xiang HD. Association of angiotensin converting enzyme gene I/D polymorphism with type 2 diabetes mellitus. Biomed Environ Sci 2006;19:323–327. [PubMed: 17044652]
27. Cheverud JM. A simple correction for multiple comparisons in interval mapping genome scans. Heredity 2001;87:52–58. [PubMed: 11678987]
28. Ramachandran V, Ismail P, Stanslas J, Shamsudin N, Moin S, Mohd JR. Association of insertion/ deletion polymorphism of angiotensin-converting enzyme gene with essential hypertension and type

2 diabetes mellitus in Malaysian subjects. J Renin Angiotensin Aldosterone Syst 2008;9:208–214. [PubMed: 19126661]

29. Ryan AS, Nicklas BJ, Berman DM, Ferrell RE. The insertion/deletion polymorphism of the ACE gene is related to insulin sensitivity in overweight women. Diabetes Care 2001;24:1646–1652. [PubMed: 11522714]
30. Thomas GN, Tomlinson B, Chan JC, Sanderson JE, Cockram CS, Critchley JA. Renin-angiotensin system gene polymorphisms, blood pressure, dyslipidemia, and diabetes in Hong Kong Chinese: a significant association of tne ACE insertion/deletion polymorphism with type 2 diabetes. Diabetes Care 2001;24:356–361. [PubMed: 11213892]
31. van Valkengoed IG, Stronks K, Hahntow IN, Hoekstra JB, Holleman F. The angiotensin converting enzyme insertion/deletion polymorphism and differences in fasting plasma glucose in Hindustani Surinamese, African Surinamese and ethnic Dutch: the population-based SUNSET-study. Diabetes Res Clin Pract 2008;81:e12–e14. [PubMed: 18439702]
32. Bonnet F, Patel S, Laville M, Balkau B, Favuzzi A, Monti LD, et al. Influence of the ACE gene insertion/deletion polymorphism on insulin sensitivity and impaired glucose tolerance in healthy subjects. Diabetes Care 2008;31:789–794. [PubMed: 18235053]
33. Ohishi M, Rakugi H, Miki T, Katsuya T, Okamura A, Kamide K, et al. Deletion polymorphism of angiotensin-converting enzyme gene is associated with postprandial hyperglycaemia in individuals undergoing general check-up. Clin Exp Pharmacol Physiol 2000;27:483–487. [PubMed: 10874503]
34. Perticone F, Ceravolo R, Iacopino S, Cloro C, Ventura G, Maio R, et al. Relationship between angiotensin-converting enzyme gene polymorphism and insulin resistance in never-treated hypertensive patients. J Clin Endocrinol Metab 2001;86:172–178. [PubMed: 11231997]
35. Akin F, Turgut S, Dursunoglu D, Turgut G, Karasu U, Gur S. ACE gene polymorphism and cardiac structure in patients with insulin resistance. Mol Biol Rep 2009;36:623–629. [PubMed: 18347926]
36. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343–1346. [PubMed: 1976655]
37. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 1995;92:1387–1388. [PubMed: 7664416]
38. Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, McKenzie CA, et al. Linkage and association analysis of angiotensin I-converting enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. Am J Hum Genet 2001;68:1139–1148. [PubMed: 11283791]
39. Villard E, Tiret L, Visvikis S, Rakotovao R, Cambien F, Soubrier F. Identification of new polymorphisms of the angiotensin I-converting enzyme (ACE) gene, and study of their relationship to plasma ACE levels by two-QTL segregation-linkage analysis. Am J Hum Genet 1996;58:1268–1278. [PubMed: 8651305]
40. Keavney B, McKenzie CA, Connell JM, Julier C, Ratcliffe PJ, Sobel E, et al. Measured haplotype analysis of the angiotensin-I converting enzyme gene. Hum Mol Genet 1998;7:1745–1751. [PubMed: 9736776]
41. Kim TH, Chang HS, Park SM, Nam BY, Park JS, Rhim T, et al. Association of angiotensin I-converting enzyme gene polymorphisms with aspirin intolerance in asthmatics. Clin Exp Allergy 2008;38:1727–1737. [PubMed: 18727619]
42. Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, et al. Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. Nature 1994;367:463–467. [PubMed: 8107805]
43. Conde L, Vaquerizas JM, Dopazo H, Arbiza L, Reumers J, Rousseau F, et al. PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. Nucleic Acids Res 2006;34:W621–W625. [PubMed: 16845085]
44. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, et al. Detection of large-scale variation in the human genome. Nat Genet 2004;36:949–951. [PubMed: 15286789]
45. ACR Group. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-

Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Collaborative Research Group. JAMA 2002;288:2981–2997. [PubMed: 12479763]

46. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat Med 2005;24:2911–2935. [PubMed: 16152135]
47. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Ahead of Print. Nat Genet. 2009
48. Dolley G, Lamarche B, Despres JP, Bouchard C, Perusse L, Vohl MC. Phosphoinositide cycle gene polymorphisms affect the plasma lipid profile in the Quebec Family Study. Mol Genet Metab 2009;97:149–154. [PubMed: 19329342]
49. Gilon P, Henquin JC. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. Endocr Rev 2001;22:565–604. [PubMed: 11588141]

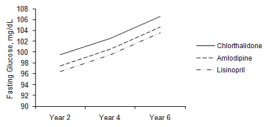

Figure 1.
Estimated mean fasting glucose by antihypertensive treatment group during follow-up

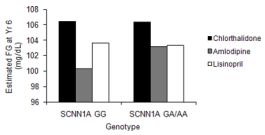

Figure 2.
Pharmacogenetic association of SCNN1A T663A genotype with fasting glucose (FG) for amlodipine vs. chlorthalidone (*P*=0.001)

Table 1

Baseline characteristics of the study population by antihypertensive treatment group

Covariate	Chlorthalidone N=4318	Amlodipine N=2554	Lisinopril N=2437	*P*
Age, y	66.3±7	66.5±7	66.4±7	0.54
Gender, % females	1785 (41)	1117 (44)	1005 (41)	0.10
Race*, n(%)				
NHW	2301 (54)	1385 (54)	1311 (54)	
NHB	1048 (24)	628 (25)	585 (24)	
HW	600 (14)	333 (13)	323 (13)	
HB	140 (3)	86 (3)	73 (3)	
O	229 (5)	122 (5)	145 (6)	0.73
Current Smoking, %	1163 (27)	635 (25)	618 (25)	0.12
Baseline fasting glucose, mg/dL	93.4±11	93.3±11	93.8±11	0.26
Baseline BMI, kg/m^2	29.0±6	28.8±6	28.8±6	0.34
Baseline SBP	144.6±14	144.2±14	144.8±14	0.31
Baseline DBP	83.9±10	83.7±10	83.9±10	0.40

* non-Hispanic White (NHW), non-Hispanic Black (NHB), Hispanic White (HW), Hispanic Black (HB), Other (O)

Table 2

Genotype counts for SNPs in hypertension candidate genes

Polymorphism	rs#	Description	Minor/Major Allele	MAF	Missing Rate
ACE	rs1799752	Intronic	I/D	46%	286 (3%)
ACE −262	rs4291	5' near gene	T/A	37%	205 (2%)
ACE T776T	rs4343	synonymous	G/A	44%	155 (2%)
ACE 20320	rs4363	Intronic	G/A	49%	144 (2%)
AGT −6	rs5051	5' UTR	G/A	42%	152 (2%)
AGT −20	rs5050	5' UTR	C/A	14%	126 (1%)
AGT M235T	rs699	missense	T/C	43%	192 (2%)
AGTR1 1166	rs5186	3' UTR	C/A	24%	195 (2%)
AGTR1 −535	rs1492078	5' near gene	A/G	48%	291 (3%)
AGTR1 −153	rs275653	5' near gene	C/T	19%	158 (2%)
α-Adducin G460T	rs4961	missense	T/G	16%	199 (2%)
GNB3 S825S	rs5443	synonymous	T/C	44%	194 (2%)
REN −1111	rs6681776	3' near gene	A/G	15%	123 (1%)
REN 2646	rs2368564	Intronic	T/C	38%	143 (1%)
GRK4 R65L	rs2960306	missense	T/G	38%	130 (1%)
GRK4 A142V	rs1024323	missense	T/C	42%	187 (2%)
GRK4 A486V	rs1801058	missense	T/C	33%	168 (2%)
NPPA V664M	rs5063	missense	A/G	5%	173 (2%)
NPPA X2238R	rs5065	missense	C/T	22%	180 (2%)
SCNNIA T663A	rs2228576	missense	A/G	23%	287 (3%)
NOS3 E298D	rs1799983	missense	T/G	26%	184 (2%)
NOS3 −690	rs3918226	intronic	T/C	7%	172 (2%)
NOS3 −922	rs1800779	intronic	G/A	31%	179 (2%)
PON1 −108	rs705379	3' near gene	T/C	40%	260 (3%)

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Table 3

Main effect associations of SNPs with fasting glucose

Gene	Model	β *	SE	*P*
ACE I/D	II/ID vs. DD	−0.93	0.41	0.02
ACE −262	AA/AT vs. TT	−1.72	0.54	**0.0014**
ACE 776	AA/AG vs. GG	−0.88	0.47	0.061
ACE 20320	AA/AG vs. GG	−0.71	0.43	0.099
AGT −6	Additive for A	0.082	0.28	0.77
AGT −20	CC/AC vs. AA	0.39	0.40	0.33
AGT M235T	Additive for C	0.07	0.28	0.81
AGTR1 1166	Additive for C	0.13	0.31	0.68
AGTR1 −535	Additive for A	0.27	0.28	0.34
AGTR1 −153	Additive for C	0.30	0.33	0.36
Adducin G460T	TT/GT v. GG	0.54	0.42	0.19
GNB3 825	Additive for C	−0.23	0.28	0.40
RENIN −1111	AA/GA v. GG	0.53	0.41	0.20
RENIN 2646	Additive for C	−0.07	0.28	0.81
GRK4 R65L	Additive for T	−0.14	0.28	0.60
GRK4 A142V	Additive for T	−0.29	0.27	0.27
GRK4 A486V	Additive for T	0.11	0.28	0.71
NPPA V664M	AA/GA v. GG	−0.36	0.62	0.56
NPPA X2238R	Additive for C	−0.05	0.33	0.88
SCNNIA T663A	AA/GA v. GG	0.25	0.39	0.53
NOS3 E298D	TT/GT v. GG	−0.14	0.30	0.65
NOS3 −690	TT/CT v. CC	−0.11	0.57	0.85
NOS3 −922	Additive for G	−0.43	0.29	0.13
PON1 −108	Additive for T	0.26	0.28	0.34

* β reflects average change in fasting glucose in mg/dL when comparing the risk genotype to the reference genotype or the heterozygote to the reference homozygote for additive models.

Models are adjusted for ethnicity and visit number.