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Antihypertensive Pharmacogenetic Effect of Fibrinogen-beta Variant -455 G>A on Cardiovascular Disease, End-Stage Renal Disease and Mortality: The GenHAT Study

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Abstract

Objective—The *FGB* gene codes for fibrinogen-beta, a polypeptide of the coagulation factor fibrinogen, which is positively associated with cardiovascular diseases. Studies show ACE inhibitors lower plasma fibrinogen concentrations, whereas diuretics and calcium channel blockers do not. Since carriers of the *FGB*-455 minor "A" allele have higher levels of fibrinogen while ACE inhibitors lower it, we hypothesize that "A" allele carriers benefit more from antihypertensive treatment with ACE inhibitors than calcium channel blockers or diuretics, relative to "GG" genotype individuals.

Methods—The GenHAT study (ancillary to ALLHAT) genotyped hypertensive participants for several hypertension-related candidate genes, making this a post-hoc analysis of a randomized trial. In total, 90.1% of the ALLHAT population was successfully genotyped for *FGB-455*. We included participants (n=30,076) randomized to one of three antihypertensive medications (lisinopril, amlodipine, chlorthalidone), with two treatment comparisons: lisinopril versus chlorthalidone and lisinopril versus amlodipine. The primary outcome of ALLHAT/GenHAT was coronary heart disease, defined as fatal CHD or non-fatal MI, and secondary outcomes included stroke, heart failure, all-cause mortality and end-stage renal disease (ESRD) with mean follow-up time of 4.9 years. Genotype-by-treatment interactions (pharmacogenetic effects) were tested with Cox regression.

Results—Stroke: Common "GG" homozygotes had higher risk on lisinopril versus amlodipine (HR=1.38, p<0.001), while minor "A" allele carriers had slightly lower risk (HR=0.96, p=0.76; p-value for interaction=0.03). Mortality: "GG" homozygotes had higher risk on lisinopril versus amlodipine (HR=1.12, p=0.02) or chlorthalidone (1.05, p=0.23), while "A" allele carriers had slightly lower risk (HR=0.92, p=0.33 for lisinopril versus amlodipine, HR=0.88, p=0.08 for lisinopril versus chlorthalidone; p-value for interactions 0.04 and 0.03, respectively). ESRD: "GG" homozygotes had higher risk on lisinopril versus chlorthalidone (HR=1.27, p=0.08), while "A" allele carriers had lower risk (HR=0.64, p=0.12; p-value for interaction=0.03).

Conclusions—There was evidence of pharmacogenetic effects of *FBG-455* on stroke, ESRD and mortality, suggesting that relative to those homozygous for the common allele, variant allele carriers of the *FGB* gene at position -455 have a better outcome if randomized to lisinopril than chlorthalidone

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(for mortality and ESRD) or amlodipine (for mortality and stroke). For the models in which a pharmacogenetic effect was observed, the outcome rates among "GG" homozygotes were higher in those randomized to lisinopril versus amlodipine or chlorthalidone, whereas minor "A" allele carriers had lower event rates when randomized to lisinopril versus the other medications.

Keywords

FGB -455; fibrinogen gene; pharmacogenetics; hypertension; antihypertensive medication; cardiovascular disease

Introduction

There are approximately 72 million people in the U.S. with hypertension.[1] Since hypertension is an important risk factor for cardiovascular disease, knowledge of how a patient will respond to a given medication could enhance efficacy and cause reduced morbidity and mortality. The goal of pharmacogenetics is to tailor pharmacologic treatment to an individual's genotype for the best possible outcome. While some progress has been made on this front for the treatment of hypertension, with the pharmacogenetic effects of renin-angiotensin-aldosterone system genes with ACE inhibitor, angiotensin II blocker and diuretic treatments being the most frequently studied, there is still much to be learned about how antihypertensive medications interact with genes to affect blood pressure-related outcomes for patients.[2]

The *FGB* gene, found on chromosome 4q28, codes for fibrinogen-beta, one of three polypeptides (alpha, beta and gamma) that make up the coagulation factor fibrinogen.[3] Fibrinogen is a plasma glycoprotein which influences clot formation. Plasma fibrinogen concentration has been shown to have a positive association with risk of cardiovascular diseases (CVD) such as coronary heart disease, myocardial infarction, ischemic stroke, as well as end-stage renal disease.[4–16] Importantly, a large meta-analysis of the association between plasma fibrinogen and CVD and mortality used individual data for 154,211 participants from 31 prospective studies to show a moderately strong prospective association between fibrinogen and risk of CHD, stroke and mortality.[17] Several variants within the *FGB* gene have been identified. The *FGB* -455 G>A variant in particular has been shown to be a functional polymorphism, having an effect on the basal rate of gene transcription and plasma fibrinogen concentration, with the minor "A" allele being associated with higher plasma fibrinogen.[5, 18–20] The Framingham Heart Study recently reported that this one variant accounts for approximately 1% of the variance in serum fibrinogen concentration (multivariable model).[21] Further, the *FGB* -455 G>A variant has been positively associated with CVD events in some, but not all, studies. For example, there was no association between FGB -455 and CAD found in one previous study [20], while the minor "A" allele was found to be associated with stroke [21] and red blood cell aggregation among coronary artery disease (CAD) patients [22] in two different studies.

Previous research has also shown that different classes of antihypertensive medication have different effects on plasma fibrinogen. Overall, studies have shown that ACE inhibitors (including lisinopril) lower plasma fibrinogen concentrations, while other classes such as diuretics and calcium channel blockers do not have this effect.[22–25] While no mechanism has been clearly elucidated to explain why ACE inhibitors lead to fibrinogen lowering, it has been postulated that it may be related to inhibition of the hepatic synthesis of fibrinogen [24]. Because of this differential effect of medication class on plasma fibrinogen, there is reason to suspect a pharmacogenetic effect of the *FGB* gene on cardiovascular disease outcomes.

Since there is evidence of an increase in plasma fibrinogen associated with the *FGB* -455 minor "A" allele, and evidence that lisinopril lowers plasma fibrinogen relative to other drug classes,

would GenHAT participants with the minor "A" allele benefit more from treatment with lisinopril, an ACE inhibitor, than treatment with amlodipine, a calcium channel blocker, or chlorthalidone, a diuretic? Because there may be an increased risk of disease among those with the minor allele, we expected participants with the minor allele to experience lower rates of CVD when assigned to lisinopril compared with amlodipine or chlorthalidone, relative to those with the major "GG" genotype, due to the potential fibrinogen-lowering affect of lisinopril.

Methods

Study Population

The Genetics of Hypertension Associated Treatment (GenHAT) study, an ancillary study to the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), was designed with the goal of understanding pharmacogenetic effects on CVD outcomes and blood pressure lowering. GenHAT genotyped 39,114 of the 42,418 ALLHAT participants (92%) with available DNA for variants in several genes implicated in hypertension and stroke etiology. Approximately half of the participants were women (46%), and about half were white non-Hispanic (47%).[26] ALLHAT was a randomized, double-blind, multi-center (623 clinical centers) clinical trial of four antihypertensive medications: a calcium channel blocker (amlodipine), an ACE inhibitor (lisinopril), an alpha-adrenergic blocker (doxazosin), and a diuretic (chlorthalidone). The ratio of medication assignment was 1 : 1 : 1 : 1.7, respectively. ALLHAT was designed to determine if the incidence of fatal coronary heart disease (CHD) and nonfatal myocardial infarction (the primary outcome was the incident of either of these two events) was lower with treatment started with one of three antihypertensive drug classes (calcium channel blocker, ACE inhibitor, alpha-adrenergic blocker) when each was compared to a diuretic-based treatment in high-risk hypertensive patients. Medication doses were titrated to attain control of blood pressure to less than 140/90 mm Hg. At the last clinic visit, the average dose for each treatment group was 7.7, 29.0, and 19.5 mg for the amlodipine, lisinopril and chlorthalidone groups, respectively (doxazosin group discontinued as described below).[27] Secondary endpoints included stroke, heart failure, all-cause mortality, end-stage renal disease (ESRD). In total, ALLHAT randomized 42,418 hypertensive participants aged 55 years and older with one or more additional risk factors for CVD.[28] Of the 30,780 genotyped participants randomized to lisinopril, amlodipine or chlorthalidone, 704 were missing genotype data for the *FGB* -455 variant. Therefore 30,076 participants were included in our analysis with a mean follow-up time of 4.9 years. An additional 2,728 participants were missing one or more of the 12 variables included as covariates in the multivariable models. Therefore, a total of 27,348 participants were included in the fully adjusted analysis of the main effect of the genotype on the outcomes.

This research was approved by local Institutional Review Boards and informed written consent was collected from all ALLHAT participants. Genetic data were made anonymous since GenHAT identifiers were unique, and the code that links the GenHAT to ALLHAT has been destroyed for participants from the Veteran Affairs' study sites. The code for the remaining subjects is offline and stored in a locked file at the ALLHAT coordinating center. Complete descriptions of both ALLHAT and GenHAT study design and rationale have been previously published.[26,28]

Outcome Ascertainment

ALLHAT randomized participants to treatment between February of 1994 and January of 1998, and the follow-up period ended in March of 2002. After a January 2000 review of the data it was decided that the doxazosin arm of the trial would be discontinued due to futility for the primary end point, and a significantly higher incidence of CVD, particularly CHF, when compared with the chlorthalidone arm. This decision was in keeping with *a priori* stopping

guidelines for ALLHAT. [29] Because of the reduced follow-up time for the doxazosin treatment group, and thus fewer clinical outcomes, we have opted not to include the doxazosin group in our analysis.

The outcomes we assessed in this study were the primary outcome of fatal CHD or non-fatal MI (herein referred to as "CHD"), and the secondary outcomes of stroke (including only fatal and hospitalized strokes [cerebral vascular accidents], with no classification by subtype), heart failure (hospitalized or treated in a non-hospital setting), all-cause mortality, and ESRD, defined as the initiation of chronic renal dialysis, kidney transplant or kidney death. The mean follow-up time was 4.9 years. All outcomes were documented and reported by clinical investigators, using a checklist completed at follow-up visits and, if needed, interim reports. For any outcome involving death or hospitalization, documentation such as a hospital discharge summary or death certificate was submitted. To allow the Endpoints Committee to confirm the accuracy of the endpoint diagnoses, the Clinical Trials Center requested more detailed information for a random sample (10%) of hospitalized myocardial infarction and stroke events, such as hospital ECGs and enzyme levels for myocardial infarction cases, and neurologist reports and CT/MRI reports for stroke cases. Detailed descriptions of outcome ascertainment for ALLHAT have been previously published.[28–31]

Genotyping

GenHAT genotyped one *FGB* variant: the *FGB* -455 G>A SNP on chromosome 4q28[32] (SNP database ID rs1800790) in the context of a multi-locus cardiovascular disease SNP panel (Roche Molecular Systems, Pleasanton, CA, USA). *FGB* -455 is a G to A transition at base -455, which is the 5'-flanking region of the *FGB* gene. DNA was isolated on FTA® paper (Fitzco Inc, Maple Plain, MN, USA) from blood samples. 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, essentially as described previously.[33] Genotype calls were made with the assistance of image processing software provided by Roche Molecular Systems.

Statistical Analysis

STATA© version 9.2 (STATA Corporation, College Station, Texas) was used for all analyses. Hardy-Weinberg (HW) equilibrium was assessed using a chi-square goodness-of-fit test. Cox proportional hazards regression was used to determine whether there was a gene-by-treatment interaction (pharmacogenetic effect) on the rates of the primary outcome of CHD, and also for secondary outcomes of stroke, heart failure, all-cause mortality, and ESRD. Due to small numbers of "AA" minor allele homozygotes and a resulting small number of events in that group, genotypes were collapsed into two categories resulting in dominant models of inheritance: "GG" homozygotes and "GA + AA" minor allele carriers. We undertook two comparisons of the randomized treatment groups: We compared the lisinopril group to those randomized to chlorthalidone, and the lisinopril group to those randomized to amlodipine. The test of interest was the statistical significance of the (gene * treatment) interaction term, which results in a ratio of hazard ratios (RHR) point estimate. We assessed the main effects of the genetic variant on outcomes using Cox regression after adjusting for age, sex, race and Hispanic status (race data was collected in 5 study-defined self-reported categories: White, Black, Asian/Pacific Islander, American Indian/Alaskan Native, "Other"; Hispanic status was collected in 3 self-reported categories: "yes", "no" and "don't know"), baseline body mass index (BMI), type 2 diabetes status (yes/no), baseline LDL and HDL cholesterol, smoking status (yes/no), baseline systolic and diastolic blood pressures, and aspirin use (yes/no). For each outcome we tested whether there was a detectable (gene * race) interaction modeled both in the 5 race categories and with participants stratified as black/non-black. Using Cox regression, we also assessed the main effect of treatment assignment on each outcome to compare the results for

this GenHAT subgroup to the previously published results for the full ALLHAT population. For the main effects of treatment assignment we compared chlorthalidone versus lisinopril, chlorthalidone versus amlodipine, and amlodipine versus lisinopril for each outcome. Since GenHAT identified sex, race and diabetes status as being of interest for sub-group analyses, we also tested for 3-way gene-treatment-sex, -race, and -diabetes interactions by adding the appropriate 3-way interaction term to the model.

Results

Baseline characteristics according to treatment assignment for participants are described in Table 1. There were no significant differences for baseline values between the groups. The *FGB -455* genotype frequencies were in HW equilibrium when assessed in a race/Hispanic-specific manner in all groups except the Black non-Hispanic group (p=0.02). The event rates per 1000 person-years for each of the outcomes ranged from 18.5–20.8 for CHD, 8.1–11.1 for stroke, 12.1–17.6 for heart failure, 25.6–30.7 for all-cause mortality, and 1.6–3.4 for end-stage renal disease, depending upon genotype/treatment category.

Main Effect of FGB Variant and Treatment Assignment

The main effect of the *FGB* -455 variant on the five outcomes can be found in Table 2. There was no evidence of an effect of the FGB -455 variant on any of the outcomes in either the minimally adjusted models (age, sex, race/Hispanic status) or the fully adjusted models (age, sex, race/Hispanic status, BMI, type 2 diabetes status, baseline LDL and HDL cholesterol, smoking status, baseline systolic and diastolic blood pressures, and aspirin). The only outcome that approached a significant association with the variant was stroke: Carriers of the minor “A” allele had higher risk of stroke than the more common “GG” homozygotes (HR=1.11 (0.98–1.26), p=0.09 in the minimally adjusted model, HR=1.14 (1.00–1.30), p=0.06 in the fully adjusted model). We also tested whether race (both in 5 categories and stratified by black/non-black status) was an effect modifier in the association between the *FGB* -455 genotype and each outcome. No such interaction was observed (data not shown).

We found evidence of a main effect of treatment assignment on the outcomes of heart failure and stroke. Assignment to chlorthalidone was protective compared to the lisinopril and the amlodipine groups for heart failure (HR=0.82 (0.74–0.91); p<0.001 for chlorthalidone vs. lisinopril; HR=0.71 (0.64–0.79), p<0.001 for chlorthalidone vs. amlodipine), with those on amlodipine being at higher risk than those on lisinopril (HR=1.15 (1.03–1.29); p=0.02 for amlodipine vs. lisinopril). Assignment to chlorthalidone or amlodipine versus lisinopril was protective for stroke (HR=0.87 (0.76–0.98); p=0.03 for chlorthalidone vs. lisinopril, HR=0.81 (0.70–0.93); p=0.004 for amlodipine vs. lisinopril) As in the full ALLHAT population, there was a difference in treatment effect between black and non-black participants for stroke in this GenHAT subpopulation, with a significant treatment effect between chlorthalidone and lisinopril found only among blacks [HR=0.69 (0.57–0.84); p<0.001 for blacks, HR=1.02 (0.87–1.21); p=0.80 for non-blacks; interaction p=0.002] (data not shown).

Pharmacogenetic Effects (Genotype-by-Treatment Interactions)

The results for tests of pharmacogenetic effects can be found in Table 3 (total number of events, event rates per 1000 person-years, genotype-specific treatment effects, and genotype-by-treatment interaction RHRs and accompanying p-values). A pharmacogenetic effect (RHR) equal to 1 indicates that the effect of the minor allele in a dominant model is equal in the two treatment groups (or likewise, the treatment effect is equal in the two genotype groups). However, a departure from the null value of 1 indicates the effect of the minor allele differs by treatment (or likewise, the effect of treatment differs by genotype). For stroke, common “GG” homozygotes had higher risk on lisinopril versus amlodipine (HR=1.38, p<0.001), while

minor “A” allele carriers had slightly lower risk (HR=0.96, p=0.76; p-value for interaction=0.03). For all-cause mortality, “GG” homozygotes had higher risk on lisinopril versus amlodipine (HR=1.12, p=0.02) or chlorthalidone (1.05, p=0.23), while “A” allele carriers had slightly lower risk (HR=0.92, p=0.33 for lisinopril versus amlodipine, HR=0.88, p=0.08 for lisinopril versus chlorthalidone; p-value for interactions=0.04 and 0.03, respectively). For ESRD, “GG” homozygotes had higher risk on lisinopril versus chlorthalidone (HR=1.27, p=0.08), while “A” allele carriers had lower risk (HR=0.64, p=0.12; p-value for interaction=0.03). There was no evidence of a similar pharmacogenetic effect on CHD or heart failure. Therefore, in the models showing evidence of a pharmacogenetic effect, when compared to “GG” homozygotes, participants with at least one copy of the minor “A” allele had a *decreased* risk of stroke, ESRD or all-cause mortality when randomized to lisinopril than when randomized to amlodipine or chlorthalidone, whereas “GG” homozygotes had an *increased* risk of stroke, ESRD or all-cause mortality when randomized to lisinopril versus amlodipine or chlorthalidone.

We found no evidence of 3-way gene-treatment-sex, -race, and -diabetes interactions (data not shown). Since recently published ALLHAT results showed that among black participants, metabolic syndrome may be an effect modifier in the relation between treatment with chlorthalidone versus lisinopril and ESRD[34], we tested whether there was a 3-way (genotype-treatment-metabolic syndrome) interaction among the black participants in this GenHAT subgroup. We observed no such interaction (data not shown).

Discussion

We report evidence of a pharmacogenetic effect of the *FGB* -455 variant on stroke, ESRD and all-cause mortality, but no similar effect on CHD or heart failure. There was no significant main effect of the *FGB* -455 variant detected for any of the outcomes in the multivariable models. The ALLHAT investigators have previously published the effect of treatment assignment on CVD outcomes.[29–31] ALLHAT showed that participants randomized to chlorthalidone had lower rates of some CVD outcomes such as stroke and heart failure than those randomized to either lisinopril or amlodipine [30]. Analogous to the ALLHAT findings, we found evidence of significant effects of treatment assignment on heart failure and stroke in this GenHAT subpopulation of 30,076 participants. However, this study also shows that antihypertensive treatment effects differed by *FGB* -455 genotype group in terms of stroke, ESRD and all-cause mortality events, thus identifying this variant as a possible treatment effect modifier. As hypothesized, carriers of the variant allele of the *FGB* gene at position -455 have lower rates of stroke, ESRD and total mortality when randomized to lisinopril than chlorthalidone or amlodipine treatment, whereas those participants who were homozygous for the common allele had higher outcome rates on lisinopril versus chlorthalidone or amlodipine in the models showing evidence of a pharmacogenetic effect. Mechanistically, this may be due to the possible fibrinogen lowering affects of lisinopril, which would be particularly beneficial for those with a genetic predisposition to higher plasma fibrinogen concentrations. We do not have plasma fibrinogen measurements for ALLHAT participants, which would have provided direct information about the *FGB* genotype-specific and treatment-specific effects on plasma fibrinogen for this study population. In addition, our analysis of the main effect of this *FGB* variant on the outcomes included in this study did not identify a clear “high risk” genotype. The minor allele carriers had a higher risk of stroke, though this association did not reach statistical significance (HR=1.14 [1.00–1.30]. Since the goal of pharmacogenetics is to identify particular sub-groups (genotype groups) for whom the best treatment option may be different than for another sub-group, it is possible to provide evidence of pharmacogenetic associations without identifying a putative allele for the overall population.

Caution must be used in generalizing these findings to a younger and healthier population, since ALLHAT included only older, hypertensive participants with other risk factors for CVD. Additionally, since only one *FGB* variant was typed by GenHAT, we cannot view this study as a complete evaluation of the pharmacogenetic effects of the *FGB* gene. Since we performed multiple tests of pharmacogenetic effects, these findings would not meet the threshold of statistical significance if corrected for multiple testing (e.g., Bonferroni correction: 0.05/10 tests would equate to a p-value of 0.005). Therefore, we cannot rule out chance as a possible explanation for the findings.

To our knowledge, this study is the first to examine the pharmacogenetic associations between the *FGB* -455 variant and hypertension treatment, with the unique benefit of a very large and diverse study population. Limitations notwithstanding, these findings underline the importance of pharmacogenetic research, and encourage further study of the pharmacogenetic effects of the *FGB* gene in other populations and for other variants within the *FGB* gene. Despite optimism that predictive pre-prescription genotyping is on the horizon, clinical applications of pharmacogenetic findings are still largely pending reproducible prospective investigations. It is particularly challenging to unravel the underlying pharmacogenetic effects of antihypertensive medications, given the complexity of hypertension and its treatment. More research such as that reported here will be necessary to meet the ultimate goal of pharmacogenetic studies, which is to tailor pharmacologic treatment to an individual's genotype for the best possible outcome for patients.

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Table 1

Baseline characteristics for participants (n=30,076) by treatment group

Characteristic	Lisinopril	Amlodipine	Chlorthalidone	p-value*
Sample size by treatment	8,189	8,112	13,775	
Age (y), mean (SD)	66.8 (7.8)	66.9 (7.7)	66.8 (7.7)	0.93
Race, n (column %)				0.86
White	4,970 (60.7)	4,916 (60.6)	8,373 (60.8)	
Black	2,811 (34.3)	2,808 (34.6)	4,708 (34.2)	
American Indian/Alaskan native	18 (0.2)	19 (0.2)	27 (0.2)	
Asian/Pacific Islander	84 (1.0)	96 (1.2)	167 (1.2)	
Other	306 (3.7)	273 (3.4)	500 (3.)	
Hispanic, n (%)	1,626 (19.9)	1,544 (19.0)	2,696 (19.6)	0.73
Women, n (%)	3,793 (46.3)	3,864 (47.6)	6,476 (47.0)	0.24
Previous antihypertensive treatment, n (%)	7,373 (90.0)	7,346 (90.6)	12,426 (90.2)	0.52
Blood pressure, mm Hg:				
All participants				
SBP, mean (SD)	146.6 (15.6)	146.2 (15.7)	146.3 (15.7)	0.27
DBP, mean (SD)	84.2 (10.0)	83.9 (10.2)	84.1 (10.1)	0.18
Treated at baseline				
SBP, mean (SD)	145.5 (15.5)	145.1 (15.6)	145.2 (15.7)	0.35
DBP, mean (SD)	83.6 (9.9)	83.3 (10.0)	83.5 (10.0)	0.12
Untreated at baseline				
SBP, mean (SD)	156.4 (12.4)	156.5 (12.2)	156.1 (12.0)	0.68
DBP, mean (SD)	89.1 (9.3)	89.7 (9.6)	89.5 (9.0)	0.46
Eligibility risk factors:				
Cigarette smoker, n (%)	1,793 (21.9)	1,792 (22.1)	3,042 (22.1)	0.94
Type 2 diabetes, n (%)	2,867 (35.0)	2,954 (36.4)	4,938 (35.9)	0.17
HDL-C < 35 mg/dL, n (%)	963 (11.8)	923 (11.4)	1,650 (12.0)	0.41
LVH by electrocardiogram, n (%)	1,325 (16.2)	1,378 (17.0)	2,219 (16.1)	0.21
BMI, kg/m2, mean (SD)	29.8 (6.2)	29.8 (6.3)	29.7 (6.1)	0.49
Aspirin use, n (%)	2,976 (36.3)	2,950 (36.4)	4,919 (35.7)	0.59
Fasting glucose, mg/dL, mean (SD)	122.4 (55.9)	122.9 (57.3)	123.3 (58.5)	0.62
LDL cholesterol, mg/dL, mean (SD)	135.9 (36.3)	135.8 (37.3)	136.0 (37.4)	0.95
HDL cholesterol, mg/dL, mean (SD)	46.6 (14.6)	47.1 (14.7)	46.7 (14.9)	0.06
Fasting triglycerides, mg/dL, mean (SD)	175.5 (138.9)	176.9 (133.2)	177.2 (132.6)	0.67
Glomerular filtration rate (GFR), mean (SD)	77.7 (19.9)	78.1 (19.6)	77.6 (19.6)	0.15
FGB -455, n (column %)				0.30
GG	5,919 (72.3)	5,817 (71.7)	10,038 (72.9)	
GA	2,030 (24.8)	2,029 (25.0)	3,330 (24.2)	
AA	240 (2.9)	266 (3.3)	407 (3.0)	

SBP = systolic blood pressure, DBP = diastolic blood pressure, HDL-C = HDL cholesterol, LVH = left ventricular hypertrophy

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

Table 2

Main effect of FGB -455 variant on outcomes, n=30,076

Outcome	Total number of events		Event rate per 1000 person years		Main effect of genotype			
					Minimally adjusted*, Hazard Ratios (95% CI), p-value		Fully adjusted models**, Hazard Ratios (95% CI), p-value	
	GG (n=21,774)	GA + AA (n=8,302)	GG	GA + AA	GG	GA + AA	GG	GA + AA
CHD	1901	759	19.0	20.1	1.00	1.00 (0.91–1.09), p=0.94	1.00	1.01 (0.92–1.11), p=0.83
Stroke	970	373	9.6	9.7	1.00	1.11 (0.98–1.26), p=0.09	1.00	1.14 (1.00–1.30), p=0.06
Heart failure	1466	534	14.6	14.0	1.00	0.95 (0.86–1.05), p=0.33	1.00	0.99 (0.89–1.10), p=0.86
All-cause mortality	3115	1117	29.1	27.7	1.00	0.98 (0.91–1.05), p=0.59	1.00	1.00 (0.93–1.08), p=0.98
End stage renal disease	306	89	2.9	2.3	1.00	0.98 (0.76–1.25), p=0.87	1.00	0.95 (0.72–1.25), p=0.73

CHD = coronary heart disease

* adjusted for age, sex, race, Hispanic status

** adjusted for age, sex, race, Hispanic status, baseline BMI, diabetes status, baseline LDL and HDL cholesterol, smoking status, baseline systolic and diastolic blood pressures, aspirin use

Table 3

Total events and event rates by genotype and treatment group, genotype-by-treatment interaction results

Outcome	FGB -455 Genotype	Total number of events			Event rate per 1000 person-years			Genotype-specific treatment effect HR (95% CI), p-value		Genotype-by-treatment interactions (pharmacogenetic effects) RHR (95% CI), p-value	
		LIS	AML	CHL	LIS	AML	CHL	LIS vs. AML	LIS vs. CHL	LIS vs. AML, GA+AA vs. GG	LIS vs. CHL, GA+AA vs. GG
CHD	GG	501	523	877	18.5	19.4	19.0	0.95 (0.84–1.08), p=0.45	0.97 (0.87–1.09), p=0.65	1.09 (0.86–1.37), p=0.49	0.98 (0.80–1.20), p=0.84
	GA+AA	204	201	354	19.8	19.1	20.8	1.04 (0.85–1.26), p=0.72	0.95 (0.80–1.13), p=0.60		
Stroke	GG	304	221	445	11.1	8.1	9.5	1.38 (1.16–1.64), p<0.001	1.17 (1.01–1.35), p=0.04	**0.70 (0.51–0.96), p=0.03**	0.95 (0.71–1.26), p=0.72
	GA+AA	105	111	157	10.1	10.5	9.1	0.96 (0.73–1.25), p=0.76	1.11 (0.87–1.42), p=0.41		
Heart failure	GG	426	473	567	15.8	17.6	12.1	0.90 (0.79–1.02), p=0.11	1.30 (1.15–1.47), p<0.001	0.89 (0.68–1.15), p=0.37	0.79 (0.61–1.01), p=0.06
	GA+AA	137	174	223	13.2	16.6	13.0	0.80 (0.64–0.99), p=0.05	1.02 (0.83–1.26), p=0.84		
All-cause mortality	GG	889	790	1436	30.7	27.5	29.1	1.12 (1.02–1.23), p=0.02	1.05 (0.97–1.14), p=0.23	**0.82 (0.68–0.99), p=0.04**	**0.83 (0.71–0.99), p=0.03**
	GA+AA	283	307	527	25.6	27.6	29.1	0.92 (0.78–1.08), p=0.33	0.88 (0.76–1.02), p=0.08		
End stage renal disease	GG	95	83	128	3.4	3.0	2.7	1.15 (0.86–1.54), p=0.36	1.27 (0.97–1.65), p=0.08	0.53 (0.27–1.04), p=0.07	**0.50 (0.27–0.93), p=0.03**
	GA+AA	17	28	44	1.6	2.6	2.5	0.61 (0.34–1.12), p=0.11	0.64 (0.36–1.12), p=0.12		

CHL= chlorthalidone, AML = amlodipine, LIS = lisinopril, CHD = coronary heart disease