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RYR3 gene polymorphisms and cardiovascular disease outcomes in the context of antihypertensive treatment

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Abstract

Nearly one-third of adults in the U.S. have hypertension, which is associated with increased cardiovascular disease (CVD) morbidity and mortality. The goal of antihypertensive pharmacogenetic research is to enhance understanding of drug response based on the interaction of individual genetic architecture and antihypertensive therapy to improve blood pressure control and ultimately prevent CVD outcomes. In the context of the Genetics of Hypertension Associated Treatment (GenHAT) study and using a case-only design, we examined whether single nucleotide polymorphisms in *RYR3* interact with four classes of antihypertensive drugs, particularly the calcium channel blocker amlodipine versus other classes, to modify the risk of coronary heart disease (CHD; fatal CHD and non-fatal myocardial infarction combined) and heart failure in high-risk hypertensive individuals. RYR3 mediates the mobilization of stored Ca^{+2} in cardiac and skeletal muscle to initiate muscle contraction. There was suggestive evidence of pharmacogenetic effects on heart failure, the strongest of which was for rs877087, with the smallest p-value =.0005 for the codominant model when comparing amlodipine versus all other treatments. There were no pharmacogenetic effects observed for CHD. The findings reported here for the case-only analysis of the antihypertensive pharmacogenetic effect of *RYR3* among 3,058 CHD cases and 1,940 heart failure cases show that a hypertensive patient's genetic profile may help predict which medication(s) might better lower cardiovascular disease risk.

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Conflict of Interest

None

Keywords

RYR3 gene; calcium channel blocker; hypertension; coronary heart disease; heart failure; genetic interaction

Introduction

Nearly one-third of adults in the U.S. have hypertension, which is associated with increased cardiovascular disease (CVD) morbidity and mortality.[1] Antihypertensive treatment is effective, but it has been estimated only 70% of those treated reach treatment goals.[2] Using genetics to characterize response to antihypertensive treatment is an important personalized medicine initiative aimed to improve drug efficacy. Specifically, the goal of antihypertensive pharmacogenetic research is to enhance the understanding of drug response based on the interaction of individual genetic architecture and antihypertensive therapy to improve blood pressure (BP) control and ultimately prevent CVD outcomes.

In the cardiovascular system, Ca^{+2} is essential for cardiac muscle contraction and relaxation, and acts as a second messenger in signal transduction pathways. Complex mechanisms regulate intracellular free calcium levels in the heart and vasculature, and a failure of these systems to maintain normal Ca^{+2} homeostasis has been linked to hypertension and other CVD outcomes.[3] A potential candidate gene for antihypertensive pharmacogenetic study is the ryanodine receptor 3 gene (*RYR3*) in chromosome 15, which belongs to a class of intracellular calcium channels. RYR3 has been shown to mediate the mobilization of stored Ca^{+2} in cardiac and skeletal muscle to initiate muscle contraction. Supporting its potential role in CVD, *RYR3* is expressed in human arterial endothelial cells.[4] In a recent genome-wide association (GWA) study of HIV-positive males receiving highly active antiretroviral therapy (HAART), two *RYR3* SNPs (non-synonymous rs2229116 and intronic rs7177922), in high linkage disequilibrium (r^2=0.97), were associated with increased common carotid intima-media thickness (IMT), a subclinical marker for atherosclerosis.[5] There are published reports of associations between RYR and heart failure (HF), and importantly there was a recent meta-analysis published showing an association between calcium channel blockers (CCBs) and HF.[6–8] This previous research provides support for a possible pharmacogenetic association with *RYR3* on CVD.

In the current study, we examined whether single nucleotide polymorphisms (SNPs) in *RYR3* interact with four classes of antihypertensive treatment, particularly a CCB versus other classes, to modify the risk of coronary heart disease (CHD) and HF in high-risk hypertensive individuals. The hypothesis is tested among participants of the largest antihypertensive trial to date, the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT).[9] Further, knowledge about the interactions of *RYR3* with antihypertensive drugs could provide insights into the specific pathways in which this gene might be involved in CVD.

Methods

Study Design and Population

The participants of this study were part of the Genetics of Hypertension Associated Treatment (GenHAT) study, an ancillary study of ALLHAT. The study design and methodology of GenHAT and ALLHAT have been previously described in detail.[9, 10] Briefly, ALLHAT was a randomized, double-blind, multicenter (623 sites) clinical trial of 42,418 hypertensive adults aged 55 years and older designed to determine if the incidence of fatal CHD and nonfatal myocardial infarction (MI) was lower among patients randomized to one of four antihypertensive drug classes: a CCB (amlodipine), an angiotensin converting enzyme (ACE) inhibitor (lisinopril), and an α-adrenergic blocker (doxazosin), each compared with a diuretic (chlorthalidone), in an assignment ratio of 1:1:1:1.7, respectively. Treatment was given once daily and was titrated to achieve BP of ≤ 140/90 mm Hg. Open label antihypertensive drugs were also added as needed to reach treatment goals. Due to early termination of the doxazosin arm owing to futility for the primary outcome and a significant increase in the secondary outcome of CVD compared with the chlorthalidone arm,[11] follow-up continued for an average of 3.2 years for the doxazosin arm and 4.9 years for all other treatment arms. In the case-only phase of GenHAT, 11,599 ALLHAT participants who experienced an adverse event (fatal CHD or nonfatal MI, stroke, HF, coronary revascularization, angina, peripheral arterial disease, end-stage renal disease, all-cause death), were successfully genotyped for about 600 polymorphisms in genes selected for their associations with BP regulation and CVD, with the goal of discovering pharmacogenetic associations with the genes.

This study focuses on two possible outcomes: the primary CHD outcome (fatal CHD and non-fatal MI), and the secondary outcome of HF (fatal or hospitalized). Outcomes were reported by clinical investigators, and documentation (death certificate, hospital discharge summary) was submitted for any outcome involving death or hospitalization. National databases (Center for Medicare and Medicaid Services, the Department of Veteran's Affairs, the National Death Index, and the Social Security Administration) were also used to identify deaths occurring among participants lost to follow-up. A complete description of outcome ascertainment has been previously published.[9, 11, 12]

Genotyping Methods

DNA was isolated on FTA® paper (Fitzco Inc, Maple Plain, MN, USA) from blood samples. Our study was ancillary to the case-only phase of GenHAT and utilized three polymorphisms (rs877087, rs2077268 and rs4780144) in the *RYR3* gene from the custom Illumina® (San Diego, CA, USA) genotyping of SNPs in several candidate genes for blood pressure regulation or CVD. Based on the role of Ca+ channel, *RYR* gene family and previous associations with cardiovascular outcomes including HF and atherosclerosis, only polymorphisms in *RYR3* gene were examined in this hypothesis-based study. The sample success rate was above 97% and reproducibility was approximately 99.99% for the three SNPs with the duplicate samples.

Statistical Methods

To test for differences in baseline measurements between treatment groups, we used ANOVA for continuous variables and chi-square tests for categorical variables. Logistic regression was used to test for pharmacogenetic (gene-by-treatment) effects among the case groups, with treatment group modeled as the dependent variable and genotype as the independent variable. The amlodipine group was compared to each of the other three treatment groups separately, as well as combined. Two genetic models were tested: co-dominant (three genotype groups, common homozygote as referent group, 2 beta-coefficient estimates – one for heterozygotes, one for rare homozygotes, 2 df test), and additive (three genotype groups, common homozygote as referent group, 1 beta-coefficient estimate, which estimates "risk" per copy of minor allele, 1 df test). All comparisons with the doxazosin treatment group were performed using a dataset limited to follow-up events to the time at which the doxazosin arm was discontinued. Separate analyses were performed for non-Hispanic whites and African-American populations based on the genetic ethnicity determined by the principal component analysis (PCA) of 64 ancestry informative markers (AIMs). A p-value of .05 was considered suggestive evidence for an association; however, Bonferroni correction was considered to adjust for multiple testing. All statistical analyses were performed using STATA© version 10.1 (STATA Corporation, College Station, Texas).

Results

A description of baseline characteristics for the 11,599 participants in the GenHAT "case-only" study is provided in Table 1, including allele frequencies for the three *RYR3* variants. The only differences between treatment groups at baseline involved BP: the group randomized to amlodipine had slightly lower mean systolic (S)BP and diastolic (D)BP (small but statistically significant) when compared to the other treatment groups (mean SBP: 146.2, 147.0, 147.4, 147.4 mmHg; mean DBP: 82.2, 82.8, 83.0, 82.5 mmHg for amlodipine, chlorthalidone, lisinopril, doxazosin, respectively; p=.03 and p=.02 for SBP and DBP, respectively). Based on the AIMs, the first three principal component values were used to discriminate individuals into four major race/ethnic groups: non-Hispanic whites, African-American, Asian, and a heterogenous Hispanic ancestry. However, based on the number of cases, only the non-Hispanic whites and African-American groups had enough statistical power to perform ethnicity-specific analysis. For the African-American group, all 3 variants were in Hardy-Weinberg equilibrium (HWE) (rs877087: p=.26, rs2077268: p=.15, rs4780144: p=.16). For the European-American group, rs877087 and rs2077268 were in HWE (p=.24 and p=.68, respectively), whereas rs4780144 was not (p=.0001). It is difficult to interpret a lack of HWE in a case-only design, because alleles associated with CHD or HF events may be more common among this group. The total number of cases of CHD and HF were 3,058 and 1,940, respectively. For the race-specific analysis, there were 1579 and 967 cases of CHD and HF, respectively for the non-Hispanic white group, and 791 and 572 cases of CHD and HF, respectively for the African-American group.

Table 2 presents the pharmacogenetic findings for the chlorthalidone, amlodipine and lisinopril groups using full follow-up data. For HF, there were suggestive pharmacogenetic

findings for rs877087 and rs2077268. For rs877087, minor allele homozygotes fared worse (and common allele carriers overall fared better) when randomized to amlodipine versus either chlorthalidone or lisinopril: minor allele homozygotes (TT) constituted 21%, 26% and 19% of cases, heterozygotes (TC) constituted 42%, 52% and 46% of cases, and common homozygotes (CC) constituted 32%, 29% and 33% of cases in the amlodipine, chlorthalidone and lisinopril groups, respectively (pharmacogenetic p-value was p=.005 for codominant model - amlodipine versus chlorthalidone; p=.01 for codominant model – amlodipine versus lisinopril and chlorthalidone). For rs2077268, there was a difference between the amlodipine and chlorthalidone groups, with the minor allele homozygotes faring worse (and common allele carriers faring better) on amlodipine versus chlorthalidone: minor allele homozygotes (TT) constituted 5% and 2% of cases, heterozygotes (TC) constituted 26%, and 26% of cases, and common homozygotes (CC) constituted 69% and 72% of cases in the amlodipine and chlorthalidone groups, respectively (pharmacogenetic p-value was p=.01 for codominant and p=.04 for additive model - amlodipine versus chlorthalidone).

Table 3 presents the pharmacogenetic findings for doxazosin comparisons including only cases occurring before the discontinuation of the doxazosin arm. For CHD, there were no suggestive findings. For HF, there were no pharmacogenetic effects observed for rs2077268 or rs4780144. The suggestive findings for rs877087, however, were made stronger with the addition of the doxazosin cases: Common allele homozygotes (CC) constituted 31%, 31%, 32%, and 29% of cases, heterozygotes (TC) constituted 39%, 49%, 47%, and 52% of cases, and minor allele homozygotes (TT) constituted 30%, 19%, 21%, and 19% of cases in the amlodipine, chlorthalidone, lisinopril, and doxazosin groups, respectively (pharmacogenetic p-values were p=.001 for codominant model - amlodipine versus doxazosin; p=.0005 for codominant model and p=.04 for additive model - amlodipine versus all other treatments).

Race-specific analyses did not yield significant race-specific pharmacogenetic findings that were not present in the full group. The only suggestive race-specific pharmacogenetic findings mirrored the findings for the full group: The findings for rs877087 for the doxazosin comparisons were suggestive among African Americans for HF when analyzed separately (p=.04 for codominant model – amlodipine versus doxazosin; p=.02 for codominant model – amlodipine versus all other treatments) (data not shown).

Discussion

Previous research has provided a biologically plausible mechanism through which *RYR3* activity could be associated with CHD and HF, [7, 8, 13–15] and the ways in which antihypertensive drugs, particularly CCBs versus other drug classes, interact with this gene to affect these outcomes is of particular interest. Given the role of *RYR3* on CA^{+2} mobilization, it is interesting that our strongest association was found for HF when comparing the CCB amlodipine to the other 3 drug classes: rs877087 minor allele homozygotes had higher risk of HF (and common allele carriers had lower risk) when randomized to amlodipine, compared to all other treatments (p=.0005 for codominant model). If we correct for multiple testing even with a stringent Bonferroni adjustment, given our 60 pharmacogenetic tests (.05/60=.0008) this finding reaches statistical significance.

Importantly, a large meta-analysis of over 156,000 hypertensive patients randomized to CCB or other classes of antihypertensive treatment (including ALLHAT) with a total of 5,049 events reported a significant increase in incident HF in patients allocated to CCBs (odds ratio 1.18, 95% confidence interval 1.07 to 1.31).[6] Future pharmacogenetic studies based on the present RYR3 genotype findings may help define a patient subgroup at even higher risk of an event who may benefit from treatment with other classes of antihypertensive agents.

The three SNPs analyzed here are not in tight linkage disequilibrium: the correlation coefficients for rs877087-rs2077268, rs877087-rs4780144 and rs4780144-rs2077268 were r^2=0.02, r^2=.004 and r^2=.005, respectively in the HapMap CEU population panel (Utah residents with Northern and Western European ancestry), and r^2=0.39, r^2=0.006 and r^2=.005, respectively in HapMap YRI population panel (Yoruba residents of Ibadan, Nigeria), as calculated using SNAP (SNP Annotation and Proxy Search).[16] We are unaware of any previous study of the antihypertensive pharmacogenetic effect of these SNPs; however, rs877087 has been associated with stroke in a GWA study.[17]

The case-only design has been demonstrated to be more efficient for detection of gene-environment interaction - including gene-treatment (pharmacogenetic) interactions - than the case-control design, and provides an unbiased estimate of the interaction under certain assumptions.[18, 19] One fundamental assumption for the case-only design is that there is no association between the genotype and the “exposure” – in this case the antihypertensive treatment. Since ALLHAT is a randomized clinical trial, genotype and treatment should be independent since treatment is randomly allocated. However, the assumption of non-dependency between genotype and treatment in the population must be verified, and from Table 1 we can confirm the lack of association between the two factors. A second assumption for the case-only design is that the outcome or disease is rare (i.e., < 5%) so that the odds ratio approximates the risk ratio. In a prospective study, such as a clinical trial, we have shown that the assumption of low disease risk is not necessary, as the interaction is a ratio of risk ratios on a multiplicative scale.[19] If these two assumptions of the case-only design are met, the case-only study is more powerful in estimating the interaction than the case-control approach for a fixed sample-size of cases.[20]

Some of the limitations of this study include the fact that ALLHAT recruited only patients aged ≥ 55 years with hypertension and other CVD risk factors, so the generalizability of these findings to a younger and healthier population may not be valid. It is possible that the role of *RYR3* is age dependent, i.e., it’s individual or interactive impact is more prominent at a younger age. At an older age, due to several other related co-morbidities, the effect could be diluted. However, the suggestive interactions in our study warrant further investigation in both younger and older populations. A limitation of the case-only design is that it is impossible to assess the effects of either the treatments or genotype on the outcomes independently. Because we only had genotype data for three *RYR3* variants, this research cannot be seen as a complete assessment of the antihypertensive pharmacogenetic effects of the *RYR3* gene. However, associations with rs877087 need to be explored further to examine if it is tagging one or multiple other functional SNPs in RYR3, a large gene spanning 15.6 kb with 104 exons.

The findings reported here for the case-only analysis of the antihypertensive pharmacogenetic effect of *RYR3* among 3,058 CHD cases and 1,940 HF cases show that a hypertensive patient's genetic profile may help predict which medication(s) could help lower their risk of CVD. Ultimately, pharmacogenetic research may assist health care providers in choosing medications most likely to benefit a patient, given their genetic profile. For complex outcomes such as CVD among hypertensives, this goal has yet to be reached in a clinical setting. While research such as that presented here may be seen as a step toward that goal, further replications in other populations and functional studies are warranted.

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Table 1

Baseline characteristics for case-only participants (n=11,599) by treatment group

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
Sample size, n (%) by treatment	4,205 (36.3)	2,516 (21.7)	2,615 (22.6)	2,263 (19.5)	.
Age (y), mean (SD)	68.9 (7.9)	68.8 (7.8)	68.9 (7.9)	68.9 (7.8)	0.99
Self-reported Race:					
White, n (col %)	2,708 (64.4)	1,627 (64.7)	1,650 (63.1)	1,461 (64.6)	
Black, n (col %)	1,328 (31.6)	807 (32.1)	885 (33.8)	739 (32.7)	
American Indian/Alaskan native, n (col %)	8 (0.2)	7 (0.3)	6 (0.2)	2 (0.1)	
Asian/Pacific Islander, n (col %)	38 (0.9)	23 (0.9)	13 (0.5)	18 (0.8)	
Other, n (col %)	123 (2.9)	52 (2.1)	61 (2.3)	43 (1.9)	0.11
Hispanic, n (%)	518 (12.3)	289 (11.5)	318 (12.2)	274 (12.1)	0.90
Women, n (%)	1,645 (39.1)	976 (38.8)	983 (37.6)	870 (38.4)	0.65
On antihypertensive treatment, n (%)	3,869 (92.0)	2,334 (92.8)	2,396 (91.6)	2,063 (91.2)	0.21
Blood pressure at baseline:					
All participants, mm Hg: SBP, mean (SD)	147.0 (16.0)	146.2 (16.1)	147.4 (16.1)	147.4 (16.4)	**0.03**
DBP, mean (SD)	82.8 (10.4)	82.2 (10.4)	83.0 (10.7)	82.5 (10.5)	**0.02**
Treated at baseline, mm Hg: SBP, mean (SD)	146.0 (15.9)	145.4 (16.1)	146.4 (16.1)	146.3 (16.3)	0.11
DBP, mean (SD)	82.3 (10.3)	81.7 (10.3)	82.6 (10.7)	81.9 (10.3)	**0.01**
Untreated at baseline, mm Hg: SBP, mean (SD)	157.8 (12.1)	157.2 (11.7)	157.8 (11.5)	158.6 (12.3)	0.73
DBP, mean (SD)	88.4 (9.3)	88.8 (9.5)	87.7 (10.2)	88.2 (11.4)	0.72
Eligibility risk factors:					
Current cigarette smoker, n (%)	975 (23.2)	535 (21.3)	613 (23.4)	536 (23.7)	0.16
Type 2 diabetes, n (%)	1,687 (40.1)	1,024 (40.7)	1,001 (38.3)	901 (39.8)	0.31
HDL-C < 35 mg/dL, n (%)	508 (12.1)	287 (11.4)	301 (11.5)	283 (12.5)	0.60
LVH by electrocardiogram, n (%)	679 (16.1)	413 (16.4)	418 (16.0)	389 (17.2)	0.67
Body mass index, mean (SD), kg/m^2	29.3 (6.3)	29.5 (6.2)	29.4 (6.0)	29.3 (5.8)	0.74
Fasting glucose, mean (SD), mg/dL	129.2 (63.8)	126.9 (60.0)	125.7 (57.8)	127.3 (60.7)	0.23
Total cholesterol, mean (SD), mg/dL	215.7 (45.6)	216.0 (45.2)	215.0 (44.5)	215.2 (43.5)	0.84

Characteristic	Chlorthalidone	Amlodipine	Lisinopril	Doxazosin	p-value*
HDL cholesterol, mean (SD), mg/dL	45.1 (14.8)	45.3 (14.2)	45.2 (14.4)	44.5 (13.6)	0.28
Fasting triglycerides, mean (SD), mg/dL	177.9 (135.2)	175.7 (131.0)	177.4 (143.7)	175.7 (164.3)	0.93
rs877087, n (col %)					
CC	1,287 (31.3)	763 (30.9)	777 (30.3)	649 (29.3)	
TC	1,932 (47.0)	1,168 (47.3)	1,242 (48.4)	1,084 (48.9)	
TT	895 (21.8)	538 (21.8)	548 (21.3)	482 (21.8)	0.72
rs2077268, n (col %)					
CC	3,030 (72.9)	1,779 (71.5)	1,860 (72.1)	1,607 (72.1)	
TC	986 (23.7)	619 (24.9)	615 (23.9)	544 (24.4)	
TT	143 (3.4)	92 (3.7)	104 (4.0)	77 (3.5)	0.78
rs4780144, n (col %)					
TT	3,145 (78.4)	1,857 (77.7)	1,898 (76.4)	1,680 (77.9)	
TC	711 (17.7)	426 (17.8)	463 (18.7)	409 (19.0)	
CC	157 (3.9)	107 (4.5)	121 (4.9)	68 (3.2)	0.06

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

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

Table 2

Genotype-by-treatment interaction results, full follow-up: number of cases by genotype and treatment group

Outcome	RYR3 Variant	Geno-type	Number of cases (col %)			Pharmacogenetic ORs and p-values <.05 (p^1=codominant genetic model, p^2=additive genetic model)		
			CHL	AML	LIS	AML vs. CHL	AML vs. LIS	AML vs. LIS+CHL
	rs877087	CC	364 (33%)	216 (32%)	196 (30%)	*	*	*
		TC	499 (45%)	314 (46%)	321 (49%)			
		TT	254 (23%)	147 (22%)	137 (21%)			
CHD	rs2077268	CC	815 (73%)	476 (70%)	482 (72%)	*	*	*
		TC	276 (25%)	179 (26%)	158 (24%)			
		TT	33 (3%)	29 (4%)	26 (4%)			
	rs4780144	TT	884 (81%)	523 (80%)	483 (75%)	*	*	*
		TC	176 (16%)	107 (16%)	125 (20%)			
		CC	38 (3%)	27 (4%)	32 (5%)			
	rs877087	CC	176 (29%)	158 (32%)	126 (33%)	**p^1=.005**	*	**p^1=.01**
		TC	311 (52%)	208 (42%)	177 (46%)			
		TT	116 (19%)	126 (26%)	82 (21%)			
Heart failure	rs2077268	CC	441 (72%)	341 (69%)	264 (67%)	**p^1=.01, p^2=.04**	*	*
		TC	159 (26%)	129 (26%)	106 (27%)			
		TT	10 (2%)	24 (5%)	24 (6%)			
	rs4780144	TT	479 (82%)	370 (78%)	279 (74%)	*	*	*
		TC	85 (15%)	83 (17%)	83 (22%)			
		CC	22 (4%)	22 (5%)	15 (4%)			

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

p-value[1]: three genotype groups, no linearity assumption, H_O=both interaction coefficients jointly equal zero (2-degrees of freedom test)

p-value[2]: common homozygotes coded 0, heterozygotes coded 1, rare homozygotes coded 2; assumption of linearity (1-degree of freedom test)

* = p-values greater than 0.05

Table 3

Genotype-by-treatment interaction results, follow-up until doxazosin discontinuation: number of cases by genotype and treatment group

Outcome	RYR3 Variant	Geno type	Number of cases (col %)				Pharmacogenetic p-values <.05 (p^1=codominant genetic model, p^2=additive genetic model)	
			DOX	CHL	AML	LIS	AML vs. DOX	AML vs. DOX+CHL+LIS
	rs877087	CC	112 (26%)	244 (34%)	131 (33%)	138 (32%)	*	*
		TC	225 (52%)	324 (45%)	184 (46%)	203 (47%)		
		TT	95 (22%)	152 (21%)	88 (22%)	88 (21%)		
CHD	rs2077268	CC	313 (72%)	525 (72%)	292 (71%)	314 (72%)	*	*
		TC	109 (25%)	183 (25%)	100 (24%)	105 (24%)		
		TT	15 (3%)	20 (3%)	17 (4%)	19 (4%)		
	rs4780144	TT	343 (80%)	578 (81%)	322 (82%)	316 (75%)	*	*
		TC	74 (17%)	112 (16%)	58 (15%)	85 (20%)		
		CC	14 (3%)	20 (3%)	15 (4%)	19 (5%)		
	rs877087	CC	103 (29%)	114 (31%)	87 (31%)	80 (32%)	**p^1=.001**	**p^1=.0005, p^2=.04**
		TC	182 (52%)	180 (49%)	109 (39%)	115 (47%)		
		TT	66 (19%)	70 (19%)	83 (30%)	52 (21%)		
Heart failure	rs2077268	CC	247 (70%)	264 (72%)	195 (69%)	168 (67%)	*	*
		TC	93 (26%)	94 (26%)	78 (28%)	70 (28%)		
		TT	13 (4%)	8 (2%)	10 (4%)	13 (5%)		
	rs4780144	TT	255 (74%)	283 (80%)	213 (80%)	179 (74%)	*	*
		TC	79 (23%)	57 (16%)	44 (16%)	55 (23%)		
		CC	10 (3%)	15 (4%)	10 (4%)	9 (4%)		

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

p-value[1]: three genotype groups, no linearity assumption, H_O=both interaction coefficients jointly equal zero (2-degrees of freedom test)

p-value[2]: common homozygotes coded 0, heterozygotes coded 1, rare homozygotes coded 2; assumption of linearity (1-degree of freedom test)

* = p-values greater than 0.05