Published in final edited form as:
Pharmacogenet Genomics. 2012 May ; 22(5): 355–366. doi:10.1097/FPC.0b013e3283516ff8.

Gene panels to help identify subgroups at high and low risk of CHD among those randomized to antihypertensive treatment: The GenHAT Study

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Abstract

Objective—To identify panels of genetic variants that predict treatment-related coronary heart disease (CHD) outcomes in hypertensive patients on one of four different classes of initial antihypertensive treatment. The goal was to identify subgroups of people based on their genetic profile who benefit most from a particular treatment.

Methods—Candidate genetic variants (n=78) were genotyped in 39,114 participants from GenHAT, ancillary to ALLHAT. ALLHAT randomized hypertensive participants (>=55 years) to one of four treatments (amlodipine, chlorthalidone, doxazosin, lisinopril). The primary outcome was fatal CHD or non-fatal MI (mean follow-up=4.9 years). A pharmacogenetic panel was derived within each of the four treatment groups. ROC curves estimated the discrimination rate between those with and without a CHD event, based on the addition of the genetic panel risk score.

Results—For each treatment group, we identified a panel of genetic variants that collectively improved prediction of CHD to a small but statistically significant extent. Chlorthalidone (A): NOS3, rs3918226; SELE, rs5361; ICAM1, rs1799969; AGT, rs5051; GNAS, rs7121; ROC comparison p=.004; Amlodipine (B): MMP1, rs1799750; F5, rs6025; NPPA, rs5065; PDE4D, rs6450512; MMP9, rs2274756; ROC comparison p=.006; Lisinopril (C): AGT, rs5051; PON1, rs705379; MMP12, rs652438; F12, rs1801020; GP1BA, rs6065; PDE4D, rs27653; ROC comparison p=.01; Doxazosin (D): F2, rs1799963; PAI1, rs1799768; MMP7, rs11568818; AGT, rs5051; ACE, rs4343; MMP2, rs243865; ROC comparison p=.007. Each panel was tested for a pharmacogenetic effect; panels A, B and D showed such evidence (p=.009, .006, and .001 respectively), panel C did not (p=.09).

Conclusion—Because each panel was associated with CHD in a specific treatment group but not the others, this research provides evidence that it may be possible to use gene panel scores as a tool to better assess antihypertensive treatment choices to reduce CHD risk in hypertensive individuals.

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Conflicts of interest: None declared

Keywords

pharmacogenetics; antihypertensive pharmacogenetics; CVD; gene panels

Introduction

Approximately 30% of Americans have hypertension, which is associated with increased cardiovascular disease (CVD) and stroke morbidity and mortality.[1] In an effort to treat hypertension, thus reducing the chance of CVD, nearly 70% of hypertensive patients use a pharmacologic antihypertensive agent; however, only 46% of hypertensive adults achieve adequate control of their blood pressure.[2] Discovering an effective treatment often requires multiple clinical visits and polypharmacy for each patient, making high blood pressure control a difficult and lengthy process.

Although evidence exists for a genetic basis for hypertension, identifying associated genes has been difficult due to the complex nature of blood pressure regulation.[3, 4] Many genes have been proposed as good candidates for blood pressure regulation, but reported results for individual genes have been difficult to replicate. It is also likely that multiple genes, the products of which working in concert, provide “checks and balances” to regulate blood pressure. Given the numbers of people being diagnosed with and treated for hypertension, pharmacogenetic research has emerged as a potentially important way to predict which treatment will have the best chance for success for each individual. However, reports of pharmacogenetic associations for individual genes have been inconsistent.[5]

Previous research has shown that it may be possible to identify gene “panels” – groups of polymorphisms that work in combination – that affect outcomes.[6, 7] A panel score that incorporates information about a set of genetic variants may more closely mirror the complex biological interactions that ultimately determine phenotypes such as high blood pressure and CVD. Translational tools with clinical utility and validity could greatly aid clinicians in deciding on appropriate hypertension therapy. Having available a simple blood test that provides an overall “score” for a panel of genes that is predictive of which drug is most beneficial could streamline the treatment process, help patients achieve target blood pressure faster, and reduce CVD risks.

Our aim for the current study was to 1) identify a panel of genetic variants associated with nonfatal myocardial infarction (MI) or CHD death (hereafter referred to as “CHD”) among hypertensive patients randomized to one of four antihypertensive agents, 2) create a summary score for each patient based on high-risk alleles from this panel, and 3) determine if the combined effect of these genetic variants differentially predicts CHD risk in each of the four randomized treatment groups.

Methods

Study Population and Design

Data were derived from the Genetics of Hypertension Associated Treatment (GenHAT) study, an ancillary study to the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT was a randomized, double-blind, multicenter (n=623) clinical trial with 42,418 hypertensive participants aged 55 years and older (46% women; 47% non-Hispanic whites) who had one or more CVD risk factors beyond hypertension. ALLHAT tested whether the incidence of fatal CHD and nonfatal MI was lower with three antihypertensive drug classes (i.e., a calcium channel blocker, an angiotensin converting enzyme (ACE) inhibitor, and an alpha-adrenergic blocker),

compared to treatment using a diuretic. Participants were randomized to treatment in a ratio of 1:1:1:1.7 for amlodipine, lisinopril, doxazosin and chlorthalidone, respectively. The goal was to achieve blood pressure less than 140/90 mm Hg by 1) titrating doses of the assigned study drug, 2) adding an open label drug (atenolol, reserpine, clonidine as step 2, hydralazine as step 3). GenHAT genotyped variants in several hypertension-related genes in 39,114 ALLHAT participants with available DNA, with the goal of understanding gene-treatment interactions on CVD outcomes. More complete descriptions of GenHAT and ALLHAT have been previously published.[8, 9] The research was approved by local Institutional Review Boards. Genetic data were anonymized.

Outcome Ascertainment

ALLHAT participants were randomized to treatment between February 1994 and January 1998. The follow-up period ended in March of 2002. In keeping with *a priori* stopping guidelines for ALLHAT, it was decided after a January 2000 data review that the doxazosin arm would be discontinued due to futility for the primary endpoint, and a significantly higher incidence of CVD, particularly CHF, when compared with chlorthalidone treatment. The outcome of interest in this analysis was CHD, defined as either fatal CHD or non-fatal MI, which was the primary endpoint for both ALLHAT and GenHAT. Outcomes were reported by clinical investigators, and documentation (death certificate, hospital discharge summary) was submitted for any outcome involving death or hospitalization. National databases were also used to identify deaths occurring among participants lost to follow-up.

Genotyping

DNA was isolated on FTA® paper (Fitzco Inc, Maple Plain, MN, USA) from blood samples. Genotyping was performed using amplified DNA products of a multiplex PCR and detected using a linear immobilized probe research assay for multiple candidate markers ("Roche strip," Roche Molecular Systems, Alameda, CA, USA) as described previously.[10] These variants were selected for inclusion by Roche because there was evidence that the biochemical pathways of the genes involved were implicated in the development and progression of CVD.

Statistical Methods

All statistical analyses were performed using STATAc version 10.1 (STATA Corporation, College Station, Texas). To test for differences in baseline measurements between treatment groups we used ANOVA for continuous variables and chi-square tests for categorical variables. Cox proportional hazards regression was used to determine which genetic polymorphisms were predictive of CHD in the four treatment-specific groups separately, using both dominant and recessive models and adjusting for sex, age, race (black/non-black), type 2 diabetes status, smoking status, history of LVH and baseline values of total cholesterol, HDL cholesterol, and systolic and diastolic blood pressure. All available genetic polymorphisms were tested (n=78; Table 2). If the p-value associated with the effect of a genetic polymorphism on CHD was less than 0.05 in the individual model, that polymorphism was included in a multi-polymorphism model which also included the adjustment variables mentioned above. A stepwise procedure using backward elimination was used to eliminate polymorphisms that were no longer significant in the multi-polymorphism model until all remaining polymorphisms were significant at a p-value less than 0.05. In this way four "panels" of genetic polymorphisms were created; gene panel A was generated using the chlorthalidone group; panel B using the amlodipine group; panel C, the lisinopril group; and panel D, the doxazosin group. The doxazosin panel creation and comparisons were completed using a separate dataset with follow-up data only to the point in time when the doxazosin arm was discontinued.

The sum of the number of higher-risk genotypes for each participant was used to create a panel score variable. For example, if the minor allele for a particular polymorphism (modeled recessively) was associated with significantly higher rates of CHD in the fully adjusted multi-polymorphism model, then the higher-risk genotype would be the minor allele homozygote at that locus. Likewise, if the common allele of a particular polymorphism (modeled dominantly) was associated with significantly higher rates of CHD, then the higher-risk genotype would be the common allele homozygote. Participants received a score of 1 or 0 for each polymorphism in the panel depending on whether they had the “higher risk” (1) or “lower risk” (0) genotype at that locus. Therefore, if there were 5 polymorphisms in the panel, the panel score value for a participant could be between 0 and 5, depending on the number of higher-risk genotypes. We did not use a weighting scheme for the polymorphisms in the creation of the panel scores, since the beta coefficients for the polymorphisms did not differ substantially (panel A: (−0.22–0.24); panel B: (−0.25–0.56); panel C: (0.21–0.88); panel D: (−0.29–0.59). Logistic regression was used to test the treatment-specific effect of the panel score variable on CHD, after adjusting for standard established predictors. No assumption of linearity was made since the score was modeled as an indicator variable, with five separate point estimates generated for scores between 1 and 5, each relative to zero. Therefore the point estimates were odds ratios (ORs), with a score of zero being the referent value.

Receiver operating characteristic (ROC) curve analysis was used to determine whether the treatment-specific gene panel score improved the prediction of CHD over standard established predictors. For each treatment group separately, the area under the ROC curve was calculated for a logistic model with sex, age, race (black/non-black), type 2 diabetes status, smoking status, history of LVH and baseline values of total cholesterol, HDL cholesterol, systolic and diastolic blood pressure as predictors of CHD (model 1). The area under the ROC curve was then calculated for a model with the treatment-specific panel score variable included along with all of the above variables (model 2). By using the probabilities of CHD generated by each of the above 2 models, we compared the areas under the ROC curves using the STATA command “roccomp”, which tests the equality of the two ROC areas. We deemed the treatment-specific gene panel scores an improvement in the prediction of CHD over the standard established predictors when the area under the curve was greater in model 2 than model 1, and when the p-value for the test of equality was less than 0.0125 (0.05/4=0.0125, since there were four such tests – one for each panel).

It was of interest whether the panel scores were also associated with blood pressure in addition to CHD within each treatment group. We tested this with linear regression using both systolic and diastolic blood pressure level at 6 months after randomization to treatment as continuous dependent variables and the panel score as the independent variable, adjusting for sex, age, race (black/non-black), type 2 diabetes status, smoking status, history of LVH and baseline values of total cholesterol and HDL cholesterol.

Results

A summary of baseline characteristics for the participants by treatment group assignment is provided in Table 1. The only difference in baseline characteristics detected between the treatment groups was for HDL cholesterol: the amlodipine group had a slightly higher mean HDL cholesterol (mg/dl) than the other treatment groups (47.2 (SD: 14.7) for amlodipine versus 46.8 (SD: 14.9), 46.6 (SD: 14.6) and 46.6 (SD: 14.4) mg/dL for chlorthalidone, lisinopril and doxazosin, respectively). The genotype frequencies for each of the genetic variants included in the panels are also shown in Table 1.

In all, there were 3,426 CHD events which occurred during follow-up (mean 4.9 years) among the GenHAT population (chlorthalidone group: 1,272; amlodipine group: 760; lisinopril group: 734; doxazosin group: 660 [early termination of this treatment arm]).

Chlorthalidone group: Creating Panel A

Among participants randomized to chlorthalidone, there were five polymorphisms that remained significant predictors of CHD after adjusting for sex, age, race, type 2 diabetes status, smoking status, history of LVH and baseline values of total cholesterol, HDL cholesterol, systolic and diastolic blood pressure in the multiple-polymorphism model, and therefore were used to create a gene panel A score. The variants included in the panel were NOS3 rs3918226 (C>T) modeled dominantly (minor allele carriers higher risk: HR=1.23 for T* vs. CC, p=.014), SELE rs5361 (A>C) modeled dominantly (common allele homozygotes higher risk: HR=0.82 for C* vs. AA, p=.017), ICAM1 rs1799969 (G>A) modeled dominantly (common allele homozygotes higher risk: HR=0.82 for A* vs. GG, p=.023), AGT rs5051 (A>G) modeled recessively (minor allele homozygotes higher risk: HR=1.18 for GG vs. A*, p=.020), and GNAS rs7121 (T>C) modeled dominantly (minor allele carriers higher risk: HR=1.14 for C* vs. TT, p=.046). Table 3 provides information on the frequency of each score, the odds ratios, event frequency and event rate by score and treatment group. The distribution of panel A score frequencies did not differ among the treatment groups (chi-square p=0.80), but when restricted to the cases only (a test of the pharmacogenetic effect) there were significant differences (chi-square p=0.009). The score was a predictor of CHD after adjustment for covariates among participants randomized to chlorthalidone (ORs for each score: 0 = 1.00, 1 = 5.03, 2 = 7.56, 3 = 8.34, 4 = 9.85, 5=14.0; p <0.0001), but not among those randomized to either amlodipine or lisinopril (p=0.89 and p=0.35, respectively). The event rate per 1000 person-years ranged from 2.9 to 33.8 among participants randomized to chlorthalidone depending on the panel score. The ROC analysis conducted within the chlorthalidone group showed that there was a small but significant improvement in the area under the curve (AUC) when adding the panel A score to a model including only the established risk factors for CHD (AUC for established risk factors (A_1)=0.6529, AUC for established risk factors + gene panel A score (A_2)=0.6601, ROC comparison (H_o: A_1=A_2), p=0.004).

Amlodipine group: Creating Panel B

Among participants randomized to amlodipine, five polymorphisms remained predictors at the p<0.05 level after adjustment: MMP1 rs1799750 (1G>2G) modeled dominantly (common allele homozygotes higher risk: HR=.80 for 2G* vs. 1G/1G, p=.007), F5 rs6025 (G>A) modeled dominantly (minor allele carriers higher risk: HR=1.46 for A* vs. GG, p=.040), NPPA rs5065 (T>C) modeled dominantly (minor allele carriers higher risk, HR=1.20 for C* vs. TT, p=.032), PDE4D rs6450512 (T>C) modeled recessively (minor allele homozygotes higher risk, HR=1.21 for CC vs. T*, p=.028), and MMP9 rs2274756 (G>A) modeled recessively (minor allele homozygotes higher risk: HR=1.59 for AA vs. G*, p=.018). The results of the gene panel B score analysis can be found in Table 4. Although there were five variants contributing to the B panel, only one participant had the “higher risk” genotype for all five variants; therefore we collapsed the score of 4 and 5 into a 4+ category. The distribution of panel B score frequencies did not differ between the treatment groups (chi-square p=0.71), but when restricted to the cases only (a test of the pharmacogenetic effect) there were significant differences (chi-square p=0.006). The score for the B panel predicted CHD among amlodipine participants, (ORs for each score: 0 = 1.00, 1 = 1.20, 2 = 1.53, 3 = 2.46, 4+ = 1.94; p<0.0001), but was not predictive among those randomized to chlorthalidone or lisinopril (p=0.06 and p=0.85, respectively). Among those randomized to amlodipine, the event rate per 1000 person-years ranged from 16.4 to 39.6 depending on the panel B score. The results of the ROC analysis indicate that among the amlodipine group,

the inclusion of the gene panel B indicator variable improves the prediction of CHD beyond the established risk factors (AUC for established risk factors (A_1)=0.6429, AUC for established risk factors + gene panel B score (A_2)=0.6548, ROC comparison (H_o: A_1=A_2), p=0.006).

Lisinopril group: Creating Panel C

For the lisinopril group, six polymorphisms remained significant in the adjusted multi-polymorphism model: AGT rs5051 (A>G) modeled recessively (minor allele homozygotes higher risk: HR=1.28 for GG vs. A*, p=.008), PON1 rs705379 (C>T) modeled dominantly (minor allele carriers higher risk, HR=1.23 for T* vs. CC, p=.014), MMP12 rs652438 (A>G) modeled recessively (minor allele homozygotes higher risk: HR=2.47 for GG vs. A*, p<.001), Factor12 rs1801020 (C>T) modeled recessively (minor allele homozygotes higher risk: HR=1.29 for TT vs. C*, p=.032), GP1BA rs6065 (C>T) modeled recessively (minor allele homozygotes higher risk: HR=1.66 for TT vs. C*, p=.017), and PDE4D rs27653 (C>A) modeled recessively (minor allele homozygotes higher risk: HR=1.26 for AA vs. C*, p=.024). Table 5 provides results of the analysis of gene panel C. Although there were six variants contributing to the C panel, no participants had a panel score of 6 and only one participant had the “higher risk” genotype for five variants; therefore we collapsed the score of 4 and 5 into a 4+ category. The distribution of panel C score frequencies differed between the treatment groups (chi-square p=0.01), with slightly fewer people in the lisinopril group falling into the higher-risk score categories relative to the other treatment groups (see Table 5), but when restricted to the cases only (a test of the pharmacogenetic effect) there were not significant differences (chi-square p=0.09). Among participants randomized to lisinopril, gene panel C was predictive of CHD (ORs for each score: 0 = 1.00, 1 = 1.07, 2 = 1.61, 3 = 2.30 and 4+ = 1.36; p <0.0001). Gene panel C did not predict CHD among those randomized to chlorthalidone or amlodipine (p=0.24 and 0.77, respectively). Among those randomized to lisinopril, the event rate ranged from 15.9 to 36.8 per 1000 person-years, depending on the panel C score. The ROC analysis indicated that for the lisinopril group, the inclusion of the gene panel C variable improves the prediction of CHD beyond the established risk factors (AUC for established risk factors (A_1)=0.6584, AUC for established risk factors + gene panel C score (A_2)=0.6693, ROC comparison (H_o: A_1=A_2), p=0.010).

Doxazosin group: Creating Panel D

For the doxazosin group, six polymorphisms remained significant in the adjusted multi-polymorphism model: F2 rs1799963 (G>A) modeled dominantly (minor allele carriers higher risk: HR=1.76 for A* vs. GG, p=.023), PAI1 rs1799768 (5G>4G) modeled dominantly (minor allele carriers higher risk: HR=1.33 for 4G* vs. 5G/5G, p=.011), MMP7 rs11568818 (A>G) modeled dominantly (common allele homozygotes higher risk: HR=0.76 for G* vs. AA, p=.005), AGT rs5050 (A>C) modeled recessively (minor allele homozygotes higher risk: HR=1.64 for CC vs. A*, p=.032, ACE rs4343 (A>G) modeled recessively (minor allele homozygotes higher risk: HR=1.31 for GG vs. A*, p=.017, and MMP2 rs243865 (C>T) modeled recessively (minor allele homozygotes higher risk: HR=1.70 for TT vs. C*, p=.007). Table 6 provides results of the analysis of gene panel D. Although there were six variants contributing to the D panel, no participants had a panel score of 6 and only eight participants had the “higher risk” genotype for five variants; therefore we collapsed the score of 4 and 5 into a 4+ category. The distribution of panel D score frequencies did not differ between the treatment groups (chi-square p=0.82), but when restricted to the cases only (a test of the pharmacogenetic effect) there were significant differences (chi-square p=0.001). Among participants randomized to doxazosin, gene panel D was predictive of CHD (ORs for each score: 0 = 1.00, 1 = 1.14, 2 = 1.74, 3 = 2.22 and 4+ = 5.56; p <0.0001). Gene panel D did not predict CHD among those randomized to chlorthalidone, amlodipine or lisinopril (p=0.16, 0.70 and 0.13, respectively). Among those randomized to doxazosin,

the event rate per 1000 person-years ranged from 14.1 to 78.9, depending on the panel D score. The ROC analysis indicated that for the doxazosin group, the inclusion of the gene panel D variable improves the prediction of CHD beyond the established risk factors (AUC for established risk factors (A_1)=0.6516, AUC for established risk factors + gene panel D score (A_2)=0.6705, ROC comparison (H_o: A_1=A_2), p=0.007).

When each panel score variable was tested for an association with systolic and diastolic blood pressure at 6 months after randomization within treatment group, there were no similar associations for Panels A, B or C, i.e. neither Panel A, B, C score was associated with 6 month systolic or diastolic blood pressure in the chlorthalidone, amlodipine, lisinopril groups, respectively. The Panel D score had no association with 6 month systolic blood pressure in the doxazosin group, but there was marginal evidence (p=0.04) of an association with diastolic blood pressure, with the mean adjusted pressure by score as follows: 0 = 80.6 (0.29); 1 = 80.4 (0.18); 2 = 79.7 (0.22); 3 = 79.3 (0.45); 4 = 79.2 (1.72).

Discussion

Our aim was to identify a panel of hypertension and CVD-related genetic variants, the combined effect of which could predict CHD among a group randomized to a particular hypertension treatment better than the standard risk factors alone. Using separate backwards stepwise procedures for each of the four ALLHAT treatment groups, a gene panel was identified for which we could calculate a patient's score based on their genotypes. This score was strongly associated with CHD in the treatment group of interest and, to a small but statistically significant extent, improved the prediction of CHD above that obtained with standard risk factors. For the chlorthalidone group (panel A), the panel variants were NOS3 rs3918226, SELE rs5361, ICAM1 rs1799969, AGT rs5051, and GNAS rs7121; for the amlodipine group (panel B) the panel variants were MMP1 rs1799750, F5 rs6025, NPPA rs5065, PDE4D rs6450512, and MMP9 rs2274756; for the lisinopril group (panel C) the panel variants were AGT rs5051, PON1 rs705379, MMP12 rs652438, F12 rs1801020, GP1BA rs6065, and PDE4D rs27653; and for the doxazosin group (panel D) the panel variants were F2 rs1799963, PAI1 rs1799768, MMP7 rs11568818, AGT rs5051, ACE rs4343, and MMP2 rs243865. The treatment-specific panel scores were not associated with CHD in the other treatment groups (for example, the panel A score was not associated with CHD in the lisinopril, amlodipine, or doxazosin groups), suggesting a pharmacogenetic effect, rather than a main effect on CHD for each gene panel score. We tested whether the panel score was associated with treatment group for the cases only using a chi-square, which is simple test of the pharmacogenetic effect, and panels A, B and D showed such evidence (p=.009, .006, and .001 respectively), whereas panel C did not (p=.09).

When we examined whether the panel scores were similarly associated with blood pressure level at 6 months after randomization to treatment, the only evidence was for an association between Panel D score and diastolic pressure in the doxazosin group. However, the direction of the association was opposite of what one might expect: the pressure *decreased* with an increasing score, whereas the odds of CHD *increased* with an increasing score. This might reflect increased arterial stiffening that resulted in lowered DBP and increased pulse pressure. However, the diastolic pressure differences were so small as to be negligible. These results suggest that any gene action through which the panels may be influencing CHD in a treatment-specific manner are independent of their action on blood pressure, i.e. the effect on CHD is not likely to be mediated through the blood pressure pathway.

The panel genes and their functions are listed in Table 7. The chlorthalidone-derived Panel A included several genes involved in endothelial function. Results of studies designed to evaluate the vascular effects of chlorthalidone have been mixed: Chlorthalidone improved

endothelial function, slowed albumin permeation, and reversed abnormal arteriolar structure, among hypertensive patients with nondiabetic metabolic syndrome in one study [11], whereas a rat study found no evidence that thiazide diuretics (including chlorthalidone) have pleiotropic protective vascular effects such as improved endothelial function independent of the effect on blood pressure.[12] Of particular interest in Panel B for the amlodipine group were the MMP variants thought to be involved in vascular remodeling, of which there were two. Previous research has shown that whereas baseline values of plasma MMP9 are decreased in hypertensive patients compared with normotensives, after 6 months of treatment with the calcium channel blocker amlodipine, patients had a significant increase in plasma concentrations of MMP9 (P=0.01) compared to before treatment.[13] Several of the lisinopril Panel C and doxazosin Panel D variants are involved in coagulation. This is of interest since ACE inhibitors have shown antithrombotic effects in rats [14], and alpha adrenergic blockers have been shown to increase bleeding and coagulation times.[15] Each of the four panels also included at least one “traditional” hypertension candidate gene (ACE, AGT, and NPPA). This suggests that genes influencing blood pressure likely interact with genes influencing endothelial function, vascular remodeling and coagulation to affect CHD in a treatment-specific manner.

Overall, the ALLHAT study showed that those randomized to the diuretic chlorthalidone had fewer CVD events than those randomized to the other study treatments [16–18]. Those findings are replicated in the GenHAT subpopulation. However, this research shows that by generating a gene panel score for a patient, it may be possible to better predict who may benefit most from a particular treatment. For example, among the group randomized to chlorthalidone, the 967 participants with a score of 0 or 1 for Panel A had a CHD event rate of 13 per 1000 person-years, whereas the 2,008 participants with a score of 4 or 5 had an event rate of 26 per 1000 person-years. Overall, the chlorthalidone group had a CHD event rate of 20 per 1000 person-years. This suggests that initial treatment with chlorthalidone may not be the best choice for those with a high score for panel A, initial treatment with amlodipine may not be the best choice for those with a high score for panel B, initial treatment with lisinopril may not be the best choice for those with a high score for panel C, and initial treatment with doxazosin may not be the best choice for those with a high score for panel D.

Lending strength to this study is the large, diverse group of participants. With nearly 40,000 hypertensive patients genotyped, about 35% of whom are African American and 47% women, the GenHAT study provides power not available to smaller pharmacogenetic studies. Using data from a randomized trial of antihypertensive drugs provides the opportunity to analyze pharmacogenetic associations with confidence that the four treatment groups are balanced with regard to measured and unmeasured confounders. However, since ALLHAT recruited patients aged 55 years or older with both hypertension and other risk factors for CVD, it is unknown whether these results apply to a younger, healthier population. Although all of the genes explored here were pre-determined to be candidates for influencing blood pressure or CVD, the panels were derived from and assessed in one patient population with many statistical tests performed; thus, replication of the findings in other populations must be achieved to validate the panels. External validity would be necessary before these gene panels in particular could be useful in guiding treatment decisions. In addition, this study should not be thought of as a comprehensive look at all of the potential gene candidates, since our analysis was limited to a pool of 78 genetic variants. If other genes were included in the initial screen for inclusion in the panels, the gene makeup of the panels may have been different.

For those seeking ways to translate pharmacogenetic research into clinically useful tools to help guide antihypertensive treatment decisions, gene panels such as those explored in this

research may be a meaningful step forward. Since the influence of each genetic variant alone is expected to be of small magnitude with many interactions between genes, the panel approach seems to be an efficacious one. Using these findings as an example, one can envision a clinical application whereby a physician, informed by a hypertensive patient's genotypes for all of the 22 panel variants, could assess the CVD risk for that patient for each of the four treatments included here using panel scores – the patient's scores may put them at high risk for one of the treatments, and at low risk for a different treatment. Of course, these panels have not been validated and therefore this only serves as a hypothetical example of how future findings could be translated into clinical applications. With the ever-growing interest in "personalized medicine" as a way to improve outcomes and reduce costs, research directed at the development of concrete, evidence-based tools for clinicians should be high on the list of priorities.

Acknowledgments

Funding: This study was supported in part by grant HL63082 (GenHAT) from the NHLBI and grant N01-HC-35130 (ALLHAT) from the NIH.

We thank Victoria Brophy, Suzanne Cheng, and colleagues at Roche Molecular Systems for kindly providing genotyping materials and technical support. We also thank Steven A Claas for editorial assistance in manuscript preparation.

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Table 1

Baseline characteristics for participants (n=39,114) by treatment group, mean (SD) unless otherwise noted

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
Sample size, n (%) by treatment	14,083 (36.0)	8,333 (21.3)	8,364 (21.4)	8,334 (21.3)	.
Age, years	66.8 (7.7)	66.9 (7.7)	66.8 (7.8)	66.7 (7.7)	0.76
Race:					
White, n (col %)	8,538 (60.6)	5,037 (60.5)	5,067 (60.6)	5,015 (60.2)	
Black, n (col %)	4,830 (34.3)	2,903 (34.8)	2,881 (34.5)	2,930 (35.2)	
American Indian/Alaskan native, n (col %)	27 (0.2)	19 (0.2)	18 (0.2)	10 (0.1)	
Asian/Pacific Islander, n (col %)	172 (1.2)	99 (1.2)	87 (1.0)	96 (1.2)	
Other, n (col %)	516 (3.7)	275 (3.3)	311 (3.7)	283 (3.4)	0.67
Hispanic, n (%)	2,740 (19.5)	1,573 (18.9)	1,648 (19.7)	1,629 (19.6)	0.91
Women, n (%)	6,615 (47.0)	3,964 (47.6)	3,870 (46.3)	3,852 (46.2)	0.24
Previous antihypertensive treatment, n (%)	12,707 (90.2)	7,551 (90.6)	7,536 (90.1)	7,521 (90.2)	0.70
Blood pressure at baseline, mm Hg:					
All participants: SBP	146.2 (15.7)	146.2 (15.7)	146.5 (15.6)	146.3 (15.7)	0.57
DBP	84.1 (10.1)	83.9 (10.2)	84.1 (10.0)	84.0 (10.0)	0.44
Treated at baseline: SBP	145.2 (15.7)	145.1 (15.6)	145.4 (15.5)	145.2 (15.6)	0.68
DBP	83.5 (10.0)	83.3 (10.0)	83.6 (9.9)	83.4 (9.9)	0.32
Untreated at baseline: SBP	156.0 (12.0)	156.6 (12.2)	156.4 (12.3)	156.8 (12.5)	0.58
DBP	89.5 (9.0)	89.7 (9.5)	89.1 (9.3)	89.4 (9.5)	0.64
Eligibility risk factors:					
Current cigarette smoker, n (%)	3,096 (22.0)	1,833 (22.0)	1,831 (22.0)	1,809 (21.7)	0.96
Type 2 diabetes, n (%)	5,041 (35.8)	3,030 (36.4)	2,931 (35.0)	2,929 (35.2)	0.24
HDL cholesterol < 35 mg/dL, n (%)	1,688 (12.0)	950 (11.4)	983 (11.8)	983 (11.8)	0.63
LVH by electrocardiogram, n (%)	2,279 (16.2)	1,423 (17.1)	1,359 (16.3)	1,371 (16.5)	0.34
Body mass index, kg/m^2	29.7 (6.1)	29.8 (6.3)	29.8 (6.2)	29.7 (5.9)	0.33
Fasting glucose, mg/dL	123.3 (58.5)	123.0 (57.3)	122.5 (55.8)	122.0 (55.9)	0.50
Total cholesterol, mg/dL	216.2 (43.4)	216.6 (44.0)	215.7 (42.3)	214.9 (42.3)	0.05

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
HDL cholesterol, mg/dL	46.8 (14.9)	47.2 (14.7)	46.6 (14.6)	46.6 (14.4)	0.04
LDL cholesterol, mg/dL	136.0 (37.3)	135.7 (37.4)	135.9 (36.4)	135.3 (36.3)	0.60
Fasting triglycerides, mg/dL	173.1 (130.6)	172.3 (129.5)	173.3 (140.1)	169.6 (135.1)	0.34
Glomerular filtration rate (GFR)	77.6 (19.7)	78.2 (19.7)	77.7 (19.9)	78.1 (19.8)	0.11
Aspirin use, n (%)	5,035 (35.8)	3,032 (36.4)	3,044 (36.4)	3,045 (36.5)	0.60
Panel A gene variant frequencies (col %)					
NOS3 rs3918226					
CC	12,306 (88.8)	7,244 (88.6)	7,293 (88.6)	7,300 (89.1)	
CT	1,477 (10.7)	897 (11.0)	905 (11.0)	864 (10.5)	
TT	79 (0.6)	32 (0.4)	36 (0.4)	33 (0.4)	0.38
GNAS rs7121					
TT	5,167 (37.3)	3,050 (37.4)	3,120 (37.9)	3,072 (37.5)	
TC	6,215 (44.9)	3,737 (45.8)	3,715 (45.2)	3,768 (46.0)	
CC	2,461 (17.8)	1,380 (16.9)	1,388 (16.9)	1,347 (16.5)	0.20
SELE rs5361					
AA	11,844 (85.6)	7,032 (86.1)	7,033 (85.6)	7,052 (86.2)	
AC	1,916 (13.8)	1,092 (13.4)	1,134 (13.8)	1,068 (13.1)	
CC	81 (0.6)	39 (0.5)	54 (0.7)	59 (0.7)	0.25
ICAM1 rs1799969					
GG	11,783 (85.2)	6,923 (84.9)	6,991 (85.0)	6,924 (84.6)	
GA	1,927 (13.9)	1,153 (14.1)	1,156 (14.1)	1,184 (14.5)	
AA	128 (0.9)	83 (1.0)	74 (0.9)	74 (0.9)	0.91
AGT rs5051					
AA	5,560 (40.1)	3,324 (40.6)	3,329 (40.5)	3,365 (40.9)	
AG	5,541 (40.0)	3,316 (40.5)	3,305 (40.2)	3,287 (40.0)	
GG	2,760 (19.9)	1,554 (19.0)	1,593 (19.4)	1,570 (19.1)	0.64
Panel B gene variant frequencies (col %)					
MMP1 rs1799750					
1G/1G	3,554 (25.8)	2,128 (26.1)	2,118 (25.9)	2,239 (27.4)	0.21

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
1G/2G	6,809 (49.4)	4,029 (49.5)	4,055 (49.5)	3,940 (48.3)	
2G/2G	3,422 (24.8)	1,990 (24.4)	2,016 (24.6)	1,987 (24.3)	
F5 rs6025					
GG	13,451 (97.1)	7,925 (97.0)	7,980 (97.0)	7,932 (96.9)	
GA	395 (2.9)	236 (2.9)	244 (3.0)	253 (3.1)	
AA	7 (0.05)	6 (0.07)	5 (0.06)	2 (0.02)	0.76
NPPA rs5065					
TT	8,278 (59.8)	5,001 (61.3)	4,919 (59.8)	4,980 (60.8)	
TC	4,565 (33.0)	2,599 (31.8)	2,700 (32.8)	2,674 (32.7)	
CC	1,006 (7.3)	565 (6.9)	608 (7.4)	533 (6.5)	0.10
PDE4D rs6450512					
TT	4,424 (33.3)	2,580 (32.9)	2,616 (33.0)	2,527 (32.2)	
TC	5,440 (40.9)	3,213 (40.9)	3,262 (41.2)	3,243 (41.4)	
CC	3,428 (25.8)	2,055 (26.2)	2,041 (25.8)	2,067 (26.4)	0.82
MMP9 rs2274756					
GG	10,004 (72.0)	5,827 (70.8)	5,916 (71.7)	5,939 (72.1)	
GA	3,564 (25.7)	2,196 (26.7)	2,121 (25.7)	2,085 (25.3)	
AA	326 (2.4)	207 (2.5)	219 (2.7)	211 (2.6)	0.33
Panel C gene variant frequencies (col %)					
F12 rs1801020					
CC	4,820 (34.9)	2,855 (35.0)	2,844 (34.7)	2,856 (34.9)	
CT	6,223 (45.0)	3,683 (45.2)	3,680 (44.8)	3,708 (45.3)	
TT	2,787 (20.2)	1,617 (19.8)	1,684 (20.5)	1,614 (19.7)	0.29
AGT rs5051					
AA	5,560 (40.1)	3,324 (40.6)	3,329 (40.5)	3,365 (40.9)	
AG	5,541 (40.0)	3,316 (40.5)	3,305 (40.2)	3,287 (40.0)	
GG	2,760 (19.9)	1,554 (19.0)	1,593 (19.4)	1,570 (19.1)	0.64
PON1 rs705379					
CC	5,646 (41.3)	3,395 (41.9)	3,508 (43.1)	3,372 (41.6)	0.24

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
CT	5,767 (42.2)	3,370 (41.6)	3,354 (41.2)	3,390 (41.9)	
TT	2,270 (16.6)	1,333 (16.5)	1,281 (15.7)	1,338 (16.5)	
MMP12 rs652438					
AA	11,029 (80.6)	6,562 (80.8)	6,652 (81.5)	6,565 (80.8)	
AG	2,423 (17.7)	1,415 (17.4)	1,400 (17.2)	1,426 (17.6)	
GG	235 (1.7)	145 (1.8)	111 (1.4)	132 (1.6)	0.31
PDE4D rs27653					
CC	5,398 (38.8)	3,276 (39.8)	3,180 (38.5)	3,153 (38.3)	
CA	6,401 (46.0)	3,819 (46.4)	3,904 (47.2)	3,884 (47.1)	
AA	2,103 (15.1)	1,134 (13.8)	1,180 (14.3)	1,204 (14.6)	0.06
GP1BA rs6065					
CC	10,482 (75.4)	6,087 (74.0)	6,194 (75.0)	6,179 (75.0)	
CT	3,082 (22.2)	1,922 (23.4)	1,853 (22.4)	1,869 (22.7)	
TT	330 (2.4)	221 (2.7)	210 (2.5)	195 (2.4)	0.29
Panel D gene variant frequencies (col %)					
F2 rs1799963					
GG	13,440 (97.6)	7,917 (97.7)	7,995 (97.9)	7,960 (97.7)	
GA	322 (2.3)	180 (2.2)	168 (2.1)	181 (2.2)	
AA	15 (0.1)	9 (0.1)	7 (0.1)	5 (0.1)	0.74
PAI1 1799768					
5G/5G	4,820 (34.9)	2,855 (35.0)	2,844 (34.7)	2,856 (34.9)	
5G/4G	6,223 (45.0)	3,683 (45.2)	3,680 (44.8)	3,708 (45.3)	
4G/4G	2,787 (20.2)	1,617 (19.8)	1,684 (20.5)	1,614 (19.7)	0.92
MMP7 rs11568818					
AA	4,212 (31.9)	2,553 (32.7)	2,577 (32.7)	2,524 (32.3)	
AG	6,310 (47.8)	3,645 (46.7)	3,702 (47.0)	3,744 (47.9)	
GG	2,677 (20.3)	1,610 (20.6)	1,592 (20.2)	1,549 (19.8)	0.59
AGT rs5050					
AA	9,707 (69.9)	5,724 (69.6)	5,777 (70.0)	5,744 (69.7)	0.99

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
AC	3,811 (27.4)	2,275 (27.7)	2,266 (27.4)	2,265 (27.5)	
CC	376 (2.7)	226 (2.8)	216 (2.6)	227 (2.8)	
ACE rs4343					
AA	4,959 (35.7)	2,833 (34.5)	2,848 (34.6)	2,841 (34.6)	
AG	6,297 (45.4)	3,819 (46.5)	3,772 (45.8)	3,731 (45.4)	
GG	2,625 (18.9)	1,558 (19.0)	1,616 (19.6)	1,642 (20.0)	0.17
MMP2 rs243865					
CC	9,737 (70.1)	5,730 (69.7)	5,741 (69.6)	5,805 (70.5)	
CT	3,624 (26.1)	2,183 (26.6)	2,207 (26.7)	2,124 (25.8)	
TT	521 (3.8)	310 (3.8)	306 (3.7)	303 (3.7)	0.86

SBP = systolic blood pressure, DBP = diastolic blood pressure, LVH = left ventricular hypertrophy

* test of differences between treatment groups: ANOVA for continuous variables, chi-square for categorical variables

Table 2

Genetic variants analyzed

SNP rs number	Gene name	Gene symbol	Alleles
1799752	angiotensin I-converting enzyme	ACE	Insertion/deletion
4363	angiotensin I-converting enzyme	ACE	A/G
4291	angiotensin I-converting enzyme	ACE	A/T
4343	angiotensin I-converting enzyme	ACE	A/G
4961	alpha adducin	ADD1	G/T
1042713	beta-2-adrenergic receptor	ADRB2	G/A
1042714	beta-2-adrenergic receptor	ADRB2	C/G
1800888	beta-2-adrenergic receptor	ADRB2	C/T
5050	angiotensin I; angiotensinogen	AGT	A/C
5051	angiotensin I; angiotensinogen	AGT	A/G
699	angiotensin I; angiotensinogen	AGT	C/T
5186	angiotensin receptor I	AGTR1	A/C
1492078	angiotensin receptor I	AGTR1	A/G
275653	angiotensin receptor I	AGTR1	T/C
676210	apolipoprotein B	APOB	C/T
1042031	apolipoprotein B	APOB	G/A
5742905	cystathionine-beta-synthase	CBS	Ile/thr
1799963	coagulation factor II	F2	G/A
6025	coagulation factor V	F5	G/A
6046	coagulation factor VII	F7	G/A
5742910	coagulation factor VII	F7	Insertion/deletion
7981123	coagulation factor VII	F7	G/T
762637	coagulation factor VII	F7	G/A
1801020	coagulation factor XII	F12	C/T
5982	coagulation factor Xll1, Al subunit	F13	C/T
5985	coagulation factor Xll1, Al subunit	F13	C/T
1800790	fibrinogen, B beta polypeptide	FGB	G/A
7121	guanine nucleotide-binding protein, alpha-stimulating polypeptide 1	GNAS	T/C
5443	guanine nucleotide-binding protein, BETA-3	GNB3	C/T
6065	platelet glycoprotein Ib (alpha polypeptide)	GP1BA	C/T
4069688	platelet glycoprotein Ib (alpha polypeptide)	GP1BA	G/T
2243093	platelet glycoprotein Ib (alpha polypeptide)	GP1BA	T/C
1024323	G protein-dependent receptor kinase 4	GRK4/GPRK2L	C/T
1129292	G protein-dependent receptor kinase 4	GRK4/GPRK2L	C/T
2960306	G protein-dependent receptor kinase 4	GRK4/GPRK2L	G/T
1799969	intercellular adhesion molecule 1	ICAM 1	G/A
1062535	integrin, alpha-2	ITGA2	G/A

SNP rs number	Gene name	Gene symbol	Alleles
5918	integrin, beta-3	ITGB3	T/C
328	lipoprotein lipase	LPL	C/G
1041981	lymphotoxin-alpha	LTA	C/A
1799750	matrix metalloproteinase 1	MMP1	1G/2G
243865	matrix metalloproteinase 2	MMP2	C/T
3025058	matrix metalloproteinase 3	MMP3	5A/6A
11568818	matrix metalloproteinase 7	MMP7	A/G
11568819	matrix metalloproteinase 7	MMP7	C/T
2664538	matrix metalloproteinase 9	MMP9	A/G
2274756	matrix metalloproteinase 9	MMP9	G/A
2276109	matrix metalloproteinase 12	MMP12	A/G
652438	matrix metalloproteinase 12	MMP12	A/G
1801131	methylenetetrahydrofolate reductase	MTHFR	A/C
1801133	methylenetetrahydrofolate reductase	MTHFR	C/T
1799983	Nitric oxide synthase 3	NOS3	G/T
3918226	Nitric oxide synthase 3	NOS3	C/T
1800779	Nitric oxide synthase 3	NOS3	A/G
5065	natriuretic peptide precursor A	NPPA	T/C
5063	natriuretic peptide precursor A	NPPA	G/A
7242	plasminogen activator inhibitor 1	PAI1/SERPINE1	T/G
1799768	plasminogen activator inhibitor 1	PAI1/SERPINE1	5G/4G
27727	phosphodiesterase 4D	PDE4D	A/G
40512	phosphodiesterase 4D	PDE4D	A/G
10074908	phosphodiesterase 4D	PDE4D	A/G
702553	phosphodiesterase 4D	PDE4D	T/A
12188950	phosphodiesterase 4D	PDE4D	G/A
6450512	phosphodiesterase 4D	PDE4D	T/C
153031	phosphodiesterase 4D	PDE4D	A/G
27653	phosphodiesterase 4D	PDE4D	C/A
456009	phosphodiesterase 4D	PDE4D	C/T
705379	paraoxonase I	PON1	C/T
6681776	renin	REN	G/A
2368564	renin	REN	C/T
5742912	sodium channel, nonvoltage-gated 1, alpha subunit	SCNNIA	T/C
2228576	sodium channel, nonvoltage-gated 1, alpha subunit	SCNNIA	G/A
5361	selectin E	SELE	A/C
5355	selectin E	SELE	C/T
361525	tumor necrosis factor	TFN	G/A
673	tumor necrosis factor	TFN	G/A

SNP rs number	Gene name	Gene symbol	Alleles
1800629	tumor necrosis factor	TFN	G/A
1800750	tumor necrosis factor	TFN	G/A

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Table 3

Gene Panel A Score

Gene panel A score*	Chlorthalidone group			Amlodipine group			Lisinopril group		
	Freq. (column %)	**CHD Event freq. (event rate per 1000 p–y)**	**CHD Odds Ratio (95% CI) (adjusted)**	**Freq. (column %)**	**CHD Event freq. (event rate per 1000 p–y)**	**CHD Odds Ratio (95% CI) (adjusted)**	**Freq. (column %)**	**CHD Event freq. (event rate per 1000 p–y)**	**CHD Odds Ratio (95% CI) (adjusted)**
0	**71 (0.5)**	**1 (2.9)**	**1.00**	39 (0.5)	4 (23.0)	1.00	36 (0.5)	5 (29.0)	1.00
1	**896 (6.6)**	**56 (13.7)**	**5.03 (0.68–37.2)**	558 (7.0)	57 (22.7)	1.03 (0.35–3.05)	569 (7.0)	52 (20.3)	0.47 (0.17–1.31)
2	**5008 (36.8)**	**413 (18.0)**	**7.56 (1.04–55.0)**	2913 (36.3)	258 (19.5)	0.92 (0.32–2.66)	2997 (37.1)	233 (17.3)	0.44 (0.17–1.18)
3	**5639 (41.4)**	**531 (20.9)**	**8.34 (1.15–60.6)**	3385 (42.2)	302 (19.4)	0.87 (0.30–2.50)	3331 (41.2)	309 (20.6)	0.52 (0.20–1.38)
4	**1827 (13.4)**	**209 (25.3)**	**9.85 (1.35–71.8)**	1025 (12.8)	95 (20.2)	0.86 (0.29–2.52)	1038 (12.8)	99 (21.0)	0.53 (0.20–1.44)
5	**181 (1.3)**	**28 (33.8)**	**14.0 (1.86–106.1)**	111 (1.4)	13 (26.8)	1.03 (0.31–3.46)	118 (1.5)	11 (20.2)	0.50 (0.16–1.58)
	.	.	**p<0.0001**	.	.	p=.89	.	.	p=0.35

ROC analysis within chlorthalidone group: AUC for established risk factors (A_1)=0.6529, AUC for established risk factors + chlorthalidone gene panel score (A_2)=0.6601, ROC comparison (H_0: A_1=A_2), p=0.004

*Scoring algorithm: NOS3 rs3918226 (C>T) minor allele carriers score +1, SELE rs5361 (A>C) common allele homozygotes score +1, ICAM1 rs1799969 (G>A) common allele homozygotes score +1, AGT rs5051 (A>G) minor allele homozygotes score +1, and GNAS rs7121 (T>C) minor allele carriers score +1

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Table 4

Gene Panel B Score

Gene panel B score*	Chlorthalidone group			Amlodipine group			Lisinopril group		
	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)
0	3849 (29.5)	349 (20.1)	1.00	**2273 (29.7)**	**170 (16.4)**	**1.00**	2319 (29.9)	190 (18.2)	1.00
1	6121 (47.0)	528 (18.9)	0.94 (0.81–1.09)	**3642 (47.5)**	**321 (19.2)**	**1.20 (0.98–1.46)**	3597 (46.4)	327 (20.3)	1.09 (0.90–1.32)
2	2655 (20.4)	272 (22.3)	1.16 (0.98–1.38)	**1507 (19.7)**	**168 (24.5)**	**1.53 (1.21–1.92)**	1565 (20.2)	137 (19.1)	1.04 (0.82–1.31)
3	389 (3.0)	34 (19.7)	0.89 (0.61–1.29)	**237 (3.1)**	**41 (39.6)**	**2.46 (1.68–3.61)**	260 (3.4)	22 (18.8)	1.07 (0.67–1.72)
4+	15 (0.1)	no events	NA	**7 (0.1)**	**1 (26.9)**	**1.94 (0.23–16.5)**	11 (0.1)	no events	NA
	.	.	p=.06	.	.	**p<.0001**	.	.	p=.85

ROC analysis within amlodipine group: AUC for established risk factors (A_1)=0.6429, AUC for established risk factors + amlodipine gene panel score (A_2)=0.6548, ROC comparison (H_0: $A_1=A_2$), p=0.006

*Scoring algorithm: MMP1 rs1799750 (1G/2G) common allele homozygotes score +1, F5 rs6025 (G>A) minor allele carriers score +1, NPPA rs5065 (T>C) minor allele carriers score +1, PDE4D rs6450512 (T>C) minor allele homozygotes score +1, and MMP9 rs2274756 (G>A) minor allele homozygotes score +1

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Table 5

Gene Panel C Score

Gene panel C score*	Chlorthalidone group			Amlodipine group			Lisinopril group		
	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)
0	3202 (24.0)	249 (17.1)	1.00	1986 (25.1)	189 (20.6)	1.00	**2080 (26.1)**	**150 (15.9)**	**1.00**
1	6306 (47.3)	555 (19.4)	1.07 (0.91–1.27)	3721 (47.1)	324 (19.1)	0.87 (0.71–1.07)	**3736 (46.9)**	**303 (18.0)**	**1.07 (0.86–1.33)**
2	3197 (24.0)	319 (22.0)	1.19 (0.99–1.44)	1869 (23.6)	176 (20.7)	0.91 (0.72–1.15)	**1832 (23.0)**	**204 (24.6)**	**1.61 (1.27–2.04)**
3	604 (4.5)	64 (23.0)	1.31 (0.97–1.77)	314 (4.0)	33 (23.3)	0.96 (0.63–1.46)	**307 (3.9)**	**47 (36.8)**	**2.30 (1.59–3.33)**
4+	27 (0.2)	3 (24.4)	1.57 (0.46–5.37)	18 (0.2)	1 (11.7)	0.73 (0.09–5.64)	**13 (0.2)**	**1 (16.4)**	**1.36 (0.17–10.7)**
	.	.	p=0.24	.	.	p=0.77	.	.	**p<0.0001**

ROC analysis within lisinopril group: AUC for established risk factors (A_1)=0.6584, AUC for established risk factors + lisinopril gene panel score (A_2)=0.6693, ROC comparison (H_0: A_1=A_2), p=0.01

*Scoring algorithm: Factor12 rs1801020 (C>T) minor allele homozygotes score +1, GP1BA rs6065 (C>T) minor allele homozygotes score +1, PDE4D rs27653 (C>A) minor allele homozygotes score +1, AGT rs5051 (A>G) minor allele homozygotes score +1, PON1 rs705379 (C>T) minor allele carriers score +1, and MMP12 rs652438 (A>G) minor allele homozygotes score +1

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Table 6

Gene Panel D Score – data from point in time when doxazosin group discontinued

Gene panel D score*	Chlorthalidone group			Amlodipine group			Lisinopril group			Doxazosin group		
	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)	Freq. (column %)	CHD Event freq. (event rate per 1000 p–y)	CHD Odds Ratio (95% CI) (adjusted)
0	2,401 (18.6)	111 (16.6)	1.00	1,411 (18.6)	73 (18.7)	1.00	1,407 (18.3)	71 (18.8)	1.00	**1,409 (18.4)**	**56 (14.1)**	**1.00**
1	5,808 (45.1)	352 (21.9)	1.32 (1.05–1.66)	3,384 (44.6)	193 (20.4)	1.03 (0.77–1.38)	3,426 (44.6)	217 (23.3)	1.16 (0.87–1.55)	**3,427 (44.9)**	**164 (17.4)**	**1.14 (0.83–1.58)**
2	3,788 (29.4)	217 (20.7)	1.20 (0.94–1.55)	2,244 (29.6)	113 (18.1)	0.87 (0.63–1.21)	2,288 (29.8)	113 (17.8)	0.90 (0.65–1.24)	**2,213 (29.0)**	**162 (26.6)**	**1.74 (1.25–2.42)**
3	820 (6.4)	54 (23.8)	1.33 (0.93–1.90)	511 (6.7)	30 (21.5)	1.03 (0.65–1.63)	509 (6.6)	39 (27.0)	1.35 (0.88–2.07)	**551 (7.2)**	**51 (34.0)**	**2.22 (1.46–3.36)**
4+	73 (0.6)	7 (34.0)	1.70 (0.75–3.86)	42 (0.6)	3 (24.6)	1.35 (0.40–4.56)	48 (0.6)	4 (28.9)	1.55 (0.53–4.54)	**40 (0.5)**	**8 (78.9)**	**5.56 (2.38–13.0)**
	.	.	p=0.16	.	.	p=0.70	.	.	p=0.13	.	.	**p<0.0001**

ROC analysis within doxazosin group: AUC for established risk factors (A_1)=0.6516, AUC for established risk factors + doxazosin gene panel score (A_2)=0.6705, ROC comparison (H_0: A_1=A_2), p=0.007

*Scoring algorithm: F2 rs1799963 (G>A) minor allele carriers score +1, PAI1 rs1799768 (5G>4G) minor allele carriers score +1, MMP7 11568818 (A>G) common allele homozygotes score +1, AGT rs5050 (A>C) minor allele homozygotes score +1, ACE rs4343 (A>G) minor allele homozygotes score +1, and MMP2 rs243865 (C>T) minor allele homozygotes score +1

Table 7

Gene function for all panel variants

Panel	Variant	RS number	Gene name	Gene function*
A	NOS3	rs3918226	Nitric oxide synthase 3	Nitric oxide synthases synthesize nitric oxide from L-arginine. Nitric oxide, a reactive free radical, mediates neurotransmission, antimicrobial and antitumoral processes.
	SELE	rs5361	Selectin E	The endothelial protein encoded by SELE is thought to mediate cell adhesion to the vascular lining at sites of inflammation.
	ICAM1	rs1799969	Intercellular adhesion molecule 1	The cell surface glycoprotein encoded by ICAM1 binds to CD11a/CD18 or CD11b/CD18 integrins, and is expressed on endothelial and immune cells.
	AGT	rs5051	Angiotensinogen	Angiotensinogen precursor, the protein encoded by this gene, is cleaved by angiotensin converting enzyme to generate the active enzyme angiotensin II, which is involved in blood pressure regulation.
	GNAS	rs7121	Guanine nucleotide-binding protein, alpha-stimulating polypeptide 1	With a very complex imprinted expression pattern, the transcripts which are derived from four alternative promoters and exons from this gene are maternally, paternally, and biallelically expressed. They may regulate imprinting in the region.
B	MMP1	rs1799750	Matrix metalloproteinase 1	The secreted enzymes encoded by this gene break down interstitial collagens type I, II, III during disease processes as well as normal processes such as tissue remodeling.
	NPPA	rs5065	Natriuretic peptide precursor A	The natriuretic proteins encoded by this gene regulate extracellular fluid volume and electrolyte homeostatis.
	PDE4D	rs6450512	Phosphodiesterase 4D	The protein encoded by this gene has 3′, 5′-cyclic-AMP phosphodiesterase activity, degrades cAMP, acting as a signal transduction molecule for various cell types.
	MMP9	rs2274756	Matrix metalloproteinase 9	The secreted enzymes encoded by this gene break down interstitial collagens type IV and V during disease processes as well as normal processes such as tissue remodeling.
	F5	rs6025	Coagulation factor V	The cofactor encoded by this gene circulates in plasma and is essential in the blood coagulation cascade.
C	AGT	rs5051	Angiotensinogen	Angiotensinogen precursor, the protein encoded by this gene, is cleaved by angiotensin converting enzyme to generate the active enzyme angiotensin II, which is involved in blood pressure regulation.
	PON1	rs705379	Paraoxonase 1	Encodes an enzyme, arylesterase, which hydrolyzes paroxon to form p-nitrophenol.
	MMP12	rs652438	Matrix metalloproteinase 12	The protein encoded by this gene is thought to be cleaved at both ends to produceactive enzyme that breaks down elastin during disease processes as well as normal processes such as tissue remodeling.
	F12	rs1801020	Coagulation factor XII	The gene product, coagulation factor XII, circulates in the blood as a zymogen. Through a cascade of processes this factor is involved in blood coagulation, fibrinolysis, and bradykinin and angiotensin production.
	GP1BA	rs6065	Platelet glycoprotein Ib (alpha polypeptide)	The platelet surface membrane glycoprotein encoded by this gene is involved in platelet activiation, thrombosis, and hemostasis.
	PDE4D	rs27653	Phosphodiesterase 4D	The protein encoded by this gene has 3′, 5′-cyclic-AMP phosphodiesterase activity, degrades cAMP, acting as a signal transduction molecule for various cell types.
D	F2	rs1799963	Coagulation factor II	In the first step of the coagulation process, which suppresses blood loss, coagulation factor II is cleaved to form thrombin.
	PAI1	rs1799768	Plasminogen activator inhibitor 1	The product of this gene inhibits fibrinolysis through the inhibition of tissue plasminogen activator and urokinase.

Panel	Variant	RS number	Gene name	Gene function*
	MMP7	rs11568818	Matrix metalloproteinase 7	The secreted enzymes encoded by this gene break down proteoglycans, fibronectin, elastin and casein during disease processes as well as normal processes such as tissue remodeling.
	AGT	rs5050	Angiotensinogen	Angiotensinogen precursor, the protein encoded by this gene, is cleaved by angiotensinconverting enzyme to generate the active enzyme angiotensin II, which is involved in blood pressure regulation.
	ACE	rs4343	Angiotensin I-converting enzyme	The enzyme encoded by this gene is a catalyst in the conversion of angiotensin I into angiotensin II, which regulates blood pressure and fluid-electorlyte balance through vasopression and aldosterone stimulation.
	MMP2	rs243865	Matrix metalloproteinase 2	The enzymes encoded by this gene break down type IV collagen, which are a structural component of basement membranes, during disease processes as well as normal processes such as tissue remodeling.

*NIH/NCBI