ORIGINAL ARTICLE

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

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Nearly one-third of adults in the United States 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 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, Ca2+ 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 Ca2+ 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.4 RYR3 has been shown to mediate the mobilization of stored Ca2+ in cardiac and skeletal muscle to initiate muscle contraction. Supporting its potential role in CVD, RYR3 mediates the mobilization of stored Ca2+ in cardiac and skeletal muscle to initiate muscle contraction. There was suggestive evidence of pharmacogenetic effects on HF, the strongest of which was for rs877087, with the smallest P-value = 0.0005 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 3058 CHD cases and 1940 HF cases show that a hypertensive patient’s genetic profile may help predict which medication(s) might better lower CVD risk.

The Pharmacogenomics Journal (2013) 13, 330–334; doi:10.1038/tpj.2012.22; published online 5 June 2012

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

INTRODUCTION

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In the cardiovascular system, Ca2+ 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 Ca2+ 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.4 RYR3 has been shown to mediate the mobilization of stored Ca2+ in cardiac and skeletal muscle to initiate muscle contraction. Supporting its potential role in CVD, RYR3 mediates the mobilization of stored Ca2+ in cardiac and skeletal muscle to initiate muscle contraction. There was suggestive evidence of pharmacogenetic effects on HF, the strongest of which was for rs877087, with the smallest P-value = 0.0005 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 3058 CHD cases and 1940 HF cases show that a hypertensive patient’s genetic profile may help predict which medication(s) might better lower CVD risk.

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MATERIALS AND 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 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.
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 was lower among patients randomized to one of four antihypertensive drug classes: a CCB (amlodipine), an angiotensin-converting enzyme 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 myocardial infarction, 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 myocardial infarction), and the secondary outcome of HF (fatal or hospitalized). Outcomes were reported by clinical investigators, and documentation (death certificate and hospital discharge summary) was submitted for any outcome involving death or hospitalization. National databases (Center for Medicare and Medicaid Services, the Department of Veterans 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, 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 BP 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 >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 χ²-tests for categorical variables. Logistic regression was used to test 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 with each of the other three treatment groups separately, as well as combined. Two genetic models were tested: co-dominant (three genotype groups, common homozygotes as referent group, one beta-coefficient estimate, which estimates ‘risk’ per copy of minor allele, 2-degrees of freedom test), and additive (three genotype groups, common homozygote as referent group, one beta-coefficient estimate, which estimates ‘risk’ per copy of minor allele, 1-degree of freedom test). All comparisons with the doxazosin treatment group were performed using a data set 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 of 64 ancestry informative markers. A P-value of 0.05 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, TX, USA).

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 (SBP) and diastolic (DBP) (small but statistically significant) when compared with the other treatment groups (mean SBP: 146.2, 147.0, 147.4, 147.4 mm Hg; mean DBP: 82.2, 82.8, 83.0, 82.5 mm Hg for amlodipine, chlorthalidone, lisinopril, doxazosin, respectively; P = 0.03 and P = 0.02 for SBP and DBP, respectively). Based on the ancestry informative markers, 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 three variants were in Hardy–Weinberg equilibrium (P = 0.32, 0.25 and 0.40 for rs877087, rs2077268 and rs4780144, respectively). The suggestive findings for rs877087 and rs4780144 were in Hardy–Weinberg equilibrium (P = 0.24 and 0.68, respectively), whereas rs4780144 was not (P = 0.0001). It is difficult to interpret a lack of Hardy–Weinberg equilibrium 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 3058 and 1940, 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 homoyzogotes 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 49% 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 = 0.005 (for codominant model—amlodipine versus chlorthalidone; P = 0.01 for codominant model—amlodipine versus lisinopril and chlorthalidone). For rs2077268, there was a difference between the amlodipine and chlorthalidone groups, with the minor allele homoyzogotes 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 = 0.01 for codominant model—amlodipine versus chlorthalidone and P = 0.04 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 = 0.001 for codominant model—amlodipine versus doxazosin; P = 0.0005 for codominant model and P = 0.04 for additive model—amlodipine versus all other treatments).
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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 RYR3 antihypertensive pharmacogenetics

| Characteristic                  | Chlorthalidone | Amlodipine | Lisinopril | Doxazosin | P-value* |
|--------------------------------|---------------|------------|------------|-----------|----------|
| Sample size, n (%) by treatment | 4205 (36.3)   | 2516 (21.7) | 2615 (22.6) | 2263 (19.5) | 0.99     |
| Age (years), mean (s.d.)       | 68.9 (7.9)    | 68.8 (7.8)  | 68.9 (7.9)  | 68.9 (7.8)  |          |
| **Self-reported race**         |               |            |            |           |          |
| White, n (col %)               | 2708 (64.4)   | 1627 (64.7) | 1650 (63.1) | 1461 (64.6) |          |
| Black, n (col %)               | 1328 (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)    |          |
| Hispanic, n (%)                | 518 (12.3)    | 289 (11.5)  | 318 (12.2)  | 274 (12.1)  | 0.90     |
| Women, n (%)                   | 1645 (39.1)   | 976 (38.8)  | 983 (37.6)  | 870 (38.4)  | 0.65     |
| **On antihypertensive treatment, n (%)** | 3869 (92.0) | 2334 (92.8) | 2396 (91.6) | 2063 (91.2) | 0.21     |
| Blood pressure at baseline     |               |            |            |           |          |
| All participants, mm Hg: SBP, mean (s.d.) | 147.0 (16.0) | 146.2 (16.1) | 147.4 (16.1) | 147.4 (16.4) | 0.03     |
| DBP, mean (s.d.)               | 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 (s.d.) | 146.0 (15.9) | 145.4 (16.1) | 146.4 (16.1) | 146.3 (16.1) | 0.11     |
| DBP, mean (s.d.)               | 82.3 (10.3)   | 81.7 (10.3) | 82.6 (10.7) | 81.9 (10.3) | 0.01     |
| Un-treated at baseline, mm Hg: SBP, mean (s.d.) | 157.8 (12.1) | 157.2 (11.7) | 157.8 (11.5) | 158.6 (12.3) | 0.73     |
| DBP, mean (s.d.)               | 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 (%)         | 1667 (40.1)   | 1024 (40.7) | 1001 (38.3) | 901 (39.8)  | 0.31     |
| LVH by electrocardiogram, n (%) | 679 (16.1)   | 413 (16.4)  | 418 (16.0)  | 389 (17.2)  | 0.67     |
| Body mass index, mean (s.d.), kg m\(^{-2}\) | 29.3 (6.3)  | 29.5 (6.2)  | 29.4 (6.0)  | 29.3 (5.8)  | 0.74     |
| Fasting glucose, mean (s.d.), mg dl\(^{-1}\) | 129.2 (63.8) | 126.9 (60.0) | 125.7 (57.8) | 127.3 (60.7) | 0.23     |
| Total cholesterol, mean (s.d.), mg dl\(^{-1}\) | 215.7 (45.6) | 216.0 (45.2) | 215.0 (44.5) | 215.2 (43.5) | 0.84     |
| HDL cholesterol, mean (s.d.), mg dl\(^{-1}\) | 45.1 (14.8) | 45.3 (14.2) | 45.2 (14.4) | 44.5 (13.6) | 0.28     |
| Fasting triglycerides, mean (s.d.), mg dl\(^{-1}\) | 177.9 (135.2) | 175.7 (131.0) | 177.4 (143.7) | 175.7 (164.3) | 0.93     |
| rs877087, n (col %)            |               |            |            |           |          |
| CC                             | 1287 (31.3)   | 763 (30.9)  | 777 (30.3)  | 649 (29.3)  | 0.72     |
| TC                             | 1932 (47.0)   | 1168 (47.3) | 1242 (48.4) | 1084 (48.9) |          |
| TT                             | 895 (21.8)    | 538 (21.8)  | 548 (21.3)  | 482 (21.8)  |          |
| rs2077268, n (col %)           |               |            |            |           |          |
| CC                             | 3030 (72.9)   | 1779 (71.5) | 1860 (72.1) | 1607 (72.1) | 0.78     |
| 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)    |          |
| rs4780144, n (col %)           |               |            |            |           |          |
| TT                             | 3145 (78.4)   | 1857 (77.7) | 1898 (76.4) | 1680 (77.9) | 0.06     |
| 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)    |          |

Abbreviations: col, column; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LVH, left ventricular hypertrophy; SBP, systolic blood pressure.

*Test of differences between genotype groups: ANOVA for continuous variables, \( \chi^2 \)-test for categorical variables.

Bold values are the ones with significant \( P \)-value (< 0.05).

The Pharmacogenomics Journal (2013), 330 – 334 © 2013 Macmillan Publishers Limited

DISCUSSION

Previous research has provided a biologically plausible mechanism through which \( RYR3 \) activity could be associated with CHD and HF, 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 \( \text{Ca}^{2+} \) mobilization, it is interesting that our strongest association was found for HF when comparing the CCB amlodipine to the other three drug classes: rs877087 minor allele homozygotes had higher risk of HF (and common allele carriers had lower risk) when randomized to amlodipine, compared with all other treatments (\( P = 0.0005 \) for codominant model). If we correct for multiple testing even with a stringent Bonferroni adjustment, given our 60 pharmacogenetic tests (0.05/60 = 0.0008) 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 5049 events reported a significant increase in incident
HF in patients allocated to CCBs (odds ratio 1.18, 95% confidence interval 1.07--1.31). 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 $r^2 = 0.02$, $r^2 = 0.004$ and $r^2 = 0.005$, 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 $r^2 = 0.005$, respectively.

### Table 2. Genotype-by-treatment interaction results, full follow-up: number of cases by genotype and treatment group

| Outcome          | RYR3 variant | Genotype | CHL (%) | AML (%) | LIS (%) | AML vs CHL | AML vs LIS | AML vs LIS+CHL |
|------------------|--------------|----------|---------|---------|---------|------------|------------|----------------|
| CHD              | rs877087     | CC       | 364 (33) | 216 (32) | 196 (30) | *          | *          | *              |
|                  |              | TC       | 499 (45) | 314 (46) | 321 (49) |            |            |                |
|                  |              | TT       | 254 (23) | 147 (22) | 137 (21) |            |            |                |
|                  | 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)   |            |            |                |
| Heart failure    | rs877087     | CC       | 176 (29) | 158 (32) | 126 (33) | $P^1 = 0.005$ | *          | $P^1 = 0.01$  |
|                  |              | TC       | 311 (52) | 208 (42) | 177 (46) |            |            |                |
|                  |              | TT       | 116 (19) | 126 (26) | 82 (21)  |            |            |                |
|                  | rs2077268    | CC       | 441 (72) | 341 (69) | 264 (67) | $P^1 = 0.01$, $P^2 = 0.04$ | *          | *              |
|                  |              | 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)   |            |            |                |

Abbreviations: AML, amlodipine; CHD, coronary heart disease; CHL, chlorthalidone; col, column; LIS, lisinopril; OR, odds ratio.

*P-value*: three genotype groups, no linearity assumption, $H_0 =$ both interaction coefficients jointly equal zero (2-degrees of freedom test).

*P-value*: common homozygotes coded 0, heterozygotes coded 1, rare homozygotes coded 2; assumption of linearity (1-degree of freedom test).

*P-values > 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 | Genotype | DOX (%) | CHL (%) | AML (%) | LIS (%) | AML vs DOX | AML vs DOX+CHL+LIS |
|------------------|--------------|----------|---------|---------|---------|---------|------------|-------------------|
| CHD              | 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)  |            |                   |
|                  | 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)   |            |                   |
| Heart failure    | rs877087     | CC       | 103 (29) | 114 (31) | 87 (31)  | 80 (32)  | $P^1 = 0.001$ | *                 |
|                  |              | TC       | 182 (52) | 180 (49) | 109 (39) | 115 (47) |            |                   |
|                  |              | TT       | 66 (19)  | 70 (19)  | 83 (30)  | 52 (21)  |            |                   |
|                  | 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)    |            |                   |

Abbreviations: AML, amlodipine; CHD, coronary heart disease; CHL, chlorthalidone; col, column; DOX, doxazosin; LIS, lisinopril.

*P-value*: three genotype groups, no linearity assumption, $H_0 =$ both interaction coefficients jointly equal zero (2-degrees of freedom test).

*P-value*: common homozygotes coded 0, heterozygotes coded 1, rare homozygotes coded 2; assumption of linearity (1-degree of freedom test).

*P-values > 0.05.
respectively in HapMap YRI population panel (Yoruba residents of Ibadan, Nigeria), as calculated using SNAP (SNP Annotation and Proxy Search). We are unaware of any previous study of the antihypertensive pharmacogenetic effect of these SNPs; however, rs877087 has been associated with stroke in a genome-wide association study.

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. 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. As ALLHAT is a randomized clinical trial, genotype and treatment should be independent as 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 (that is, <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. 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.

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, that is, its 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. As 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 3058 CHD cases and 1940 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 help 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. Although 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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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

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