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CYP3A4 and *CYP3A5* Polymorphisms and Blood Pressure Response to Amlodipine among African-American Men and Women with Early Hypertensive Renal Disease

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Key Words

Pharmacogenetics · Hypertension · Amlodipine · Renal failure · *CYP3A* polymorphisms · AASK · African-Americans

Abstract

Purpose: To explore the association between *CYP3A4* and *CYP3A5* gene polymorphisms and blood pressure response to amlodipine among participants from the African-American Study of Kidney Disease and Hypertension Trial randomized to amlodipine (n = 164). ***Methods:*** Cox proportional hazards models were used to determine the risk of reaching a target mean arterial pressure (MAP) of ≤107 mm Hg by *CYP3A4* (A–392G and T16090C) and *CYP3A5* (A6986G) gene polymorphisms, stratified by MAP randomization group (low or usual) and controlling for other predictors for blood pressure response. ***Results:*** Women randomized to a usual MAP goal with an A allele at *CYP3A4* A–392G were more likely to reach a target MAP of 107 mm Hg. The adjusted hazard ratio (AA/AG compared to GG) with 95% confidence interval was 3.41 (1.20–9.64; p = 0.020). Among participants randomized to a lower MAP goal, those with the C allele at *CYP3A4* T16090C were more likely to reach target MAP: The adjusted hazard ratio was 2.04 (1.17–3.56; p = 0.010). After adjustment for multiple testing using a threshold significance level of p = 0.016, only the *CYP3A4* T16090C SNP remained significant. *CYP3A5* A6986G was not associated with blood pressure response. ***Conclusions:*** Our findings suggest that blood pressure response to amlodipine among high-risk African-Americans appears to be determined by *CYP3A4* genotypes, and sex specificity may be an important consideration. Clinical applications of *CYP3A4* genotype testing for individualized treatment regimens warrant further study.

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Introduction

Approximately 16.8% of the adult US population is diagnosed with chronic kidney disease, and those with preexisting hypertension are almost twice as likely to develop chronic renal disease [1]. African-Americans are at highest risk for end-stage renal disease from hypertension [1], and this disparity remains despite adjustment for age, gender, and co-morbid illness [2–5]. Genetic factors may influence response to antihypertensive drug treatment, resulting in variability between ethnic groups or races.

CYP3A is a subfamily of the cytochrome P450 superfamily of genes involved in the metabolism of endogenous compounds and over 50% of pharmaceutical agents; *CYP3A4* is largely responsible for adult drug metabolism [6–8], including antihypertensive drug disposition [9–11]. *CYP3A4* and *CYP3A5* polymorphisms have been shown to vary between ethnic (racial) groups, and *CYP3A4* polymorphisms may account for much of the observed variability in drug efficacy and toxicity. While *CYP3A5* is highly polymorphic, variation at this gene does not appear to result in significant differences in hepatic adult drug metabolism [7, 8]. Hepatic P450 gene regulation is also influenced by endogenous steroid hormones, resulting in gender-specific differences in *CYP3A* expression and drug handling [7, 12].

Amlodipine is a long-acting antihypertensive dihydropyridine calcium channel blocker (CCB) that prevents the influx of calcium ions in vascular smooth muscle cells, thereby acting primarily as a peripheral vasodilator. It is slowly absorbed and is extensively metabolized in the liver, mainly by *CYP3A4* [11, 13]. CCBs have been seen to be more effective in African-Americans than in other ethnic/ancestral groups, suggesting that variation in *CYP3A4* and possibly *CYP3A5* function or expression might account for differences in CCB drug response between such ethnic groups [14–16]. However, CCB pharmacokinetics and efficacy have not as yet been conclusively shown to vary by *CYP3A4* and *CYP3A5* polymorphisms (table 1) [17–19]. In this study, we explored the association of polymorphisms on the *CYP3A4* and *CYP3A5* genes with blood pressure response to amlodipine in African-American men and women with early hypertensive nephrosclerosis based on randomized trial data from the NIDDK African-American Study of Kidney Disease and Hypertension Study (AASK) [20].

Methods

Participants and Study Design

Details of the AASK study [21, 22] and details of the AASK Genetics Study [23, 24] have been previously published. Informed consent was received from AASK participants at the time of the original study and the study protocol approved by institutional human subjects review boards. Briefly, the original trial included of 1,094 self-identified African-American men and women between the ages of 18 and 70 years and diagnosed with hypertensive kidney disease (glomerular filtration rate between 20 and 65 ml/min per 1.73 m^2), randomized to either an angiotensin-converting enzyme inhibitor, a β-adrenergic receptor blocker or a dihydropyridine CCB. Participants were also randomized to either a low mean arterial pressure (MAP) (≤92 mm Hg) or a usual MAP (102–107 mm Hg) group. Unlike the drug randomization group that was double blinded, the MAP group was known by both investigators and research subjects, and medications were titrated based on the MAP treatment group. Of those eligible and consented to participate in the AASK Genetics Study (n = 850), 159 (19.04%) were randomized to the CCB amlodipine. Ten of these observations were removed due to incomplete or missing genotype data; 149 observations were used in the present analysis.

Table 1. *CYP3A* polymorphisms: previously observed effects

Polymorphism	Effects on gene transcription or antihypertensive drug pharmacokinetics
CYP3A5 A6986G	G-allele associations (also referenced as *CYP3A5**3C)
Ref SNP 776746	No differences in felodipine pharmacokinetics [17]
Intron-3	No significant differences in nifedipine metabolism [18]
CYP3A4 A–392G	G-allele associations (also referenced as *CYP3A4**1B)
Ref SNP 2740574	Increased gene transcription [30]
Promoter region	No significant effect on nifedipine metabolism [19]
CYP3A4 T16090C	C-allele associations
Ref SNP 2246709	Interaction with CYP2D6; higher metabolism of [26] and lower sympathetic activity from yohimbine [43]
Intron-7	No significant association with *CYP3A4* enzyme activity [37, 44]

Genomics

Details of DNA extraction and handling have been previously published [23, 24]. Two *CYP3A4* and one *CYP3A5* single nucleotide gene polymorphisms (SNPs) located on chromosome 7q21 [25] known to influence the metabolism of several medications were selected (table 1): (1) *CYP3A4* T16090C, a T to C polymorphism within intron-7, 16,090 base pairs from the *CYP3A4* cap site (RefSNP rs2246709) [26], (2) *CYP3A4* A–392G, a functional A to G polymorphism, and also the most frequently studied genetic variant of *CYP3A4* [27], located in the 5′-flanking region (also known as *CYP3A4*1B;* RefSNP rs2740574) [8], and (3) *CYP3A5* A6986G, a functional A to G polymorphism located in intron-3 of *CYP3A5,* responsible for alternative splicing and protein truncation (also known as *CYP3A5*3;* RefSNP rs776746) [6, 10, 25]. Genotype assignments were made by capturing images with a flatbed scanner and using proprietary research software developed by Roche Molecular Systems (Pleasanton, Calif., USA). Hardy-Weinberg equilibrium was assessed by using the Pearson goodness of fit (χ^2) test statistic.

Statistical Analysis

Because of multiple comparison testing (3 genetic variants), statistical significance was determined by an α-level of 0.016. Descriptive statistics were performed to assess baseline characteristics and preliminary data among CCB genotypes, between males and females, and between low and usual target MAP groups using one-way ANOVAs or t tests. To eliminate any potential for obscuring the genotype-drug response relationship as a result of progressive renal disease, analyses were restricted to the first year after randomization. Haplotypes could not be inferred with reasonable certainty for many individuals; therefore, analyses were limited to individual genotypes.

As described in our previous studies, the main outcome was number of days to reach a target MAP of ≤107 mm Hg. Cox proportional hazards models were used to determine the hazard (relative risk) of reaching target MAP of 107 mm Hg. Cox proportional hazards assumptions were tested and, if violated, the model was stratified by a third variable. A stepwise approach was used to explore the effects of other potential predictors of blood pressure response and variables found to contribute significantly to the overall model were included in the adjusted models. These variables included baseline demographics (i.e., age, insurance status, education), anthropometrics (body mass index, baseline MAP, heart rate), renal function (glomerular filtration rate), other biochemical laboratory measures (serum lipids, creatinine, blood urea nitrogen, liver function tests, etc.) and co-morbid disease such as coronary artery disease. It should be noted that other antihypertensive medications (other than one of the three study drugs) were used to manage blood pressure and were systematically added according to a standardized protocol across study sites. Therefore, the average number of daily medications during the first year of follow-up was used as a proxy measure to control for antihypertensive medication use.

With blood pressure randomization (low or usual MAP) and gender stratification, this study had 80% power to detect a hazard ratio of 2.6. Statistical analyses were performed using SAS statistical software, version 9.1, programming package (SAS Institute, Inc., Cary, N.C., USA) and STATA statistical software, release 9.2 and 10.0 (StataCorp LP, College Station, Tex., USA).

Drug Specificity and Population Stratification

The metabolism and mechanism of action for amlodipine on blood pressure is different from angiotensin enzyme inhibitors. Therefore, participants randomized to ramipril (n = 302) were used as specificity controls. Previously, we had also shown that population stratification did not contribute to blood pressure response in this dataset using a generalized analysis of molecular variance (GAMOVA) [23, 28].

Results

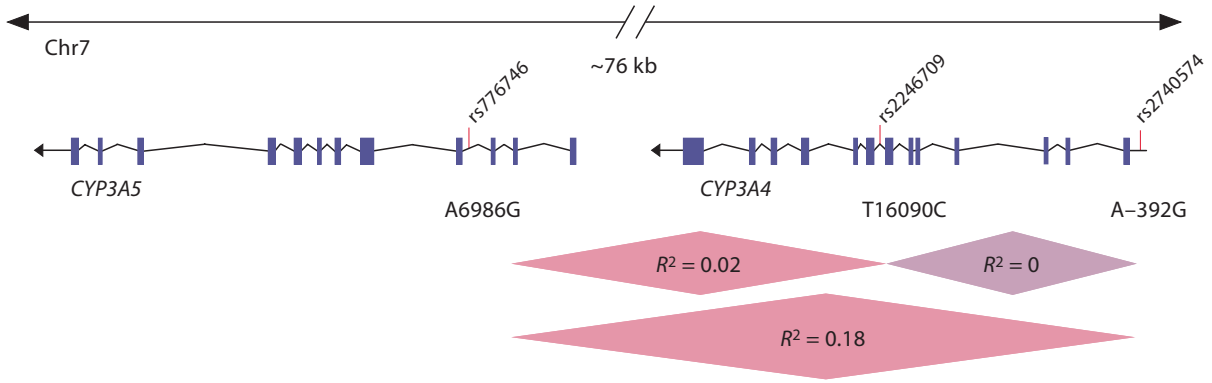
Genomics

Of the 164 participants randomized to amlodipine, 137 (84%) were genotyped at *CYP3A4* A–392G, 143 (87%) at *CYP3A4* T16090C and 145 (88%) at *CYP3A5* A6986G. All three polymorphisms were in Hardy-Weinberg equilibrium and allele frequencies were similar to frequencies listed on dbSNP (http://www.ncbi.nlm.nih.gov/SNP/; fig. 1). However, there were too few A/A homozygotes at A–392G (n = 10) for a meaningful analysis; homozygous A/A were combined with heterozygous A/G individuals, resulting in an A-dominant model for analysis. Similarly, there were too few homozygous C/C individuals at T16090C (n = 13) and too few G/G individuals at A6986G (n = 8), resulting in C- and G-dominant models for analyses at these sites, respectively. Some linkage disequilibrium was detected between *CYP3A5* A6986G and *CYP3A4* A–392G (R^2 = 0.18) and between *CYP3A5* A6986G and *CYP3A4* T16090C (R^2 = 0.02); however, *CYP3A4* A–392G and *CYP3A4* T16090C were not in linkage disequilibrium in this population (R^2 = 0) (fig. 1).

Ancillary AASK Study Participants: Characteristics and Preliminary Outcomes

Baseline characteristics were similar between our amlodipine group and the original AASK study cohort [29]. There was complete follow-up in the first year and 11,811 days at risk for analysis. There were 76 men (mean age 52 ± 11) and 61 women (mean age 53 ± 10). Of the 135 participants, only 11 did not reach a target MAP of 107 mm Hg.

There were no significant baseline differences by genotype (appendix 1). There were more men who did not reach target MAP compared to females (12 vs. 1.5%; p = 0.04). Those randomized to lower MAP had lower total cholesterol (206 vs. 222 mg/dl; p = 0.04), required more daily medications (3.89 vs. 3.16 medications; p = 0.0003) and had a lower average MAP in the first year after randomization (96 vs. 104 mm Hg; $p < 0.0001$). Men had higher average MAPs in the first year after randomiza-



Population	*CYP3A5* A6986G		*CYP3A4* T16090C		*CYP3A4* A–392G	
	A	G	T	C	A	G
Caucasians	0.06	0.94	0.73	0.27	0.97	0.03
African-Americans	0.65	0.35	0.67	0.33	0.32	0.68
AASK	0.72	0.28	0.62	0.38	0.34	0.66

Fig. 1. *CYP3A4* and *CYP3A5* polymorphisms population frequencies. *CYP3A4* and *CYP3A5* are located in tandem on chromosome 7q21. Two *CYP3A4* and one *CYP3A5* SNP were selected: (1) *CYP3A4* T16090C, a T to C polymorphism within intron-7, 16,090 base pairs from the *CYP3A4* cap site (rs2246709); (2) *CYP3A4* A–392G, an A to G polymorphism, and also the most common variant of *CYP3A4,* located in the 5′-flanking region (rs2740574), and (3) *CYP3A5* A6986G, an A to G polymorphism located in intron-3, responsible for alternative splicing and protein truncation (rs776746). The extent of linkage disequilibrium between loci is represented as R-squared (R^2). Allele frequencies in Caucasian and African-Americans made available dbSNP (http://www.ncbi.nlm.nih.gov/SNP) or the Pharmacogenomics Knowledge Base (http://www.pharmgkb.org) and in the AASK population are also shown.

tion (102 vs. 98 mm Hg; p = 0.003) had higher measures of serum creatinine (2.39 vs. 1.70 mg/dl; p < 0.0001), and on average used more daily medications (3.81 vs. 3.20 medications; p = 0.05; appendix 2).

Survival Analyses: Time to Reach Target MAP (≤107 mm Hg)

Hazard ratios ('relative risk' of reaching target MAP ≤107 mm Hg) and 95% confidence intervals were estimated using a Cox proportional hazards model (table 2). Inclusion of low and usual MAP groups violated the Cox proportional hazards assumption; hence, these groups were analyzed in separate models. The effects of gender were also explored by gender stratification.

CYP3A4 A–392G

There were no significant differences in the risk of reaching a target MAP ≤107 mm Hg among men and women (combined) randomized to a low MAP goal by *CYP3A4* A–392G genotypes (table 2). However, gender stratification suggested that women with an A/A or A/G randomized to a usual MAP genotype were more likely to reach a target MAP of 107 mm Hg. The hazard ratio (A/A and A/G compared to G/G), with 95% confidence interval, was 2.81 (0.93–5.59; p = 0.07). After adjusting for average number of daily medications, this association was stronger, though of borderline significance with correction for multiple comparisons: 3.41 (1.20–9.64; p = 0.02) (fig. 2).

CYP3A4 T16090C

No significant differences were found by *CYP3A4* T16090C genotypes among those randomized to the usual MAP group (table 2). However, among those randomized to the low MAP group, individuals with a C/C or T/C genotype were twice as likely to reach a target MAP 107 mm Hg compared to T/T individuals: 2.04 (1.17–3.56; adjusted p = 0.01). Similar results were found

Table 2. Cox proportional hazards model in amlodipine-treated subjects, stratified by *CYP3A4* and *CYP3A5* genotypes

Model	*CYP3A4* A–392G (A/A or A/G vs. G/G) (n = 135)			*CYP3A4* T16090C (C/C or T/C vs. T/T) (n = 145)			*CYP3A5* A6986G (G/G or A/G vs. A/A) (n = 146)		
	hazard ratio	95% CI	p*	hazard ratio	95% CI	p*	hazard ratio	95% CI	p*
Low MAP									
Overall									
Unadjusted	0.92	0.56, 1.54	0.76	2.18	1.27, 3.76	**0.005**	0.81	0.50, 1.29	0.37
Adjusted*	0.81	0.48, 1.38	0.44	2.04	1.17, 3.56	**0.01**	0.99	0.60, 1.61	0.95
Males									
Unadjusted	1.01	0.45, 2.30	0.98	1.94	0.95, 4.20	0.07	0.84	0.42, 1.68	0.61
Adjusted*	1.10	0.46, 2.65	0.832	2.22	1.03, 4.79	**0.04**	0.85	0.42, 1.73	0.66
Females									
Unadjusted	0.87	0.43, 1.78	0.70	2.22	1.01, 5.01	**0.05**	1.06	0.50, 2.24	0.88
Adjusted*	0.67	0.31, 1.42	0.29	2.33	1.01, 5.40	**0.05**	1.20	0.55, 2.59	0.649
Usual MAP									
Overall									
Unadjusted	0.91	0.53, 1.56	0.72	0.81	0.49, 1.32	0.39	0.85	0.53, 1.37	0.51
Adjusted*	1.48	0.82, 2.69	0.19	0.77	0.46, 1.31	0.34	0.60	0.35, 1.02	0.06
Males									
Unadjusted	0.97	0.45, 2.10	0.94	0.77	0.39, 1.49	0.43	0.75	0.39, 1.42	0.38
Adjusted*	1.31	0.58, 2.93	0.51	0.82	0.46, 1.85	0.92	0.73	0.38, 1.38	0.32
Females									
Unadjusted	2.81	0.93, 5.59	0.07	0.95	0.46, 2.09	0.95	1.44	0.64, 3.21	0.38
Adjusted*	3.41	1.20, 9.64	0.02	1.39	0.59, 3.27	0.46	1.16	0.52, 2.60	0.72

$p \leq 0.05$ bolded, marginal significance; $p \leq 0.016$ significant after adjustment for three multiple comparisons.

* Adjustment for baseline MAP, average number of daily antihypertensive medications, age, gender (for combined male and female models) and/or serum creatinine.

among men and women: 2.22 (1.03–4.79; adjusted p = 0.04) and 2.33 (1.01–5.40; adjusted p = 0.048), respectively (fig. 3).

CYP3A5 A6986G

There were no associations between *CYP3A5* A6986G genotypes and risk of reaching target MAP of 107 mm Hg in this dataset.

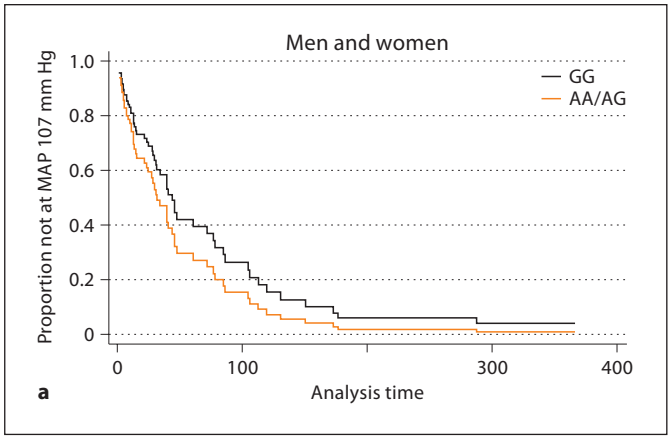
Drug Specificity

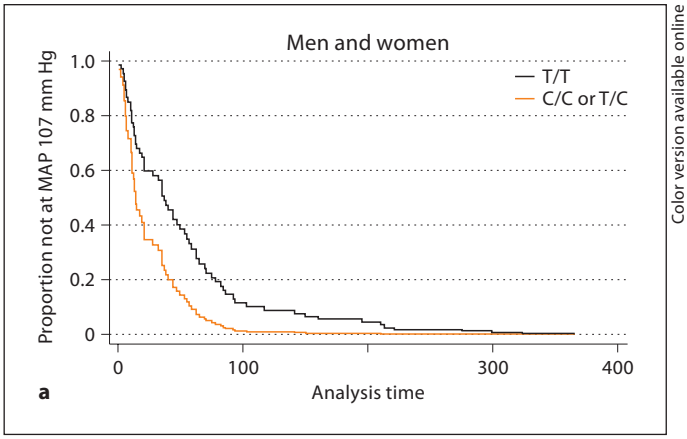
Among those randomized to ramipril, there were no associations between *CYP3A4* A–392G, *CYP3A4* T16090C, or *CYP3A5* A6986G genotypes and time to reach target MAP among men or women randomized to a low or usual MAP.

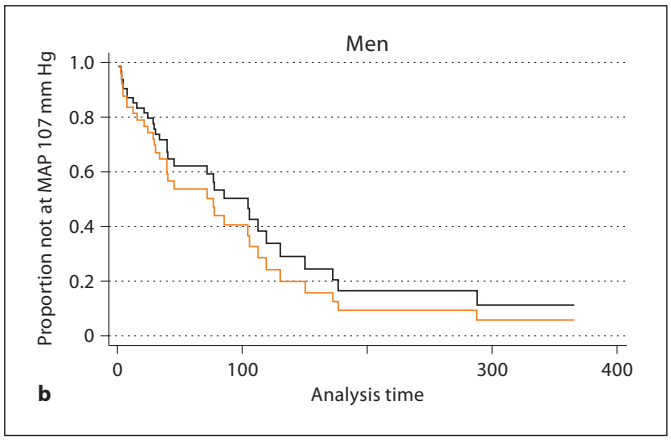
Discussion

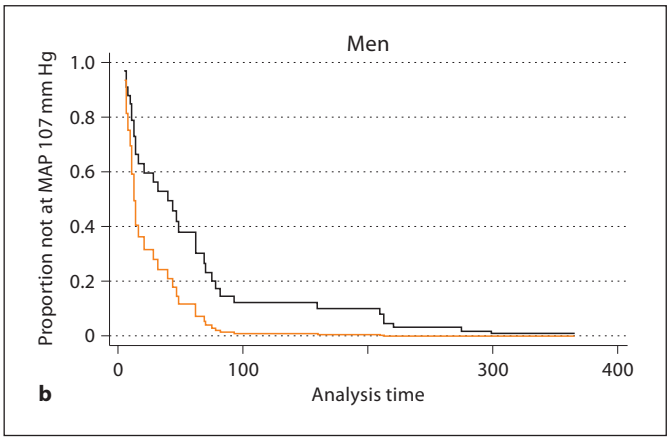
Study Overview and Main Results

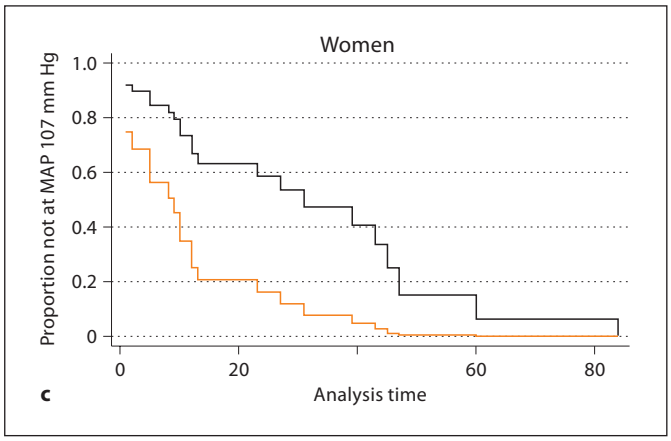
The *CYP3A4* A–392G promoter polymorphism was predictive of blood pressure response among African-American women with early hypertensive nephrosclerosis randomized to a usual MAP group (102–107 mm Hg); though of marginal significance after correction for multiple comparisons, women with an A allele were over 3 times more likely to reach a target MAP of 107 mm Hg (p = 0.02). A less studied *CYP3A4* T16090C intronic SNP [26] was significantly associated with blood pressure response to amlodipine among those randomized to a lower MAP group (≤92 mm Hg). Men and women with a C allele at T16090C were twice as likely to reach target MAP 107 mm Hg (p = 0.01). *CYP3A4* genotype and blood pressure response appeared to be specific to amlodipine since there were no associations among men or women randomized to ramipril. As shown in our previous studies,











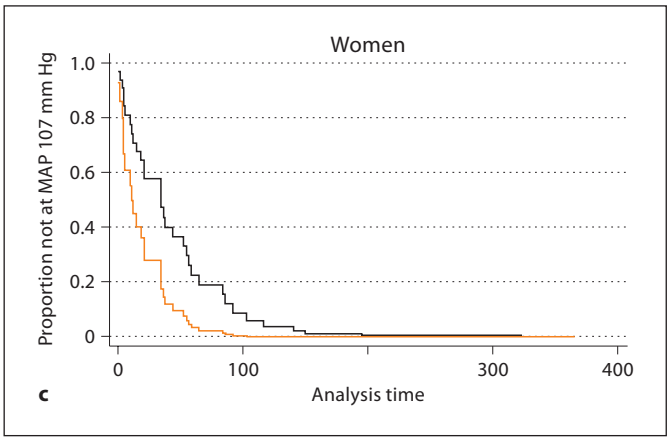


Fig. 2. Adjusted Cox regression survival curves by *CYP3A4* promoter A–392G (usual MAP goal 102–107 mm Hg treatment group). Among men and women randomized to usual MAP treatment goal, there was not a significant difference in rate of reaching a target MAP of 107 mm Hg by A–392G genotypes (**a**; adjusted p = 0.77). Upon gender stratification, there was no difference among men (**b**; adjusted p = 0.82); however, women with A/A or A/G (compared to G/G) genotype were over 3 times more likely to reach a target MAP of 107 mm Hg (**c**; adjusted p = 0.02).

Fig. 3. Adjusted Cox regression survival curves by *CYP3A4* T16090C (low MAP goal <92 mm Hg). Among more aggressively managed men and women, those with a C/C or T/C compared to a T/T genotype were twice as likely to reach a target MAP of 107 mm Hg (**a**; adjusted p = 0.01). Stratified analyses suggested similar results among men (**b**; adjusted p = 0.05) and women (**c**; adjusted p = 0.05).

population stratification did not appear to be contributing to the relationship between genotype and blood pressure response in this dataset [23, 24]. *CYP3A5* A6986G was not associated with blood pressure response in this study.

CYP3A4 G–392A

G–392A is a functional promoter polymorphism [27] and is a likely candidate for the regulation of gene transcription [30]. Based on this study, women with an A-allele were more likely to respond to amlodipine, suggesting that the G-allele carriers were more rapid metabolizers of amlodipine. The G-allele at A–392G has been associated with higher drug dose requirements in some [31–33] but not all studies [19, 34]. However, most studies did not examine the relationship in a sex-specific manner. We found that the effects of *CYP3A4* A–392G genotype on therapeutic response to amlodipine differed in men and women, suggesting differential CYP activity in the male versus female hormonal milieu. P-glycoprotein levels, a multidrug transporter that mediates cellular transport of several drugs, have been shown to be 2–3 times higher in men in comparison to women. P-glycoprotein-mediated efflux of drugs such as amlodipine may result in higher intracellular concentrations in women, resulting in more variation in metabolism according to *CYP3A4* genotype [35, 36]. Another *CYP3A4* variant (rs464637, in close proximity to T16090C) also affected CYP expression and activity in a sex-dependent way [37], and *CYP3A4* gene polymorphisms have been associated with early onset of puberty in females [38–40] and hormone-dependent cancers (e.g. endometrial, breast, prostate) [27, 41, 42].

CYP3A4 T16090C

Men and women with a C-allele who were more aggressively treated (i.e., randomized to the low MAP of $\leq$92 mm Hg) were more likely to respond to amlodipine (fig. 3), suggesting that carriers of the C-allele may be poorer metabolizers of amlodipine. Associations for men and women were of similar magnitude and direction, suggesting that the effect of T16090C genotypes is not sex-specific. The intronic *CYP3A4* T16090C SNP was first studied by Le Corre et al. [26]. In this original study, the metabolism of yohimbine (α_2-adrenergic antagonist that results in increased sympathetic activity) was shown to be dependent on both *CYP3A4* T16090C and *CYP2D6*. Among those in the fast *CYP2D6* metabolizer group, the C-allele was associated with faster metabolism of yohimbine in comparison to the T-allele, suggesting functionality at (or in close proximity to) *CYP3A4* T16090C. In concordance with these findings, Bharucha et al. [43] found higher sympathetic activity with yohimbine among those classified as poor metabolizers (in comparison to fast metabolizers) according to *CYP2D6* and *CYP3A4* T16090C status. While our results suggest decreased metabolic activity with the C-allele at *CYP3A4* T16090C, epistatic effects or interactions with *CYPD26* were not explored here. Other studies have not found significant effects of the T16090C polymorphism on gene expression [37, 44] or N-dealkylation of midazolam or verapamil [37, 45].

CYP3A5 A6986G

While CCBs are also *CYP3A5* substrates, the lack of an association between blood pressure response to amlodipine and *CYP3A5* A6986G is consistent with previous studies suggesting no difference in the pharmacokinetics of nifedipine or felodopine (dihydropyridine CCBs, similar to amlodipine) by *CYP3A5* A6986G genotypes [17, 18]. *CYP3A5* is highly polymorphic and *CYP3A5* variants result in decreased CYP3A5 protein expression. However, *CYP3A5* does not appear to contribute significantly to adult hepatic metabolism in comparison to *CYP3A4*. Furthermore, the *CYP3A5* A6986G allele is not common among African-Americans (28% minor allele frequency in our study population), resulting in limited power to study the effects of this polymorphism with this dataset [46].

Study Advantages and Limitations

The AASK Genomics Study presented a unique opportunity to explore associations between genotype and antihypertensive blood pressure responses in the powerful setting of a large randomized clinical trial using a novel application of survival methodology to the analysis of blood pressure and to explore gene-by-gender interactions with respect to blood pressure response to amlodipine. While we considered adjustment for multiple comparison testing by the three genotypes (significant p value 0.016), we did not adjust for data stratification by MAP randomization group (low and usual MAP) and gender. Results from this study must also be confirmed in independent populations, in other clinical trials or other ethnic/racial groups. TagSNPs (markers for a haplotype block) could also be used in future studies to more comprehensively cover the region of the genome around these sites. Given the modest number of AASK subjects randomized to amlodipine, this study was not designed to detect small differences in genotype-dependent blood pressure response, nor was it adequately powered to study the effects of rare alleles.

Conclusions

Blood pressure response to amlodipine among high-risk African-American patients appears to be partly determined by *CYP3A4* genotypes, and sex specificity may be an important consideration. These initial results are provocative and suggest that *CYP3A4* genotype testing may be clinically predictive of blood pressure response to amlodipine and perhaps other CCBs. The clinical use of *CYP3A4* genotype testing for individualized treatment regimens should be further explored in larger hypertension clinical studies.

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Appendix 1. Baseline characteristics and preliminary outcomes stratified by *CYP3A* genotypes

	CYP3A4 A–392G (n = 135)				*CYP3A4* T16090C (n = 145)				*CYP3A5* A6986G (n = 146)			
	A/A (n = 10)	A/G (n = 70)	G/G (n = 55)	p	C/C (n = 13)	T/C (n = 79)	T/T (n = 53)	p	A/A (n = 74)	A/G (n = 64)	G/G (n = 8)	p
Number randomized to low MAP (%)	4 (40)	31 (44)	32 (58)		7 (53)	40 (49)	28 (53)		39 (52)	31 (47)	5 (63)	
Number of men (%)	6 (60)	48 (69)	20 (37)		8 (61)	45 (55)	29 (55)		33 (44)	41 (62)	8 (100)	
Number who did not reach target (%)	0	9 (13)	2 (4)	0.26	1 (8)	3 (4)	4 (8)	0.66	1 (1)	6 (9)	1 (12)	0.07
Age, years	53 ± 10	53 ± 11	52 ± 11	0.96	53 ± 12	52 ± 11	53 ± 10	0.90	53 ± 11	53 ± 11	49 ± 13	0.74
Baseline MAP, mm Hg	106 ± 21	114 ± 19	115 ± 15	0.38	117 ± 22	115 ± 17	114 ± 18	0.69	114 ± 16	115 ± 20	120 ± 18	0.58
Body mass index, kg/m^2	31 ± 7	30 ± 7	32 ± 6	0.52	29 ± 7	31 ± 7	31 ± 6	0.36	31 ± 7	30 ± 6	30 ± 5	0.30
Serum creatinine, mg/dl	2.24 ± 0.82	2.21 ± 0.90	1.88 ± 0.72	0.07	2.03 ± 1.0	2.01 ± 0.74	2.11 ± 0.88	0.56	1.99 ± 0.78	2.01 ± 0.83	2.8 ± 0.66	0.09
Glomerular filtration rate ml/min per 1.73 m^2	42 ± 15	45 ± 13	47 ± 12	0.39	46 ± 12	47 ± 13	45 ± 12	0.54	46 ± 12	47 ± 13	39 ± 14	0.55
Total cholesterol, mg/dl	227 ± 54	207 ± 50	219 ± 46	0.26	202 ± 29	212 ± 50	218 ± 48	0.28	216 ± 47	211 ± 47	216 ± 57	0.69
Average number of daily medications	2.89 ± 1.17	3.76 ± 1.34	3.41 ± 1.12	0.07	3.3 ± 1.1	3.5 ± 1.3	3.6 ± 1.3	0.36	3.5 ± 1.2	3.6 ± 1.3	3.3 ± 1.4	0.78
Average MAP in first year after randomization	99 ± 6	101 ± 7	100 ± 6	0.55	101 ± 10	100 ± 6	100 ± 7	0.94	100 ± 6	101 ± 7	98 ± 9	0.94

Appendix 2. Baseline characteristics and outcomes stratified by gender or MAP group

AASK participant characteristic	All (n = 149)	Gender			MAP group		
		males (n = 82)	females (n = 67)	p	low MAP (≤ 92 mm Hg) (n = 75)	usual MAP (≤ 107 mm Hg) (n = 74)	p
Number randomized to low MAP (%)	75 (50)	39 (52)	36 (48)		–	–	
Number of men (%)	82 (55)	–	–		39 (52)	43 (58)	
Number who did not reach target (%)	11 (7)	10 (12)	1 (1.5)	**0.04**	4 (5)	7 (10)	0.46
Age at randomization, years	53 ± 11	52 ± 11	54 ± 11	0.27	53 ± 11	53 ± 11	0.93
Baseline MAP, mm Hg	115 ± 18	117 ± 20	112 ± 15	**0.05**	116 ± 20	112 ± 15	0.13
Body mass index, kg/m^2	31 ± 7	31 ± 6	30 ± 7	0.63	31 ± 6	31 ± 7	0.82
Serum creatinine, mg/dl	2.05 ± 0.82	2.33 ± 0.89	1.70 ± 0.55	**<0.0001**	1.94 ± 0.73	2.15 ± 0.89	0.13
Glomerular filtration rate, ml/min per 1.73 m^2	46 ± 13	47 ± 13	45 ± 12	0.17	46 ± 12	46 ± 13	0.82
Total cholesterol, mg/dl	214 ± 47	211 ± 48	217 ± 47	0.46	206 ± 39	222 ± 54	**0.04**
Average number of daily medications	3.53 ± 1.25	3.81 ± 1.24	3.20 ± 1.19	**0.005**	3.89 ± 1.15	3.16 ± 1.25	**0.0003**
Average MAP in first year after randomization	100 ± 7	102 ± 7	98 ± 5	**0.003**	96 ± 6	104 ± 5	**<0.0001**

p < 0.05 bolded.

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