Pharmacogenetic Associations of MMP9 and MMP12 Variants with Cardiovascular Disease in Patients with Hypertension

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Abstract

Objectives: MMP-9 and -12 function in tissue remodeling and may play roles in cardiovascular disease (CVD). We assessed associations of four MMP polymorphisms and three antihypertensive drugs with cardiovascular outcomes.

Methods: Hypertensives (n = 42,418) from a double-blind, randomized, clinical trial were randomized to chlorthalidone, amlodipine, lisinopril, or doxazosin treatment (mean follow up, 4.9 years). The primary outcome was coronary heart disease (CHD). Secondary outcomes included combined CHD, all CVD outcomes combined, stroke, heart failure (HF), and mortality. Genotype-treatment interactions were tested.

Results: There were 38,698 participants genotyped for at least one of the polymorphisms included here. For MMP9 R668Q (rs2274756), lower hazard ratios (HRs) were observed for AA subjects for most outcomes when treated with chlorthalidone versus amlodipine (eg., CCHD: GG = 1.00, GA = 1.01, AA = 0.64; P = 0.038). For MMP9 R279Q (rs17576), modest pharmacogenetic findings were observed for combined CHD and the composite CVD outcome. For MMP12 N122S (rs652438), lower HRs were observed for CHD in subjects carrying at least one G allele and being treated with chlorthalidone versus lisinopril (CHD: AA = 1.07, AG = 0.80, GG = 0.49; P = 0.005). In the lisinopril-amlodipine comparison, higher HRs were observed for participants having at least one G allele at the MMP12 N122S locus (CHD: AA = 0.94, AG = 1.19, GG = 1.93; P = 0.041). For MMP12 —82A>G (rs2276109), no pharmacogenetic effect was found for the primary outcome, although lower HRs were observed for AA homozygotes in the chlorthalidone-amlodipine comparison for HF (P = 0.015).

Conclusions: We observed interactions between antihypertensive drugs and MMP9 and MMP12 for CHD and composite CVD. The data suggest that these genes may provide useful clinical information with respect to treatment decisions.

Introduction

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases which play a role in connective tissue remodeling. Circulating levels of MMPs are associated with many cardiovascular diseases (CVDs), including atherosclerosis, myocardial infarction (MI), and heart failure [1,2,3]. MMP-9 (gelatinase-B) functions in the degradation of type IV and V collagens, and its levels are raised in individuals with hypertension, acute coronary syndrome, and acute MI [2,4,5]. MMP-12, (macrophage metalloelastase) is up-regulated in atherosclerotic lesions and aneurysms and may contribute to the activation of other MMPs, which, in turn, degrade other extracellular matrix proteins [6]. A recent study found that MMP-12 production by macrophages plays a role in the transition from fatty acids to fibrous plaques during the progression of atherosclerosis [7]. Given MMPs’ associations and possible causal connections with CVD, these enzymes represent potential drug targets for CVD treatment and prevention. In fact, amlodipine and lercanidipine have been shown to influence MMP-9 plasma levels and activity [8,9,10]. ACE inhibitors may also affect MMP levels [11,12,13,14]. Attempts have been made to assess associations of MMP gene variants with disease and risk.

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Competing Interests: BD has twice served as a consultant on the Takeda Pharmaceuticals Data Safety Monitoring Board and has received one honoraria for work done for Forest Laboratories. VB is employed by Roche Molecular Systems, Inc., a company with no current business interests in cardiovascular disease. These potential competing interests in no way compromise the authors' adherence to PLoS ONE data and material sharing policies. All other authors disclosed no potential conflicts of interest.

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phenotypes [15,16,17,18,19]. For example, individuals carrying the MMP9 279Q allele had higher plasma levels of MMP-9 and higher risk of cardiovascular events than patients homozygous for the 279R allele (P = 0.02) [16]. The MMP9 R279Q polymorphism is a glutamine to arginine substitution located in the catalytic domain of MMP-9 [16,30] and plausibly represents a loss-of-function mutation [21]. The R668Q variant of MMP9 lies within exon 12. This missense mutation lies in the hemopexin-like domain and probably functions in substrate binding [19]. The MMP12 −82A>G variant, which lies in the gene’s promoter region, affects transcription factor binding affinity, with the A allele associated with increased promoter activity [15,18]; this variant has been shown to affect coronary artery luminal diameter [15]. The MMP12 N122S variant lies within the gene’s coding region; although this variant has been associated with endpoints such as breast cancer prognosis [22] and diabetic nephropathy [23], the functional significance of the single-nucleotide polymorphism (SNP) is unknown. Given these and other MMP genetic associations with cardiovascular phenotypes and known molecular interactions between antihypertensive drugs and some MMPs, MMP genes may modify rates of CVD outcomes differently for different antihypertensive treatments. We tested whether hypertensive participants in the Genetics of Hypertension Associated Treatment (GenHAT) Study genotyped for MMP9 variants R279Q and R668Q and for MMP12 variants N122S and −82A>G and randomized to either the diuretic chlorthalidone, the calcium channel blocker amlodipine (CCB), or the angiotensin converting enzyme (ACE) inhibitor lisinopril had different outcome rates with regard to six CVD phenotypes. (The doxazosin treatment arm was not included in the pharmacogenetic analyses. See below.) All three of these drugs are known to effectively lower blood pressure and all three are commonly prescribed. The effect of treatment assignment on CVD outcomes has been previously published [24,25]. We sought to determine whether genotype for these variants interacted with treatment assignment to produce a pharmacogenetic association with CVD outcomes.

Methods

Ethics statement

Participants recruited during the parent Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) signed informed consent documents; the GenHAT study was approved by the University of Minnesota Institutional Review Board, the University of Alabama at Birmingham Institutional Review Board for Human Use, and the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects.

Study Population

Study participants were part of the GenHAT study, the primary objective of which was to determine whether variants in hypertension susceptibility genes interact with antihypertensive drugs to modify coronary heart disease risk in hypertensive patients [26]. GenHAT is ancillary to the ALLHAT study, a randomized, double-blind clinical trial examining 42,418 hypertensive patients age 55 or older (47% non-White and 46% female) resulting in hazard ratios (HR) and ratios of hazard ratios point estimates, respectively. To test the main effects of MMP genotypes on the outcomes as well as the genotype-by-treatment interactions (the pharmacogenetic effects), resulting in hazard ratios (HR) and ratios of hazard ratios point estimates, respectively. To test the main effects of the genotypes on the outcomes, we adjusted for age; gender; ethnicity (Hispanic status); race (5 self-reported categories: White, Black, American Indian/Alaskan native, Asian/Pacific Islander, and “other”); smoking status; diabetes status; aspirin use; and baseline values for body mass index (BMI), systolic and diastolic blood pressures, and HDL and LDL cholesterol. To test for genotype-by-treatment
Table 1. Baseline characteristics for participants (n = 38,698) by treatment group, mean (SD) unless otherwise noted.

| Characteristic                        | Chlorothalidone | Amlodipine | Lisinopril | Doxazosin | P-value* |
|---------------------------------------|-----------------|------------|------------|-----------|----------|
| Sample size, n (%) by treatment       | 13,928 (36.0)   | 8,243 (21.3) | 8,273 (21.4) | 8,254 (21.3) |          |
| Age (y)                               | 66.8 (7.7)      | 66.9 (7.7) | 66.8 (7.8) | 66.7 (7.7) | 0.74     |
| Race                                  |                 |            |            |           |          |
| White, n (col %)                      | 8,451 (60.7)    | 4,999 (60.7) | 5,025 (60.7) | 4,976 (60.3) |          |
| Black, n (col %)                      | 4,770 (34.3)    | 2,859 (34.7) | 2,839 (34.3) | 2,890 (35.0) |          |
| American Indian/Alaskan native, n (col %) | 26 (0.2)   | 19 (0.2) | 18 (0.2) | 10 (0.1) |          |
| Asian/Pacific Islander, n (col %)     | 170 (1.2)       | 97 (1.2)   | 85 (1.0) | 95 (1.2) |          |
| Other, n (col %)                      | 511 (3.7)       | 269 (3.3)  | 306 (3.7) | 283 (3.4) | 0.64     |
| Hispanic, n (%)                       | 2,722 (19.5)    | 1,558 (18.9) | 1,639 (19.8) | 1,618 (19.6) | 0.86     |
| Women, n (%)                          | 6,552 (47.0)    | 3,926 (47.6) | 3,831 (46.3) | 3,817 (46.2) | 0.22     |
| Previous antihypertensive treatment, n (%) | 12,571 (90.3) | 7,472 (90.7) | 7,454 (90.1) | 7,447 (90.2) | 0.66     |
| Blood pressure at baseline, mm Hg     |                 |            |            |           |          |
| All participants                      |                 |            |            |           |          |
| SBP                                   | 146.2 (15.7)    | 146.2 (15.7) | 146.5 (15.6) | 146.3 (15.8) | 0.67     |
| DBP                                   | 84.1 (10.0)     | 83.9 (10.2) | 84.1 (10.0) | 83.9 (10.0) | 0.41     |
| Treated at baseline                   |                 |            |            |           |          |
| SBP                                   | 145.2 (15.6)    | 145.1 (15.6) | 145.4 (15.5) | 145.2 (15.7) | 0.75     |
| DBP                                   | 83.5 (10.0)     | 83.3 (10.1) | 83.6 (9.9) | 83.4 (9.9) | 0.30     |
| Untreated at baseline                 |                 |            |            |           |          |
| SBP                                   | 156.1 (12.0)    | 156.6 (12.2) | 156.4 (12.3) | 156.8 (12.5) | 0.57     |
| DBP                                   | 89.5 (9.0)      | 89.7 (9.5)  | 89.1 (9.3) | 89.4 (9.5) | 0.63     |
| Eligibility risk factors              |                 |            |            |           |          |
| Current cigarette smoker, n (%)       | 3,061 (22.0)    | 1,810 (22.0) | 1,817 (22.0) | 1,795 (21.8) | 0.98     |
| Type 2 diabetes, n (%)                | 4,983 (35.8)    | 3,008 (36.5) | 2,899 (35.0) | 2,891 (35.0) | 0.15     |
| HDL cholesterol <35 mg/dL, n (%)      | 1,666 (12.0)    | 939 (11.4)  | 976 (11.8) | 973 (11.8) | 0.65     |
| LVH by electrocardiogram, n (%)       | 2,255 (16.2)    | 1,398 (17.0) | 1,342 (16.2) | 1,354 (16.4) | 0.47     |
| Body mass index, kg/m²                | 29.7 (6.1)      | 29.8 (6.3)  | 29.8 (6.2) | 29.7 (5.9) | 0.32     |
| Fasting glucose, mg/dL                | 123.4 (58.6)    | 123.1 (57.5) | 122.4 (55.6) | 121.9 (55.9) | 0.35     |
| LDL cholesterol, mg/dL               | 136.0 (37.3)    | 135.7 (37.3) | 135.8 (36.4) | 135.2 (36.3) | 0.60     |
| HDL cholesterol, mg/dL               | 46.8 (14.9)     | 47.2 (14.7) | 46.6 (14.6) | 46.6 (14.4) | 0.04     |
| Fasting triglycerides, mg/dL          | 176.7 (130.9)   | 176.6 (132.8) | 175.5 (138.5) | 174.1 (137.6) | 0.53     |
| Aspirin use, n (%)                    | 4,986 (35.8)    | 3,002 (36.4) | 3,021 (36.5) | 3,026 (36.7) | 0.54     |
| MMP9 R279Q (A>G) (rs17576)            |                 |            |            |           |          |
| AA                                    | 5,955 (42.8)    | 3,422 (41.6) | 3,596 (43.6) | 3,550 (43.1) |          |
| AG                                    | 6,257 (45.0)    | 3,783 (46.0) | 3,633 (44.0) | 3,675 (44.6) |          |
| GG                                    | 1,691 (12.2)    | 1,026 (12.5) | 1,024 (12.4) | 1,013 (12.3) | 0.21     |
| MMP9 R668Q (G>A) (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     |
| MMP12 82A>G (rs2276109)               |                 |            |            |           |          |
| AA                                    | 11,648 (84.0)   | 6,899 (84.0) | 6,903 (83.8) | 6,926 (84.3) |          |
| AG                                    | 2,083 (15.0)    | 1,242 (15.1) | 1,269 (15.4) | 1,216 (14.8) |          |
| GG                                    | 137 (1.0)       | 68 (0.8)    | 68 (0.8)   | 78 (1.0)   | 0.74     |
| MMP12 N122S (A>G) (rs652438)          |                 |            |            |           |          |
| AA                                    | 11,029 (80.6)   | 6,562 (80.8) | 6,652 (81.5) | 6,655 (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     |

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

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

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interactions, we created a genotype x treatment parameter and did three separate comparisons: chlorthalidone versus amlodipine, chlorthalidone versus lisinopril, and lisinopril versus amlodipine. We tested three genetic models for the pharmacogenetic effects: additive (3 genotype categories with a 2 degrees of freedom \( df \) interaction test), dominant (collapsing minor homozygote group with heterozygote group resulting in 2 genotype groups with a 1 df interaction test), and recessive (collapsing common homozygote group with heterozygote group resulting in 2 genotype groups with a 1 df interaction test).

The previously published GenHAT design paper outlined six primary, \textit{a priori} hypotheses; however, these hypotheses did not include testing the pharmacogenetic effect of \textit{MMP9} or \textit{MMP12} variants. Therefore, secondary investigations such as this MMP study are considered exploratory and, as such, are not adjusted for multiple comparisons. Since we performed multiple statistical tests of the pharmacogenetic effects of \textit{MMP9} and \textit{MMP12} variants, caution must be exercised in pronouncing any findings reported here as statistically significant.

**Results**

Table 1 shows the baseline characteristics of the study subjects \((n = 38,698, \text{participants with at least one variant included in these analyses})\). There were no significant differences between groups except for HDL cholesterol \((P = 0.04)\), which was higher in those randomized to amlodipine versus chlorthalidone or lisinopril.

The \textit{MMP9} R279Q and \textit{MMP12} −82A>G genotype frequencies were in Hardy-Weinberg equilibrium (HWE) when tested within

| Genotype | CHD | CCHD | CCVD | ACM | Stroke | CHF |
|----------|-----|------|------|-----|--------|-----|
| MMP9 R279Q (rs17576) |
| AA | 1373 | 2573 | 4368 | 2268 | 777 | 1149 |
| (19.0) | (37.3) | (68.1) | (29.0) | (10.6) | (15.9) |
| GA | 1478 | 2738 | 4529 | 2319 | 731 | 1158 |
| (19.5) | (37.8) | (67.2) | (28.1) | (9.5) | (15.3) |
| GG | 420 | 767 | 1275 | 618 | 202 | 347 |
| (20.3) | (38.9) | (69.3) | (27.4) | (9.6) | (16.8) |
| \( P \) | 0.99 | 0.92 | 0.51 | 0.20 | 0.04 | 0.10 |

| Genotype | CHD | CCHD | CCVD | ACM | Stroke | CHF |
|----------|-----|------|------|-----|--------|-----|
| MMP9 R668Q (rs2274756) |
| GG | 2360 | 4376 | 7311 | 3738 | 1240 | 1897 |
| (19.6) | (38.0) | (68.3) | (28.5) | (10.2) | (15.7) |
| GA | 817 | 1538 | 2579 | 1323 | 425 | 688 |
| (18.6) | (36.6) | (66.1) | (27.7) | (9.5) | (15.7) |
| AA | 94 | 164 | 275 | 133 | 45 | 67 |
| (22.4) | (41.0) | (74.0) | (29.3) | (10.5) | (16.0) |
| \( P \) | 0.16 | 0.34 | 0.31 | 0.59 | 0.04 | 0.98 |

| Genotype | CHD | CCHD | CCVD | ACM | Stroke | CHF |
|----------|-----|------|------|-----|--------|-----|
| MMP12 −82A>G (rs2276109) |
| AA | 2692 | 4991 | 8409 | 4409 | 1445 | 2161 |
| (19.1) | (37.0) | (67.0) | (28.7) | (10.1) | (15.3) |
| AG | 535 | 1008 | 1624 | 736 | 245 | 449 |
| (20.8) | (41.4) | (71.9) | (26.5) | (9.4) | (17.5) |
| GG | 35 | 67 | 116 | 47 | 19 | 32 |
| (22.4) | (45.2) | (87.3) | (27.9) | (12.0) | (20.6) |
| \( P \) | 0.87 | 0.63 | 0.24 | 0.20 | 0.53 | 0.36 |

| Genotype | CHD | CCHD | CCVD | ACM | Stroke | CHF |
|----------|-----|------|------|-----|--------|-----|
| MMP12 N122S (rs652438) |
| AA | 2651 | 4969 | 8210 | 4143 | 1334 | 2126 |
| (19.7) | (38.7) | (68.8) | (28.4) | (9.8) | (15.8) |
| AG | 503 | 911 | 1621 | 893 | 321 | 432 |
| (17.2) | (32.4) | (61.9) | (28.2) | (10.9) | (14.9) |
| GG | 60 | 102 | 169 | 82 | 29 | 45 |
| (22.5) | (40.1) | (71.2) | (27.3) | (10.7) | (16.7) |
| \( P \) | 0.04 | 0.01 | 0.12 | 0.83 | 0.39 | 0.64 |

CHD, coronary heart disease defined as fatal CHD or nonfatal myocardial infarction; CCHD, combined CHD death or non-fatal myocardial infarction plus revascularization procedures plus hospitalized angina; CCVD, combined cardiovascular disease defined as CHD death or non-fatal MI plus stroke, revascularization procedures, angina, heart failure, stroke, and peripheral arterial disease; ACM, all-cause mortality; CHF, chronic heart failure.

*adjusted for age, sex, race, Hispanic status, smoking status, diabetes status, aspirin use, and baseline values for BMI, SBP, DBP, HDL- and LDL-cholesterol.

**Table 2. Main effects of MMP genetic variants on clinical outcomes, all treatments combined.**
were in HWE for all groups except Black non-Hispanics (minor groups. The American Indian/Alaskan native group included no Pacific islander and “other” groups, but not for the Black or White MMP12 N122S genotype frequencies were in HWE for the Asian/Hispanic Whites, but not for Hispanic Whites (P = 0.04). After stratifying Whites by Hispanic status, the MMP12 N122S variant and stroke was modestly significant, with participants carrying the minor G allele having decreased risk of stroke (HR = 1.00 for AA, 0.87 for GA, 0.91 for GG; P = 0.04, additive genetic model). The associations between the MMP12 N122S variant and CHD and CCHD were also modestly significant (P = 0.04; for CCHD HR = 1.00 for AA, 0.95 for AG, 1.36 for GG; P = 0.04, for CCHD HR = 1.00 for AA, 0.95 for AG, 1.31 for GG; P = 0.01). The effects of the MMP9 R668Q and MMP12 −82A>G variants were not significant for any outcome.

race/ethnicity groups. The MMP9 R668Q genotype frequencies were in HWE equilibrium for the Black, American Indian/Alaskan native, Asian/Pacific islander, and “other” groups, but not for the White group (P = 0.04). After stratifying Whites by Hispanic status, the MMP9 R668Q genotype frequencies were in HWE for non-Hispanic Whites, but not for Hispanic Whites (P = 0.001). The MMP12 N122S genotype frequencies were in HWE for the Asian/Pacific islander and “other” groups, but not for the Black or White groups. The American Indian/Alaskan native group included no minor MMP12 N122S homozygotes (ie, GG). After stratifying Whites and Blacks by Hispanic status, MMP12 N122S genotype frequencies were in HWE for all groups except Black non-Hispanics (P = 0.0003).

Main effects of MMP9 and MMP12 variants on clinical outcomes

The effects of ALLHAT treatment assignments on CVD outcomes have been published elsewhere [24,25,27]. Hazard ratios for main effects of genetic variants on ALLHAT clinical outcomes in GenHAT are shown in Table 2. After adjusting for baseline characteristics, the association between the MMP9 R279Q variant and stroke was modestly significant, with participants carrying the minor G allele having decreased risk of stroke (HR = 1.00 for AA, 0.87 for GA, 0.91 for GG; P = 0.04, additive genetic model). The associations between the MMP12 N122S variant and CHD and CCHD were also modestly significant (P = 0.04 and P = 0.01, respectively), with participants with the minor allele homozygote (GG) genotype having a 30% increased risk of CHD or CCHD compared to participants having the common AA genotype (for CHD HR = 1.00 for AA, 0.95 for AG, 1.36 for GG; P = 0.04; for CCHD HR = 1.00 for AA, 0.95 for AG, 1.31 for GG; P = 0.01). The effects of the MMP9 R668Q and MMP12 −82A>G variants were not significant for any outcome.

Pharmacogenetic effects of MMP9 and MMP12 variants on clinical outcomes

Tables 3, 4, 5, 6, 7, 8 show ALLHAT clinical outcome frequencies and rates by genotype and treatment group for CHD,

### Table 3. Pharmacogenetic effects of MMP genetic variants on CHD.

| Genotype | Number of events (Event rate per 1000 p-y) | Genotype-specific treatment effects (Hazard Ratio (95% CI)) | Pharmacogenetic effects* |
|----------|---------------------------------------------|----------------------------------------------------------|--------------------------|
|          | CHL | AML | LIS | C vs. A | C. vs. L | L vs. A | P_A | P_D | P_R |
| **MMP9 R279Q (rs17576)** |     |     |     |        |         |       |      |      |      |
| AA       | 544 | 285 | 311 | 1.12   | 1.06    | 1.06   | P_A = 0.11 | P_D = 0.18 | P_R = 0.56 |
|          | (20.0) | (17.9) | (18.9) | (0.97–1.29) | (0.92–1.21) | (0.90–1.24) |      |      |      |
| AG       | 550 | 343 | 307 | 0.97   | 1.03    | 0.94   | P_D = 0.07 | P_D = 0.39 | P_D = 0.38 |
|          | (19.0) | (19.7) | (18.5) | (0.84–1.11) | (0.89–1.18) | (0.81–1.10) |      |      |      |
| GG       | 137 | 100 | 101 | 0.84   | 0.81    | 1.04   | P_R = 0.13 | P_R = 0.07 | P_R = 0.78 |
|          | (17.7) | (21.1) | (22.0) | (0.65–1.08) | (0.62–1.04) | (0.79–1.37) |      |      |      |
| **MMP9 R668Q (rs2274756)** |     |     |     |        |         |       |      |      |      |
| GG       | 902 | 523 | 517 | 1.01   | 1.02    | 0.98   | P_A = 0.13 | P_A = 0.45 | P_A = 0.61 |
|          | (19.7) | (19.5) | (19.2) | (0.91–1.12) | (0.92–1.14) | (0.87–1.11) |      |      |      |
| GA       | 303 | 177 | 180 | 1.07   | 0.99    | 1.08   | P_D = 0.97 | P_D = 0.53 | P_D = 0.60 |
|          | (18.3) | (17.2) | (16.6) | (0.89–1.29) | (0.82–1.19) | (0.88–1.33) |      |      |      |
| AA       | 25  | 27  | 24  | 0.59   | 0.74    | 0.84   | P_R = 0.05 | P_R = 0.22 | P_R = 0.50 |
|          | (16.9) | (28.5) | (23.8) | (0.34–1.02) | (0.42–1.30) | (0.48–1.46) |      |      |      |
| **MMP12 −82A>G (rs2276109)** |     |     |     |        |         |       |      |      |      |
| AA       | 1006 | 610 | 591 | 0.98   | 1.00    | 0.98   | P_A = 0.52 | P_A = 0.19 | P_A = 0.13 |
|          | (18.8) | (19.2) | (18.8) | (0.88–1.08) | (0.90–1.10) | (0.88–1.10) |      |      |      |
| AG       | 200 | 108 | 125 | 1.14   | 0.98    | 1.16   | P_D = 0.25 | P_D = 0.77 | P_D = 0.44 |
|          | (20.9) | (18.4) | (21.3) | (0.90–1.44) | (0.79–1.23) | (0.89–1.50) |      |      |      |
| GG       | 18  | 9   | 3   | 1.00   | 3.25    | 0.33   | P_R = 0.96 | P_R = 0.07 | P_R = 0.10 |
|          | (28.9) | (28.3) | (9.3) | (0.45–2.22) | (0.96–1.10) | (0.09–1.22) |      |      |      |
| **MMP12 N122S (rs652438)** |     |     |     |        |         |       |      |      |      |
| AA       | 1003 | 593 | 563 | 1.01   | 1.07    | 0.94   | P_A = 0.91 | P_A = 0.005 | P_A = 0.041 |
|          | (19.8) | (19.6) | (18.5) | (0.91–1.12) | (0.97–1.19) | (0.84–1.06) |      |      |      |
| AG       | 182 | 113 | 128 | 0.96   | 0.80    | 1.19   | P_D = 0.66 | P_D = 0.005 | P_D = 0.037 |
|          | (16.3) | (17.0) | (20.3) | (0.76–1.21) | (0.64–1.01) | (0.93–1.54) |      |      |      |
| GG       | 21  | 13  | 19  | 0.97   | 0.49    | 1.93   | P_R = 0.89 | P_R = 0.019 |
|          | (19.7) | (20.6) | (40.9) | (0.48–1.93) | (0.26–0.91) | (0.95–3.92) |      |      |      |

*P* = P-value for additive genetic model, *P_D* = P-value for dominant genetic model, *P_R* = P-value for recessive genetic model.

**CHD**, coronary heart disease defined as fatal CHD or nonfatal myocardial infarction; **CHL**, chlorthalidone treatment group; **AML**, amlodipine treatment group; **LIS**, lisinopril treatment group.

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CCHD, CCVD, ACM, stroke, and CHF, respectively. Also shown are genotype-specific treatment effects and gene-by-treatment interaction $P$-values for chlorthalidone versus amlodipine, chlorthalidone versus lisinopril, and lisinopril versus amlodipine. Treatment effects differed by genotype for several outcomes.

**Table 4. Pharmacogenetic effects of MMP genetic variants on CCHD.**

| Genotype | Number of events (event rate per 1000 p-y) | Genotype-specific treatment effects: Hazard Ratio (95% CI) | Pharmacogenetic effects* |
|----------|------------------------------------------|----------------------------------------------------------|--------------------------|
|          | CHL | AML | LIS | C vs. A | C. vs. L | L vs. A | $P_A$ | $P_D$ | $P_R$ |
| **MMP9 R279Q (rs17576)** | | | | | | | | | |
| AA | 952 | 556 | 569 | 1.00 | 1.01 | 0.99 | $P_A = 0.22$ | $P_A = 0.12$ | $P_A = 0.71$ |
| (36.5) | (36.6) | (36.3) | (0.90–1.11) | (0.91–1.12) | (0.88–1.11) |
| AG | 1022 | 602 | 599 | 1.03 | 0.97 | 1.06 | $P_D = 0.91$ | $P_D = 0.29$ | $P_D = 0.40$ |
| (37.2) | (36.1) | (38.1) | (0.93–1.14) | (0.88–1.08) | (0.94–1.18) |
| GG | 256 | 182 | 186 | 0.85 | 0.81 | 1.06 | $P_R = 0.09$ | $P_R = 0.045$ | $P_R = 0.78$ |
| (34.5) | (40.6) | (42.9) | (0.70–1.03) | (0.67–0.97) | (0.86–1.30) |
| **MMP9 R668Q (rs2274756)** | | | | | | | | | |
| GG | 1615 | 946 | 970 | 1.00 | 0.97 | 1.03 | $P_A = 0.12$ | $P_A = 0.30$ | $P_A = 0.84$ |
| (36.9) | (36.9) | (37.9) | (0.92–1.08) | (0.90–1.05) | (0.94–1.12) |
| GA | 565 | 350 | 346 | 1.01 | 0.96 | 1.05 | $P_D = 0.65$ | $P_D = 0.54$ | $P_D = 0.89$ |
| (35.9) | (35.7) | (37.6) | (0.88–1.15) | (0.84–1.09) | (0.91–1.22) |
| AA | 45 | 44 | 43 | 0.64 | 0.71 | 0.93 | $P_R = 0.038$ | $P_R = 0.13$ | $P_R = 0.60$ |
| (31.6) | (49.2) | (45.4) | (0.42–0.97) | (0.47–1.08) | (0.61–1.42) |
| **MMP12 –82A>G (rs2276109)** | | | | | | | | | |
| AA | 1817 | 1109 | 1120 | 0.97 | 0.95 | 1.03 | $P_A = 0.48$ | $P_A = 0.18$ | $P_A = 0.76$ |
| (35.5) | (36.5) | (37.6) | (0.90–1.05) | (0.88–1.02) | (0.95–1.12) |
| AG | 370 | 218 | 226 | 1.04 | 1.00 | 1.04 | $P_D = 0.35$ | $P_D = 0.31$ | $P_D = 0.95$ |
| (40.8) | (39.3) | (40.8) | (0.88–1.23) | (0.85–1.18) | (0.86–1.25) |
| GG | 32 | 12 | 9 | 1.35 | 1.91 | 0.72 | $P_R = 0.33$ | $P_R = 0.08$ | $P_R = 0.46$ |
| (54.1) | (39.4) | (29.1) | (0.70–2.63) | (0.91–4.01) | (0.30–1.71) |
| **MMP12 N122S (rs652438)** | | | | | | | | | |
| AA | 1831 | 1092 | 1092 | 1.00 | 1.00 | 1.00 | $P_A = 0.45$ | $P_A = 0.023$ | $P_A = 0.14$ |
| (38.0) | (37.8) | (37.8) | (0.93–1.08) | (0.93–1.08) | (0.92–1.09) |
| AG | 319 | 209 | 227 | 0.90 | 0.79 | 1.15 | $P_D = 0.39$ | $P_D = 0.008$ | $P_D = 0.10$ |
| (29.7) | (32.9) | (37.7) | (0.76–1.07) | (0.66–0.93) | (0.95–1.38) |
| GG | 40 | 20 | 24 | 1.18 | 0.74 | 1.58 | $P_R = 0.52$ | $P_R = 0.29$ | $P_R = 0.14$ |
| (39.1) | (33.1) | (53.5) | (0.69–2.01) | (0.45–1.23) | (0.87–2.85) |

$P_A = P$-value for additive genetic model, $P_D = P$-value for dominant genetic model, $P_R = P$-value for recessive genetic model.

* $P$-value for gene-by-treatment interaction.

CCHD, combined CHD defined as CHD death or non-fatal myocardial infarction plus revascularization procedures plus hospitalized angina; CHL, chlorthalidone treatment group; AML, amlodipine treatment group; LIS, lisinopril treatment group. doi:10.1371/journal.pone.0023609.t004

A similar effect was observed for the CCHD outcome, with only the chlorthalidone versus lisinopril comparison reaching a $P$-value $< 0.05$ ($P = 0.045$ for the recessive model).

Participants having the **MMP12 N122S** variant GG genotype had significantly increased rates (40.9 per 1000 p-y) of CHD if taking lisinopril versus any other genotype-treatment combination (16.3–20.6 per 1000 p-y). This difference led to a detectable pharmacogenetic effect for both chlorthalidone versus lisinopril and lisinopril versus amlodipine comparisons ($P$-values for pharmacogenetic effect ranged from 0.005 to 0.041 depending on genetic model). Results for CCHD and CCVD were consistent with this observation.

For the **MMP12 –82A>G** variant, there was evidence of a pharmacogenetic effect on CCVD for the chlorthalidone versus lisinopril comparison. Homozygotes for the minor G allele had increased risk of CCVD when randomized to chlorthalidone versus lisinopril ($HR = 1.75$), while the common A allele carriers had slightly reduced risk ($HR = 0.90$ for AA group, $HR = 0.95$ for AG group; pharmacogenetic effect $P = 0.025$).
### Table 5. Pharmacogenetic effects of MMP genetic variants on CCVD.

| Genotype   | Number of events (event rate per 1000 p-y) | Genotype-specific treatment effects: Hazard Ratio (95% CI) | Pharmacogenetic effects* |
|------------|-------------------------------------------|----------------------------------------------------------|--------------------------|
|            | CHL | AML | LIS | C vs. A | C vs. L | L vs. A | P<sub>A</sub> | P<sub>D</sub> | P<sub>A</sub> |
| **MMP9 R279Q (rs17576)** |
| AA         | 1552 | 952 | 963 | 0.94   | 0.96   | 0.98   | P<sub>A</sub> = 0.038 | P<sub>D</sub> = 0.05 | P<sub>A</sub> = 0.15 |
| AG         | 1631 | 978 | 1012 | 1.01  | 0.91   | 1.11   | P<sub>A</sub> = 0.67 | P<sub>D</sub> = 0.10 | P<sub>A</sub> = 0.06 |
| GG         | 413  | 301 | 303 | 0.81   | 0.78   | 1.04   | P<sub>A</sub> = 0.024 | P<sub>D</sub> = 0.026 | P<sub>A</sub> = 0.98 |

| **MMP9 R668Q (rs2274756)** |
| GG         | 2602 | 1600 | 1617 | 0.94   | 0.93   | 1.01   | P<sub>A</sub> = 0.36 | P<sub>D</sub> = 0.33 | P<sub>A</sub> = 0.16 |
| GA         | 907  | 562 | 597 | 1.00   | 0.87   | 1.15   | P<sub>A</sub> = 0.52 | P<sub>D</sub> = 0.17 | P<sub>A</sub> = 0.07 |
| AA         | 82   | 65  | 69  | 0.80   | 0.78   | 1.02   | P<sub>A</sub> = 0.28 | P<sub>D</sub> = 0.35 | P<sub>A</sub> = 0.88 |

| **MMP12 −82A>G (rs2276109)** |
| AA         | 2942 | 1857 | 1890 | 0.93   | 0.90   | 1.04   | P<sub>A</sub> = 0.21 | P<sub>D</sub> = 0.06 | P<sub>A</sub> = 0.35 |
| AG         | 588  | 343 | 371 | 1.05   | 0.95   | 1.10   | P<sub>A</sub> = 0.28 | P<sub>D</sub> = 0.16 | P<sub>A</sub> = 0.74 |
| GG         | 53   | 23  | 17  | 1.16   | 1.75   | 0.70   | P<sub>A</sub> = 0.41 | P<sub>D</sub> = 0.025 | P<sub>A</sub> = 0.19 |

| **MMP12 N122S (rs652438)** |
| AA         | 2903 | 1800 | 1822 | 0.96   | 0.94   | 1.02   | P<sub>A</sub> = 0.52 | P<sub>D</sub> = 0.06 | P<sub>A</sub> = 0.19 |
| AG         | 566  | 350 | 388 | 0.96   | 0.81   | 1.18   | P<sub>A</sub> = 0.26 | P<sub>D</sub> = 0.024 | P<sub>A</sub> = 0.08 |
| GG         | 59   | 44  | 37  | 0.76   | 0.70   | 1.10   | P<sub>A</sub> = 0.25 | P<sub>D</sub> = 0.19 | P<sub>A</sub> = 0.83 |

P<sub>A</sub> = P-value for additive genetic model, P<sub>D</sub> = P-value for dominant genetic model, P<sub>A</sub> = P-value for recessive genetic model.

*P-value for gene-by-treatment interaction.

CCVD, combined cardiovascular disease defined as CHD death or non-fatal MI plus stroke, revascularization procedures, angina, heart failure, stroke, and peripheral arterial disease; CHL, chlorthalidone treatment group; AML, amlopidine treatment group; LIS, lisinopril treatment group.

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**ACM.** Participants with the **MMP12 N122S AG** and **GG** genotypes experienced substantially increased ACM (39% and 28%, respectively) on lisinopril compared to those on amlopidine, while no differences in ACM were found with the common AA genotype-treatment combinations. The *P*-value for the pharmacogenetic effect was 0.013 when modeled additively and 0.004 when modeled dominantly. For the chlorthalidone versus amlopidine comparison, GG participants had reduced risk on chlorthalidone (HR = 0.81), while AG heterozygotes had increased risk (HR = 1.32). There was no treatment effect among the common AA homozygotes (HR = 1.08), whereas participants with the AA genotype had a 3.65-fold increased risk of stroke when randomized to chlorthalidone versus amlopidine. However, there was a stronger protective effect of chlorthalidone versus amlopidine among those with the common AA genotype compared to G allele carriers (HR = 0.67 for AA group, HR = 0.95 for AG group, HR = 0.77 for GG genotype).

**Stroke.** Those subjects having **MMP9 R668Q common GG** genotype randomized to chlorthalidone had a slightly increased risk of stroke over those with the same genotype randomized to amlopidine (HR = 1.08), whereas participants with the AA genotype had a 3.65-fold increased risk of stroke when randomized to chlorthalidone versus amlopidine. There was no treatment effect among heterozygotes (HR = 0.99). The *P*-value for the pharmacogenetic effect was marginally significant (*P* = 0.048).

**HF.** A pharmacogenetic effect is suggested for the **MMP12 −82A>G** polymorphism with both the chlorthalidone versus amlopidine group and the chlorthalidone versus lisinopril group comparisons. All subjects with data for the **MMP12 −82A>G** polymorphism, regardless of genotype, showed decreased risk of HF on chlorthalidone versus amlopidine. However, there was a stronger protective effect of chlorthalidone versus amlopidine among those with the common AA genotype compared to G allele carriers (HR = 0.67 for AA group, HR = 0.95 for AG group, HR = 0.77 for GG genotype).
Discussion

In this study we evaluated the pharmacogenetic effects of MMP9 and MMP12 variants on CHD, stroke, HF, combined

Table 6. Pharmacogenetic effects of MMP genetic variants on ACM.

| Genotype | CHL P-y | AML P-y | LIS P-y | Number of events (event rate per 1000 P-y) | Genotype-specific treatment effects: Hazard Ratio (95% CI) | Pharmacogenetic effects* |
|----------|---------|---------|---------|-------------------------------|----------------------------------------------------------|--------------------------|
|          | C vs. A | C vs. L | L vs. A | P = 0.72 | P = 0.87 | P = 0.76 |
| MMP9 R279Q (rs17576) |        |         |         | 1.08 | 1.00 | 1.07 |
| AA       | (29.6) | (27.5) | (29.5) | (0.96–1.21) | (0.90–1.12) | (0.95–1.22) |
| AG       | (28.6) | (27.5) | (29.6) | (0.93–1.16) | (0.87–1.08) | (0.96–1.22) |
| GG       | (27.6) | (28.0) | (27.6) | (0.79–1.21) | (0.81–1.24) | (0.77–1.24) |
| MMP9 R668Q (rs2274756) |        |         |         | 1.05 | 0.97 | 1.08 |
| GG       | (29.1) | (27.6) | (29.8) | (0.96–1.15) | (0.89–1.06) | (0.98–1.19) |
| GA       | (28.4) | (27.0) | (27.8) | (0.91–1.21) | (0.89–1.18) | (0.87–1.21) |
| AA       | (30.0) | (30.1) | (29.7) | (0.63–1.57) | (0.67–1.66) | (0.58–1.58) |
| MMP12 −82A>G (rs2276109) |        |         |         | 1.03 | 0.96 | 1.07 |
| AA       | (28.9) | (28.1) | (29.9) | (0.95–1.11) | (0.89–1.04) | (0.98–1.16) |
| AG       | (29.7) | (148)  | (168)  | 1.22  | 1.09  | 1.12  |
| GG       | (21)    | (14)   | (5)    | 0.74  | 2.04  | 0.37  |
| MMP12 N122S (rs652438) |        |         |         | 1.01 | 1.00 | 1.01 |
| AA       | (28.5) | (28.3) | (28.5) | (0.93–1.10) | (0.93–1.09) | (0.92–1.10) |
| AG       | (365)   | (164)  | (220)  | 1.32  | 0.95  | 1.39  |
| GG       | (29)    | (22)   | (21)   | 0.81  | 0.63  | 1.28  |

P values for gene-by-treatment interaction.

ACM, all-cause mortality; CHL, chlorthalidone treatment group; AML, amlodipine treatment group; LIS, lisinopril treatment group.

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GG group: P = 0.015 for pharmacogenetic effect when modeled dominantly). Among those with the GG genotype, a 3.76-fold increased risk of HF was found for those subjects randomized to chlorthalidone vs. lisinopril, whereas for those with the common AA genotype the risk of HF was reduced in the chlorthalidone group versus the lisinopril group (HR = 0.78). There was no difference in treatment effect among AG heterozygotes (HR = 0.98). Although this pharmacogenetic effect is significant (P = 0.040 for the additive model, P = 0.044 for the dominant model, P = 0.048 for the recessive model), it should be noted that there were few events (n = 2) in the GG genotype-lisinopril group.

Implications of Hardy-Weinberg disequilibrium

Because MMP12 N122S and MMP9 R668Q variants were not in HWE for all ethnic subgroups, we tested whether the suggested pharmacogenetic effects would be similar if we omitted the subgroups in HW disequilibrium. Therefore, for the MMP12 N122S analysis we omitted the Black-non-Hispanic participants (31% of the overall population), and for the MMP9 R668Q analysis we omitted the White-Hispanic participants (13% of the overall population). The results of these analyses showed that for all the findings presented in Tables 3, 4, 5, 6, 7, 8 for these two variants with a P-value less than 0.05, the pharmacogenetic effect (ratio of hazard ratios) was in the same direction and, for the most part, of similar magnitude (data not shown). Due in part to reduced sample sizes, the P-values varied in most cases. In some cases the association was slightly strengthened. In cases where association was attenuated, the most disparate finding when omitting the subgroup in HW disequilibrium was found for the pharmacogenetic effect of the MMP9 R668Q variant on combined CHD when comparing chlorthalidone to amlodipine: for the full group modeled recessively, the ratio of hazard ratios was 0.64 (P = 0.04), whereas it was 0.71 (P = 0.13) when omitting the White-Hispanic group.
CHD and CVD, and ACM. Our data provide evidence of pharmacogenetic associations between variants in the MMP9 and MMP12 genes and treatment for a variety of these cardiovascular outcomes: MMP9 R668Q variant for both combined CHD and stroke; MMP9 R279Q variant for the combined CVD outcome; and MMP12 N122S variant for CHD, combined CHD, combined CVD, and ACM. Specifically, we found that patients having the MMP9 R668Q AA genotype who were treated with chlorthalidone had lower risk of combined CHD but an increased risk of stroke compared to patients treated with amlodipine. Patients with the MMP9 R279Q GG genotype treated with chlorthalidone had lower risk of combined CVD than patients treated with amlopidine or lisinopril. For those having either MMP12 N122S AG or GG genotype, lisinopril treatment increased the risk of CHD, combined CHD, and combined CVD when compared to patients treated with chlorthalidone or a higher risk of CHD and ACM when compared to patients treated with amlopidine. In aggregate, these results indicate these variants may be useful in selecting appropriate antihypertensive agents to reduce risk of CVD.

Because some MMP12 and MMP9 variants were not in HWE for all ethnic subgroups, we investigated whether the pharmacogenetic effects would be similar if we omitted the subgroups in HW disequilibrium. These analyses showed that the pharmacogenetic effect was in the same direction and of similar magnitude with the reduced sample. We note that both the Black and Hispanic groups likely had diverse ancestry given that participants were recruited in the US, Canada, and the Caribbean; therefore, some degree of population substructure is expected. However, it should be emphasized that even if population substructure in the data leads to HW disequilibrium, which can lead to an increase in false-positive findings for main effects of genetic variants on outcomes, this should not explain any pharmacogenetic findings because participants were randomized to treatment; therefore, confounding from population substructure should be controlled because randomization in the context of a large sample size results in the same degree

### Table 7. Pharmacogenetic effects of MMP genetic variants on stroke.

| Genotype | Number of events (event rate per 1000 p-y) | Genotype-specific treatment effects: Hazard Ratio (95% CI) | Pharmacogenetic effects* |
|----------|------------------------------------------|----------------------------------------------------------|--------------------------|
|          | CHL | AML | LIS | C vs. A | C vs. L | L vs. A | C vs. A | C vs. L | L vs. A |
| **MMP9 R279Q (rs17576)** | | | | | | | | | |
| AA       | 288 | 146 | 180 | 1.15 | 0.96 | 1.20 | P_A = 0.63 | P_A = 0.29 | P_A = 0.47 |
|          | (10.4) | (9.0) | (10.8) | (0.94–1.40) | (0.80–1.16) | (0.96–1.49) | | | |
| AG       | 253 | 145 | 185 | 1.05 | 0.78 | 1.35 | P_O = 0.41 | P_O = 0.18 | P_O = 0.68 |
|          | (8.6) | (8.2) | (11.1) | (0.86–1.29) | (0.65–0.94) | (1.08–1.67) | | | |
| GG       | 70  | 45  | 45  | 0.95 | 0.93 | 1.02 | P_R = 0.46 | P_R = 0.75 | P_R = 0.34 |
|          | (8.9) | (9.4) | (9.6) | (0.65–1.38) | (0.64–1.35) | (0.68–1.55) | | | |
| **MMP9 R668Q (rs2274756)** | | | | | | | | | |
| GG       | 456 | 247 | 286 | 1.08 | 0.93 | 1.16 | P_A = 0.12 | P_A = 0.16 | P_A = 0.15 |
|          | (9.8) | (9.1) | (10.5) | (0.93–1.26) | (0.81–1.08) | (0.98–1.37) | | | |
| GA       | 139 | 87  | 114 | 0.99 | 0.71 | 1.39 | P_O = 0.99 | P_O = 0.10 | P_O = 0.15 |
|          | (8.3) | (8.4) | (11.6) | (0.76–1.30) | (0.56–0.92) | (1.05–1.84) | | | |
| AA       | 17  | 3   | 11  | 3.65 | 1.04 | 3.50 | P_R = 0.048 | P_R = 0.62 | P_R = 0.11 |
|          | (11.3) | (3.1) | (10.7) | (1.07–12.5) | (0.49–2.23) | (0.98–12.6) | | | |
| **MMP12 N82A> >G (rs2276109)** | | | | | | | | | |
| AA       | 507 | 283 | 356 | 1.06 | 0.83 | 1.28 | P_A = 0.42 | P_A = 0.18 | P_A = 0.29 |
|          | (9.3) | (8.8) | (11.2) | (0.92–1.23) | (0.73–0.95) | (1.10–1.50) | | | |
| AG       | 98  | 48  | 53  | 1.25 | 1.14 | 1.09 | P_O = 0.57 | P_O = 0.07 | P_O = 0.29 |
|          | (10.1) | (8.1) | (8.9) | (0.88–1.76) | (0.82–1.60) | (0.74–1.62) | | | |
| GG       | 6   | 5   | 2   | 0.59 | 1.58 | 0.43 | P_R = 0.31 | P_R = 0.50 | P_R = 0.16 |
|          | (9.2) | (15.8) | (6.1) | (0.18–1.94) | (0.32–7.81) | (0.08–2.25) | | | |
| **MMP12 N122S (rs652438)** | | | | | | | | | |
| AA       | 483 | 261 | 315 | 1.10 | 0.92 | 1.20 | P_A = 0.62 | P_A = 0.40 | P_A = 0.43 |
|          | (9.4) | (8.5) | (10.2) | (0.95–1.28) | (0.80–1.06) | (1.02–1.42) | | | |
| AG       | 108 | 62  | 83  | 1.04 | 0.74 | 1.41 | P_O = 0.56 | P_O = 0.22 | P_O = 0.60 |
|          | (9.7) | (9.3) | (13.1) | (0.76–1.42) | (0.56–0.99) | (1.01–1.96) | | | |
| GG       | 11  | 9   | 5   | 0.72 | 1.01 | 0.71 | P_R = 0.36 | P_R = 0.80 | P_R = 0.32 |
|          | (10.2) | (14.2) | (10.1) | (0.30–1.74) | (0.35–2.91) | (0.24–2.11) | | | |

P_A = P-value for additive genetic model, P_O = P-value for dominant genetic model, P_R = P-value for recessive genetic model.

*P-value for gene-by-treatment interaction.

CHL, chlorthalidone treatment group; AML, amlodipine treatment group; LIS, lisinopril treatment group.

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Because some MMP12 and MMP9 variants were not in HWE for all ethnic subgroups, we investigated whether the pharmacogenetic effects would be similar if we omitted the subgroups in HW disequilibrium. These analyses showed that the pharmacogenetic effect was in the same direction and of similar magnitude with the reduced sample. We note that both the Black and Hispanic groups likely had diverse ancestry given that participants were recruited in the US, Canada, and the Caribbean; therefore, some degree of population substructure is expected. However, it should be emphasized that even if population substructure in the data leads to HW disequilibrium, which can lead to an increase in false-positive findings for main effects of genetic variants on outcomes, this should not explain any pharmacogenetic findings because participants were randomized to treatment; therefore, confounding from population substructure should be controlled because randomization in the context of a large sample size results in the same degree
ACE inhibitor and found supplemental treatment with the diuretic examined the effects of diuretic therapy in combination with an effects of chlorthalidone on MMP activity. However, Seeland et al. stood, and we are not aware of studies evaluating independent Chlorthalidone’s antihypertensive mechanism is not fully under-
to greatly increased sodium and chloride excretion [32,33]. Like diuretic, acts on the Na⁺Cl⁻ symporter in the kidney, leading
potentially influence MMP-9 function. Chlorthalidone, a thiazide-
like diuretic, acts on the Na⁺Cl⁻ symporter in the kidney, leading
to greatly increased sodium and chloride excretion [32,33]. Chlorthalidone’s antihypertensive mechanism is not fully under-
stood, and we are not aware of studies evaluating independent effects of chlorthalidone on MMP activity. However, Seeland et al. examined the effects of diuretic therapy in combination with an ACE inhibitor and found supplemental treatment with the diuretic furosemide did not provide additional benefit on extracellular matrix remodeling compared to ACE inhibitor alone [34]. Amlodipine is a CCB of the dihydropyridine class. It inhibits vascular smooth muscle contractions, causing increased blood flow to the heart and decreased peripheral arterial resistance and blood pressure [35]. Zervoudaki et al. reported that amlodipine increases plasma MMP-9 levels in hypertensive patients [8,9]. However, a study involving lercanidipine (another dihydropyridine CCB) found decreased MMP-9 activity in hypertensives and suggested a mechanism involving the antioxidant effects of lercanidipine, which are shared by amlodipine [10]. A possible explanation for this discrepancy is that the amlodipine study used ELISA to measure MMP-9 level, whereas the more recent lercanidipine study used gel zymography [10]. Like other ACE inhibitors, lisinopril blocks the production of angiotensin II (a vasoconstric-
tor), thereby decreasing blood pressure. Like the MMPs, ACE is a zinc-dependent endopeptidase [11]. Studies have shown that ACE inhibition may also inhibit MMP levels [1,12,13,14,36,37,38]. Yamamoto et al. identified two potential interaction mechanisms of population substructure in each treatment group. We conclude that, although unmeasured population substructure may be present, it is unlikely that the HW disequilibrium in some ethnic subgroups is driving our suggestive results.

MMP-9 may have a direct effect on plaque destabilization and may also serve as a biomarker predictive of mortality in patients with CVD [16]. As previously mentioned, elevated MMP-9 levels have been observed following acute coronary events [5]. Based on our findings, chlorthalidone and amlodipine could during healing processes as opposed to causing rupture of plaques [16]. However, Johnson et al. suggested MMP-9 is activated in plasma MMP-9 levels in hypertensive patients [5,9] and found decreased MMP-9 activity in hypertensives and suggested a mechanism involving the antioxidant effects of lercanidipine, which are shared by amlodipine [10]. A possible explanation for this discrepancy is that the amlodipine study used ELISA to measure MMP-9 level, whereas the more recent lercanidipine study used gel zymography [10]. Like other ACE inhibitors, lisinopril blocks the production of angiotensin II (a vasoconstric-
tor), thereby decreasing blood pressure. Like the MMPs, ACE is a zinc-dependent endopeptidase [11]. Studies have shown that ACE inhibition may also inhibit MMP levels [1,12,13,14,36,37,38]. Yamamoto et al. identified two potential interaction mechanisms

| Genotype | CHL | AML | LIS | Hazard Ratio (95% CI) | Pharmacogenetic effects* |
|----------|-----|-----|-----|-----------------------|--------------------------|
| **MMP9 R279Q (rs17576)** | | | | | |
| AA       | 359 | 283 | 239 | 0.73                  | PA = 0.53                |
|          | (13.1) | (17.8) | (14.5) | (0.63–0.86) | (0.76–1.06) | (0.69–0.97) |
| AG       | 346 | 281 | 255 | 0.73                  | PD = 0.68                |
|          | (11.9) | (16.2) | (15.5) | (0.63–0.86) | (0.65–0.90) | (0.80–1.13) |
| GG       | 97  | 95  | 70  | 0.61                  | PR = 0.26                |
|          | (12.4) | (20.2) | (15.2) | (0.46–0.81) | (0.60–1.11) | (0.55–1.03) |
| **MMP9 R668Q (rs2274756)** | | | | | |
| GG       | 591 | 463 | 403 | 0.74                  | PA = 0.57                |
|          | (12.8) | (17.3) | (15.0) | (0.65–0.83) | (0.75–0.97) | (0.76–0.99) |
| GA       | 192 | 175 | 149 | 0.67                  | PD = 0.34                |
|          | (11.5) | (17.2) | (15.4) | (0.55–0.82) | (0.60–0.93) | (0.72–1.12) |
| AA       | 17  | 19  | 14  | 0.57                  | PR = 0.49                |
|          | (11.5) | (20.1) | (13.8) | (0.30–1.10) | (0.41–1.68) | (0.35–1.37) |
| **MMP12 –82A>G (rs2276109)** | | | | | |
| AA       | 628 | 546 | 467 | 0.67                  | PA = 0.05                |
|          | (11.6) | (17.3) | (14.9) | (0.60–0.76) | (0.69–0.88) | (0.76–0.98) |
| AG       | 154 | 99  | 96  | 0.95                  | PD = 0.015               |
|          | (16.0) | (17.0) | (16.4) | (0.74–1.22) | (0.76–1.26) | (0.73–1.28) |
| GG       | 14  | 9   | 7   | 0.77                  | PR = 0.048               |
|          | (22.4) | (28.8) | (6.1) | (0.33–1.78) | (0.85–16.6) | (0.04–0.95) |
| **MMP12 N122S (rs652438)** | | | | | |
| AA       | 639 | 522 | 450 | 0.72                  | PA = 0.98                |
|          | (12.5) | (17.3) | (14.8) | (0.64–0.81) | (0.75–0.95) | (0.76–0.97) |
| AG       | 131 | 108 | 96  | 0.72                  | PD = 0.03                |
|          | (11.7) | (16.4) | (15.2) | (0.55–0.92) | (0.59–1.00) | (0.70–1.22) |
| GG       | 14  | 12  | 12  | 0.88                  | PR = 0.84                |
|          | (12.9) | (19.2) | (24.8) | (0.31–1.46) | (0.24–1.13) | (0.59–2.91) |

PA = P-value for additive genetic model, PD = P-value for dominant genetic model, PR = P-value for recessive genetic model.
*P-value for gene-by-treatment interaction.
CHF: chronic heart failure; CHL, chlorthalidone treatment group; AML, amlodipine treatment group; LIS, lisinopril treatment group.
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Table 8. Pharmacogenetic effects of MMP genetic variants on CHF.
between lisinopril and the active site of MMP-9 [38]. Lisinopril was shown to be stabilized in the active site by specific hydrogen bonds, and its hydrophobic group interacted preferentially with the S1 site compared to the S1’ site [38]. In their subsequent work, Yamamoto et al. and Takai et al. also found that different ACE inhibitors show differential binding affinities for MMP-9 [13,36,37,38]. Sakata et al. observed that ACE inhibition directly lowers MMP activity in rats, thus preventing left ventricular remodeling [39]. The MMP9 R279Q polymorphism is a glutamine to arginine substitution located in the catalytic domain of MMP-9 [16,20] and may affect substrate binding [16]. The MMP9 R668Q polymorphism is in the hemopexin-like domain and probably also functions in substrate binding, since removal of this domain disables the cleavage of triple-helix collagen [19].

MMP-12 is expressed in macrophages and epithelial cells and has been implicated in the progression of atherosclerosis, wound repair, and certain cancers [40,41]. Like MMP-9, MMP-12 activity is increased following vascular injury [42]. Plasma MMP-12 levels are increased in patients with coronary artery disease and may, therefore, be an independent risk factor for coronary artery disease [43]. Morgan et al. observed higher MMP-12 levels in thin-cap atherosclerotic plaques (those considered more prone to rupture) versus thick-cap plaques. MMP-12 levels were also elevated in already ruptured plaques, suggesting that MMP-12 has a role in plaque stability [44]. Based on our findings, chlorthalidone, lisinopril, and amlodipine could potentially affect MMP-12 activity. To our knowledge, studies have not evaluated the effects of diuretics, ACE inhibitors, or CCBs on MMP-12. However, based on the finding that lercanidipine may inhibit macrophage function and the fact that macrophages secrete MMP-12, we hypothesize that CCBs may decrease MMP-12 levels [40,45]. The MMP12 −82A>G polymorphism lies in the MMP12 gene promoter [15]. The MMP12 −82A>G polymorphism affects AP-1 binding affinity, with the A allele showing a higher affinity for the protein and, therefore, higher MMP12 promoter activity [15,18]. MMP12 N122S is an asparagine to serine substitution located in the coding region of the MMP12 hemopexin domain, which is responsible for gene activity [17,18]. To our knowledge, no pharmacogenetic studies have been done on this combination of polymorphisms, drugs, and outcomes.

Our data suggest associations between the MMP9 R279Q polymorphism and stroke and between the MMP12 N122S polymorphism and combined CHD. The MMP9 R279Q polymorphism has been associated with increased intima-media thickness [46], an independent predictor of MI and stroke risk [47]. Blankenberg et al. found that MMP9 R279Q had no effect on plasma MMP-9 concentration but was associated with future cardiovascular events in patients with stable angina [16]. This polymorphism also influences aortic stiffness, which is a determinant of cardiovascular risk [48]. We also found a pharmacogenetic effect of the MMP9 R668Q polymorphism to increased stroke risk, although this association may be due to the small number of events (17 in the chlorthalidone group, 3 in the amlodipine group). The MMP12 −82A>G polymorphism has been associated with coronary atherosclerosis [15,49,50]. A study by Jormajo et al. suggests this polymorphism is associated with narrowing of coronary arteries in diabetic patients with CHD [15,50].

GenHAT is an ancillary study of ALLHAT. The ALLHAT study population included only those over age 55 with hypertension. As a result, our findings may not be generalizable to younger age groups. An additional limitation is that we looked at only four MMP polymorphisms. There may be unknown polymorphisms in these genes or combinations of polymorphisms affecting the findings. Since we performed multiple tests of pharmacogenetic effects, these findings would not meet the threshold of statistical significance if corrected for multiple testing (Bonferroni correction: 0.05/72 tests would equate to a P value of 0.0007). However, given the possibility of linkage disequilibrium between variants and non-mutually exclusive outcomes, this threshold is overly conservative. Nevertheless, the likelihood that these findings are false positives is not negligible, and independent replication is necessary. At this time, there are too other large clinical trials that are outcome-based such as GenHAT in which results can be replicated. Our findings and the findings of other studies suggest that future studies might fruitfully investigate the functional interactions of MMP-9 and MMP-12 with diuretics, ACE inhibitors, and CCBs. This study has several strengths. The ALLHAT trial was a large, double-blind randomized trial. Additionally, the study population showed exceptional ethnic and gender diversity (about 50% non-White, about 50% female) [26]. Our results were not sensitive to departures from HWE in various ethnic groups.

Author Contributions
Conceived and designed the experiments: JE BD CF EB DA.Performed the experiments: EB. Analyzed the data: RT AL. Contributed reagents/materials/analysis tools: VB. Wrote the paper: RT DA AL.

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