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Angiotensin-converting enzyme gene polymorphism predicts the time-course of blood pressure response to angiotensin converting enzyme inhibition in the AASK trial

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Abstract

Objective—It has yet to be determined whether genotyping at the angiotensin-converting enzyme (ACE) locus is predictive of blood pressure response to an ACE inhibitor.

Methods—Participants from the African American Study of Kidney Disease and Hypertension trial randomized to the ACE inhibitor ramipril ($n = 347$) were genotyped at three polymorphisms on *ACE*, just downstream from the *ACE* insertion/deletion polymorphism (Ins/Del): G12269A, C17888T, and G20037A. Time to reach target mean arterial pressure (≤ 107 mmHg) was analyzed by genotype and *ACE* haplotype using Kaplan–Meier survival curves and Cox proportional hazard models.

Results—Individuals with a homozygous genotype at G12269A responded significantly faster than those with a heterozygous genotype; the adjusted (average number of medications and baseline mean arterial pressure) hazard ratio (homozygous compared to heterozygous genotype) was 1.86 (95% confidence limits 1.32–3.23; $P < 0.001$ for G12269A genotype). The adjusted hazard ratio for participants with homozygous *ACE* haplotypes compared to those heterozygous *ACE* haplotypes was 1.40 (1.13–1.75; $P = 0.003$ for haplotype). The *ACE* genotype effects were specific for ACE inhibition (i.e., not seen among those randomized to a calcium channel blocker), and were independent of population stratification.

Conclusions—African-Americans with a homozygous genotype at G12269A or homozygous *ACE* haplotypes responded to ramipril significantly faster than those with a heterozygous genotype or heterozygous haplotypes, suggesting that heterosis may be an important determinant of responsiveness to an ACE inhibitor. These associations may be a result of biological activity of this polymorphism, or of linkage disequilibrium with nearby variants such as the *ACE* Ins/Del, perhaps in the regulation of *ACE* splicing.

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Keywords

angiotensin-converting enzyme; hypertension; polymorphisms; renal failure

Introduction

Approximately 8 000 000 adults in the United States have renal insufficiency or chronic renal failure, defined by a glomerular filtration rate (GFR) below 60 ml/min per 1.73 m² [1]. While up to 10% of people in the general population are expected to develop renal insufficiency [2], over 57% of hypertensive patients will develop some degree of renal insufficiency [3]. The outcome for African Americans is particularly daunting because they are approximately six times more likely than whites to develop end stage renal disease (ESRD) [3]. While both systolic and diastolic pressures are strongly associated with the risk of developing ESRD [4], the majority of hypertensive patients are not adequately treated despite numerous therapeutic options [5–8].

The African American Study of Kidney Disease and Hypertension (AASK) trial, started in 1995, is a multisite trial sponsored by the US National Institutes of Health; details for the design of this study have been previously published and are briefly presented below [9]. The AASK Genomics Committee has also been systematically collecting DNA samples, allowing for a unique opportunity to explore the relationship between genetic predictors of antihypertensive drug response. In this study, we focused on the relationship between angiotensin-converting enzyme (ACE) gene polymorphisms (located on chromosome 17q23, consisting of 26 exons) and blood pressure response to an ACE inhibitor (ACE-I) ramipril. While some studies have begun to explore the relationship between the ACE insertion/deletion (Ins/Del) polymorphism on intron 16 and blood pressure response to ACE-Is, there is inconclusive evidence that this polymorphism results in a clinically distinct response phenotype [10]. Most studies, including the recently published Genetics of Hypertension-Associated Treatment (GenHAT) Study, compared blood pressure measurements before and after treatment initiation [11]. This approach, however, may not effectively capture blood pressure response over time. Therefore, we used a relatively novel approach and applied survival methodology by focusing on the time to reach a target blood pressure goal (in this case, a mean arterial pressure or MAP of ≤ 107 mmHg). We also concentrated on specific polymorphisms in the region of the gene encoding the catalytically active domain, and just downstream from the Ins/Del that have been previously related to ACE plasma levels [12], as well as alternative splicing of the ACE mRNA [13].

Methods

The AASK Genomics Committee enrolled volunteers through primary sites (human subjects' approval by individual study sites, including Mount Sinai School of Medicine and the University of California San Diego). Briefly, the original AASK cohort included 1094 African-American men and women between the ages of 18 and 70 years with a clinical diagnosis of hypertensive nephrosclerosis (patients with a GFR between 20 and 65 ml/min per 1.73 m² were included and patients with a history of type 1 or 2 diabetes were excluded). Participants were randomized to treatment with either the ACE-I ramipril, the β -adrenergic receptor blocker (beta-blocker) metoprolol, or the dihydropyridine calcium channel blocker (CCB) amlodipine [14]. Beginning in 2002, 994 participants were eligible (still alive and not lost to follow-up) for the AASK Genomics Study. Of these, 850 consented to participate with DNA samples (85% ascertainment); 839 had adequate DNA samples for genotyping, 347 of whom were randomized to treatment with ramipril (Fig. 1).

It should be noted here that blood pressure was systematically collected across all study sites. Blood pressures were measured using a Hawksley random zero sphygmomanometer to minimize observer bias, and observers underwent periodic training to avoid digit preference in blood pressure reports; all measurements were seated, after a 5-min minimum rest period. The average of the last two of three consecutive blood pressure measurements was used. Details of this protocol have been previously published [9,14,15].

Genetics

Three ACE gene polymorphisms, spanning approximately 8 kb and located downstream from the ACE Ins/Del (on intron 16) previously shown to predict in-vivo ACE plasma levels [12], and one of which is near a potential alternative ACE mRNA splice site [13], were selected: G12269A, a G to A polymorphism 12269 base pairs from the ATG translational start site (RefSNP or rs4344, located on intron 18 and the closest single nucleotide polymorphism (SNP) to the ACE Ins/Del on intron 16); C17888T, (rs4359, located on intron 23); and G20037A, (rs4363, located on intron 25 and near the splice acceptor site for terminal exon 26, which encodes the membrane-spanning carboxyl-terminus) [13] (Table 1 and Fig. 2). Genomic DNA was extracted from whole blood using the PureGene blood DNA kit (Gentra Biosystems, Minneapolis, Minnesota, USA). Genotype assays for SNPs were developed based on flanking genomic DNA sequence (website: <http://www.ncbi.nlm.nih.gov/SNP/>) and each subject was genotyped using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry after primer extension across the variant base (Sequenom, San Diego, California, USA) [16,17]. Deviation from Hardy–Weinberg equilibrium was tested using the Pearson goodness of fit (χ^2) test statistic. Linkage disequilibrium coefficients (D') between polymorphism were determined [18] and ACE gene haplotypes were generated using the HAP algorithm [19].

Statistical analysis

This is an ancillary study to AASK; all analyses were performed by the authors of this paper, and not by the AASK Data Coordinating Center.

Baseline characteristics and preliminary outcomes between ACE genotypes, low and usual randomization group, and those who did and did not reach target MAP were first explored. Analyses were then done by genotype and then by the three most common haplotypes, as discussed further. For this study, MAP was analyzed because medications were titrated based on MAP as an integrated index of cardiovascular risk (rather than systolic and diastolic blood pressures, SBP and DBP respectively). MAP, however, is a composite of SBP and DBP: $MAP = DBP + 1/3(SBP - DBP)$.

Since this cohort had early renal insufficiency, the analysis focused on the first year of randomization because progressive renal disease could obscure any relationship between genotype and drug response. Time, or number of days after randomization to reach a target MAP of 107 mmHg or lower (a clinically reasonable goal corresponding to a blood pressure of about 140/90 mmHg), in the first year after randomization was then determined for each study subject. In order to achieve this goal, two consecutive MAPs had to be at or below 107 mmHg, and the average of all remaining MAPs in the first year had to be at or below 107 mmHg. Participants who reached target MAP were censored (i.e. target goal achieved) on the first of the 2 days at or below target MAP; those who did not reach target MAP in the first year of randomization were considered treatment failures. In addition to randomization to one of the three study drugs, AASK participants were also randomized (50 : 50) to either a low MAP (≤ 92 mmHg) or a usual MAP (≤ 107 mmHg). Unlike the primary double-blinded drug, this MAP goal categorization was known to both investigators and patients.

Kaplan–Meier survival curves were generated by all three diploid ACE genotypes at each SNP locus, and the difference tested (Log-rank test for equality). Because of multiple comparison testing, we considered $P < 0.005$ significant (as noted in power calculations below). A specific model of genetic expression was not assumed *a priori*; these models – dominant/recessive or heterosis (i.e. heterozygote deviation from the two homozygous genotypes) – were explored depending on the main results.

We verified our results in two ways. First, we analyzed MAP response to ACE inhibition as a continuous variable (mixed effects, uneven time intervals) among subjects stratified by ACE genotype class and MAP goal (usual or low) within the first year of randomization. Because the mechanism of action for ACE-Is on blood pressure is quite different from that for CCBs, we were also able to use participants randomized to amlodipine ($n = 159$; 20% of subjects randomized to this drug) as a specificity control for the effect of genotype on response.

A Cox proportional hazards model was then used to determine the ‘hazard’ or rate of reaching target MAP while controlling for other potential predictors of blood pressure response such as renal function (serum creatinine or GFR), age, body mass index (BMI) and sex. Other predictors of blood pressure were then included in the model if the parameter appeared to be contributing to the model (i.e. the hazard rate ratio for the covariate was significant as determined by the confidence interval or P -value, or the parameter resulted in an increased maximum likelihood or an overall increase in model significance). The analyses described above were repeated by the number of haplotypes (per diploid genome).

Finally, it should be noted that other antihypertensive drugs (except ACE-Is, beta-blockers or CCBs) were used to manage blood pressure based on a standardized protocol with a typical sequence of additions: diuretic, alpha adrenergic antagonist, central alpha adrenergic agonist [9]. Both the number of antihypertensive medications and the duration of treatment (with a particular combination of medications) were systematically collected and the average number of daily medications used to manage blood pressure was determined for each participant.

Based on a two-sided test of proportions, this study ($n = 347$, half each in usual versus low MAP goal groups) had at least 80% power to detect moderate (50%) response differences between groups, assuming a minor allele frequency of at least 25% (as is the case for each polymorphism in this study); a conservative α of 0.005 was used to account for multiple covariates. Statistical analyses (including power analyses) were done using STATA 9.2 (StataCorp LP, College Station, Texas, USA).

Population stratification

In addition to the analyses described above, we were concerned about false-positive associations resulting from genetic admixture within African Americans [20]. A generalized analysis of molecular variance (GAMOVA) [21] was used to test for and quantify the relationship between the genetic background of the subjects randomized to ramipril ($n = 347$) and blood pressure response (day to reach target MAP, a quantitative measure) using an identity-by-state distance matrix based on additional genotypes of 86 biallelic markers.

Results

Genetics

Of the 347 AASK study participants randomized to ramipril, 323 (96%) were successfully genotyped at G12269A, 319 (92%) at C17888T, and 325 (93%) at G20037A. The first two polymorphisms (G12269A and C17888T) were in strong linkage disequilibrium ($D' = 0.88$). Based on this primary genotyping data, ACE haplotypes were inferred for 339 participants

using HAP [19]. Although seven ACE haplotypes were generated, the majority had one or two copies of the following three most frequent ACE haplotypes: ACA, GTG and GCA (Table 2).

Ancillary AASK study participant characteristics

There were no baseline differences (in, for example, age, sex, BMI, blood pressure, or renal function) between our ramipril cohort with available DNA ($n = 347$), the original cohort randomized to ramipril ($n = 436$), or the original AASK study cohort ($n = 1094$), suggesting that we had a representative sample of the AASK study population [14,15]. There were 217 men (mean age 53 ± 11 years) and 130 women (mean age 55 ± 10 years). There was complete follow-up in the first year of randomization, with 28 treatment failures, and a total of 27–303 days at risk.

Characteristics and preliminary outcomes by ACE genotypes—Table 3 shows baseline characteristics and preliminary outcomes by the three ACE genotypes. There was an approximate 50 : 50 randomization to the low and usual MAP groups across all ACE genotypes. There were more participants who did not reach a target MAP of 107 mmHg among those who were heterozygous at G12269A (11% of heterozygotes at G12269A did not reach target MAP) and C17888T (12%). There were marginal differences in age by C17888T and G20037A genotypes ($P = 0.03$ and 0.02 , respectively). Heterozygotes at C17888T had a higher baseline MAP ($P < 0.005$); there were also marginal differences in baseline MAP at G20037A ($P = 0.02$). BMI appeared to vary by G20037A genotypes ($P = 0.008$). There were marginal differences in medications at G12269A and G20037A ($P = 0.02$ in both cases). Finally, homozygotes at C17888T also reached target MAP faster, though initial results are also only marginally significant ($P = 0.02$, Log-rank test).

Characteristics and preliminary outcomes of those randomized to low and usual mean arterial pressure—There were no significant baseline differences between those who were randomized to low or usual MAP (Table 4). Those randomized to low MAP, however, required more medications (3.85 versus 3.40 average number of daily medications, $P < 0.001$), had a lower average MAP in the first year of randomization (96 versus 104 mmHg, $P < 0.001$), and reached a target MAP of ≤ 107 mmHg faster (the majority were at target by 86 versus 125 days, $P = 0.05$; Table 4). Among those randomized to a low MAP, 11 (6%) did not reach a target MAP of 107 mmHg; this compares to 17 (10%) among those randomized to a usual MAP (results not shown). Because of these differences, the low and usual MAP groups were analyzed separately.

Characteristics and preliminary outcomes of those who did not reach target mean arterial pressure (≤ 107 mmHg)—Participants who did not reach target MAP ($n = 28$) in the first year of randomization appeared to be different from those who did reach target MAP ($n = 319$). Compared to those who did reach target MAP, those who did not were younger (48 versus 54 years, $P = 0.003$), had a higher baseline MAP (124 versus 113 mmHg, $P < 0.001$), and had a higher BMI (33 versus 31 kg/m², $P = 0.04$; Table 3). This group also required more medications to control their blood pressures and had a higher average MAP in the first year after randomization (Table 4).

Results by genotypes and haplotypes: time to reach target mean arterial pressure (≤ 107 mmHg)—Kaplan–Meier curves by ACE genotypes and the three most common ACE haplotypes are shown in Figs 3 and 4. Adjusted hazard ratios (risk of reaching target MAP) with corresponding 95% confidence intervals derived from the Cox models are summarized in Table 5 by genotype and final haplotype model (described below).

Genotypes

Among those randomized to usual MAP (≤ 107 mmHg), there appeared to be differences in the rate of reaching blood pressure control when individuals were stratified by G12269A or C17888T genotypes ($P = 0.09$ and $P = 0.007$, respectively, Log-rank test). In both cases, those with a homozygous (i.e. a G/G or A/A at position 12269) genotype responded faster than those with a heterozygous (i.e. G/A at 12269) genotype ($P = 0.03$ and $P = 0.003$ respectively; Fig. 3).

When we analyzed the MAP response to ACE inhibition as a continuous variable (mixed effects, uneven time intervals), the model for G12269A (heterozygotes compared to homozygotes) was marginally significant only among those randomized to usual MAP only ($P = 0.03$). As noted with the Cox model below, the contribution of genotype was small ($\eta^2 = 0.03$) in comparison to baseline MAP and average number of medications. As a specificity control, there were also no associations between ACE genotypes and time to reach target MAP among those randomized to a drug acting by an independent mechanism: the CCB amlodipine (results not shown).

The unadjusted hazard rates (i.e. the rate of reaching a target MAP of ≤ 107 mmHg) derived from Cox models comparing the two groups with homozygous genotypes to the group with a heterozygous genotype at G12269A or C17888T were significant for participants randomized to usual MAP (Table 5, results for usual MAP shown): 1.42 (95% confidence interval 1.02–1.98; $P = 0.03$ for G12269A genotype coefficient) and 1.65 (1.18–2.33; $P = 0.004$ for C17888T genotype coefficient). The average number of medications (including diuretics) and baseline MAP were both significant predictors of blood pressure response, adjustment for these covariates improved the significance of the Cox models (each $P < 0.0001$). The adjusted hazard rates for G12269A and C17888T were 1.86 (1.32–3.32; $P < 0.001$ for G12269A coefficient) and 1.49 (1.01–2.13; $P = 0.02$ for C17888T coefficient; Table 5).

It is important to note here that heterozygotes at C17888T had higher baseline MAPs and required more medications (as discussed above, Table 3); adjustment for these covariates decreased the significance of C17888T ($P = 0.02$) and increased the significance of C12269A ($P < 0.001$). Covariates in the Cox model met the proportional hazards assumptions (Schoenfeld, global $P = 0.63$). Sex, age, BMI, serum creatinine, GFR, total cholesterol, history of heart disease, smoking status and other sociodemographic factors such as income and insurance status did not significantly change the Cox model.

By contrast, G20037A genotypes were not significant predictors of the rate to reach target MAP based on unadjusted or adjusted Cox models (Table 5). (Though not significant, results comparing homozygous to heterozygous genotypes at G20037A are also shown in Table 5 for consistency.) There were no significant associations between ACE genotypes and time to reach target MAP among those randomized to low MAP (results not shown).

Haplotypes

Among those randomized to usual MAP, Kaplan–Meier analyses for haplotypes ACA ($n = 97$), GTG ($n = 94$) and GCA ($n = 48$) approached marginal significance. Participants with two copies of haplotypes ACA, GTG or GCA (haplotype homozygotes) appeared to respond faster than participants with one copy (haplotype heterozygotes) of the corresponding haplotype ($P = 0.06$, 0.05 and 0.08 , respectively; results not shown). The adjusted hazard rates (haplotype homozygotes compared to heterozygotes) were also marginally significant for haplotypes ACA and GTG but not for GCA: 1.78 (1.11–2.86, $P = 0.02$ for ACA haplotype coefficient), 1.81 (1.08–3.04, $P = 0.03$ for GTG haplotype coefficient) and 0.90 (0.33–2.46, $P = 0.85$ for GCA

haplotype coefficient; results not shown). There were no response differences between ACE haplotypes among those randomized to low MAP (results not shown).

The final haplotype model comparing all ACA, GTG and GCA haplotype homozygotes to all ACA, GTG and GCA haplotype heterozygotes ($n = 168$) was marginally significant ($P = 0.01$; Fig. 4). Among those randomized to a usual MAP, the adjusted hazard ratio was 1.40 (1.13–1.75, $P = 0.003$ for haplotype coefficient; bottom of Table 5). There were no response differences by ACE haplotypes among those randomized to a low MAP (≤ 92 mmHg, results not shown).

Population stratification

GAMOVA [21] showed no significant relationship between overall genetic profile (at 86 single nucleotide variants) and the blood pressure response trait (days to target MAP), indicating that population stratification did not contribute to this phenotype ($P = 0.34$).

Discussion

Genotypes

In this study, we used a novel application of survival methodology to analyze the relationship between the time to reach a target MAP (≤ 107 mmHg) in the first year after randomization to ramipril and ACE polymorphisms, G12269A, C17888T and G20037A. Significant differences by G12269A and C17888T genotypes were found among those randomized to the usual (≤ 107 mmHg) MAP goal. Participants with a homozygous genotype at either of these two sites responded to ramipril faster than those with a heterozygous genotype. Baseline MAP and number of medications, however, were important co-variables, especially since these parameters also varied by C17888T genotypes. After adjustment using a Cox model, the association was most significant at G12269A ($P < 0.001$) and only marginally significant at C17888T ($P = 0.02$). Consistent results were found using a mixed effects model. There were also no associations by ACE genotypes among those randomized to amlodipine, suggesting that the associations between ACE genotypes and response to ramipril were not likely due to confounding by other factors associated with drug response such as, renal function or severe hypertension.

Haplotypes

Haplotype analyses may be used to explore the effects of and interactions between multiple polymorphisms at a gene locus; alternatively, because haplotypes span a larger gene region, haplotype associations may help identify biologically important sites not represented by individual polymorphisms [22]. Haplotype analysis also reduces the number of comparisons, thereby reducing the probability of type 1 statistical errors. Our initial haplotype associations approached marginal significance, partly a result of a decreased sample size. The comparison of all haplotype homozygotes to heterozygotes (usual MAP, $n = 168$), however, suggested that haplotype homozygotes responded significantly faster than heterozygotes, adjusted hazard ratio 1.40 (1.13–1.75; $P = 0.003$ for haplotype coefficient). However, it is not clear whether the haplotype association is simply a result of having homozygous alleles or heterozygous alleles at G12269A.

These associations were also only observed among those randomized to the usual MAP goal (≤ 107 mmHg). Those randomized to a lower MAP (≤ 92 mmHg) reached a MAP of 107 mmHg earlier, and were managed more aggressively (Table 4). Genotypic variation was not a significant predictor in this group, suggesting that the contribution of a genotype was probably quite small in comparison to other factors, such as the number of medications or medication dosing; alternatively, this study may be underpowered to detect an association in this group. Finally, we choose to focus on reaching a blood pressure of 107 mmHg (and not 92 mmHg in

the low MAP group) because a MAP of 107 corresponded to a generally acceptable goal in clinical practice (i.e., a blood pressure around 140/90 mmHg); furthermore, lowering blood pressure to a very low MAP of 92 mmHg did not result in significantly different long-term outcomes in the AASK trial [14].

Unique study features

Heterosis

After adjustment for baseline MAP and medications, responsiveness to an ACE-I appeared to be most influenced by ACE diploid genotypes at G12269A among participants randomized to a usual MAP (≤ 107 mmHg). While we cannot be certain why this polymorphism is important, haplotype analyses suggested that having two copies of the same ACE haplotype (ACA, GTG or GCA in our dataset) was an important determinant of responsiveness to ACE-I treatment; this may be an example of heterosis, whereby the phenotypes of individuals with homozygous alleles (major or minor) appear to be different from those with heterozygous alleles. Based on our preliminary results (not shown here), G12269A was in nearly complete linkage disequilibrium with the ACE Ins/Del polymorphism ($D' = 0.98$). It has been suggested that the Ins/Del polymorphism may regulate alternative ACE mRNA splicing [13]. If this were so, individuals with an Ins/Ins or Del/Del homozygous diploid genotype would express identical copies of the same ACE mRNAs and, ultimately, ACE proteins; those with an Ins/Del genotype, on the other hand, might express two different versions of the ACE protein.

If an enzyme typically exists in the homodimeric state, then two isoforms (copies of a qualitatively different protein) might form heterodimers, with consequently different catalytic properties from the homodimer, or such different isoforms might not efficiently heterodimerize at all [23]. In the case of ACE, ACE-inhibitors have been shown to convert ACE monomers to homodimers [24], and this dimerization process may be important for disrupting ACE activity, or perhaps intracellular signaling by membrane-spanning ACE in endothelial cells. Finally, heterosis is best documented during association with a three-state diploid genotype, while attempts at association based solely on the two-state presence or absence of an allele may not detect the phenomenon [11]; thus previous attempts at two-state 'allelic' association at ACE may partially explain the inconclusive findings on ACE-I reported to date [10].

Time-to-event strategy

Our approach was also different from previous studies because we analyzed whether the time to reach target MAP was related to ACE genotypes, rather than simply evaluating two blood pressures in each subject (blood pressure change before and after treatment). Such a time-to-event analysis may be a superior approach since it takes advantage of more comprehensive sampling of the available blood pressure data, a parameter that changes gradually with time. For example, pretreatment and posttreatment changes at a specified time interval may inaccurately classify responders (who may have an isolated lower blood pressure reading) and nonresponders (who may have isolated higher readings, or who may respond at a later date not captured by the specified time interval). Simply taking the two-state, pre-approach and post-approach would also result in null findings in our study because AASK participants were treated with increasing medication doses, and added medication categories, in order to achieve a particular goal MAP; therefore, one would not expect an association between genotype and change in blood pressure. As noted, all but 28 of the 347 ramipril study subjects eventually reached a target MAP of ≤ 107 mmHg.

Therefore in order to avoid the pitfalls of measuring change over a specified time period, we used criteria that reflected mean blood pressures over a period of time (in this case, 1 year). Our approach required that participants remain at target MAP for the duration of observation

in order to be classified as responders. While strict, this approach allowed us to efficiently use all of our data in a 1-year period, likely resulting in a more accurate classification of responders and nonresponders. We were, however, required to impose structure on fluctuating blood pressure readings but applied the same criteria to all participants, resulting in a consistent ascertainment of the time to reach a target MAP of ≤ 107 mmHg. Of note, the time to control of blood pressure may be an especially useful formulation for clinicians managing the trait.

The AASK trial

Finally, unique features of the AASK trial need to be emphasized. This trial was based on multiple sites, and included extensive, systematic information on responses: longitudinal blood pressures before and after randomization to ramipril, other biomedical parameters (age, renal function, BMI), and other antihypertensive medications. Therefore, as a result of focusing on the time to reach target MAP, and having access to meticulously collected outcome and phenotypic data, we were able to differentiate responsiveness to ACE inhibition by ACE genotypes.

Study limitations and population stratification

The AASK Genomics Study is a representative subset of the original AASK cohort (not differing in baseline age, sex, blood pressure, or renal function). While there has been 85% ascertainment, how loss to follow-up or death might bias these results is difficult to determine. Our results are not necessarily generalizable to other ethnic groups or to patients without hypertensive nephrosclerosis. Although the number of medications and baseline MAP contributed most to blood pressure response, the effect of C17888T genotypes remained significant ($P < 0.005$) after adjustment for these parameters.

The original study was designed to explore the relationship between antihypertensive drug class and the progression of renal disease, and not designed to study the relationship between genotype and blood pressure response to an antihypertensive drug class. As a result, this study was not adequately powered to detect small differences in blood pressure response or to determine the effects of rare polymorphisms. Among those randomized to a low MAP, the contribution of genotype may have been quite small and we probably did not have adequate power to detect associations between genotype and blood pressure response in this group. Similarly, while we did not find any significant associations between ACE genotypes and amlodipine, we may not have had adequate power to detect differences in this group because of the smaller sample size (the AASK study protocol randomized 20% of participants to amlodipine). While we were able to control for number of medications, it should be noted that this is a proxy measure for specific antihypertensive medication classes administered according to a specified protocol across study sites. Our analysis was also limited to the first year of randomization because worsening renal function might alter the ultimate relationship between genotype and blood pressure response.

There are several interesting observations from our dataset that we can only note here. Those who did not respond in the first year of randomization were younger and had higher baseline MAPs (Table 4). Also, as noted previously, there were significant differences in baseline MAP (and accordingly, number of medications) by C17888T genotypes. After controlling for these parameters, there was only a marginal association between C17888T genotypes and blood pressure response to ramipril ($P = 0.02$). Although there was no association between BMI and blood pressure response, our data also suggested an association between BMI and G20037A.

Finally, population stratification may confound the differential effects of particular genotypes [25], and this may be problematic within the African American population because of genetic admixture [20]; however, using GAMOVA [21] at 86 biallelic loci, we did not find any

significant relationship between population stratification and blood pressure response ($P = 0.34$).

In conclusion, African-Americans randomized to a usual MAP (≤ 107 mmHg) who are homozygous at G12269A on the ACE gene responded to treatment with an ACE-I almost twice as fast than those with heterozygous genotypes; the majority of homozygotes responded within the first 3.6 months compared to 6.3 months for heterozygotes. This latter group is probably not as responsive to ACE-I treatment, an important clinical consideration for patients with a high risk of hypertensive cardiac, cerebrovascular and renal complications. This study provides preliminary evidence that ACE genotype testing at G12269A may be a predictive marker of responsiveness to ACE-I in African-Americans; the clinical use of genotype testing at G12269A warrants further study.

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Fig. 1.

Summary of African American Study of Kidney Disease and Hypertension Genomics Study. One thousand and ninety-four participants were recruited for the AASK Trial. Of these, 853 participated in the AASK Genomics Study, 347 of whom had adequate DNA and randomized to treatment with an angiotensin-converting enzyme inhibitor, ramipril.

Fig. 2. Angiotensin-converting enzyme (ACE) gene, and the polymorphisms evaluated in this study. Base pairs are numbered with respect to the translational start codon (ATG). Solid boxes represent exons (numbered 1–26), while joining diagonal lines are introns. The extensively studied ACE Ins/Del polymorphism is located in intron 16.

Fig. 3.

Days to target mean arterial pressure (MAP) (≤ 107 mmHg) by ACE genotypes, randomized to usual MAP (≤ 107 mmHg). Kaplan-Meier curves by ACE polymorphism genotypes for those randomized to a usual MAP (≤ 107 mmHg) are shown here. Those with a homozygous genotype at G1269A or C1788A responded faster than those with a heterozygous genotype ($P = 0.03$ and 0.003 , respectively). The day on which 25, 50 and 75% of participants reached target MAP is shown in tabular form beside the graph.

Fig. 4.

Combined model: days to target mean arterial pressure (MAP) (≤ 107 mmHg) by ACE haplotypes, randomized to usual MAP (≤ 107 mmHg). Combined Kaplan–Meier curves, by ACE haplotypes (ACA, GTG or GCA), for those randomized to a usual MAP (≤ 107 mmHg) are shown here. Those with two copies of the same ACE haplotype (i.e. homozygous haplotypes) responded faster than those with one copy (i.e. heterozygous haplotypes; $P = 0.01$). The day on which 25, 50 and 75% of participants reached target MAP is shown in tabular form beside the graph.

Table 1

Angiotensin-converting enzyme (ACE) polymorphisms

Polymorphism name used in text	Nucleotide change on sense (plus) strand	Location from ATG translational start site	ACE intron location	Location from Zhu <i>et al.</i>	Ref SNP number
G12269A	G to A	12269	18	15214	rs4344
C17888T	C to T	17888	23	20833	rs4359
G20037A	G to A	20037	25	22982	rs4363

SNP, single nucleotide polymorphism.

Table 2

Angiotensin-converting enzyme haplotype distribution among chromosomes and individuals

Haplotype	Number (%) of a haplotype (<i>n</i> = 678 chromosomes)	Number of haplotypes among individuals (<i>n</i> = 339)	
		0	12
ACA	253 (37%)	138 (41%)	149 (44%)52 (15%)
GTG	242 (36%)	141 (42%)	154 (45%)44 (13%)
GCA	116 (17%)	234 (69%)	94 (28%)11 (3%)

Table 3

Characteristics and preliminary outcomes, stratified by ACE genotypes

	G12269A				C17888T				G20003A			
	AA (n = 58)	AG (n = 157)	GG (n = 118)	P*	CC (n = 109)	CT (n = 150)	TT (n = 70)	P*	AA (n = 119)	AG (n = 144)	GG (n = 62)	P*
Number randomized to low MAP (%)	27 (45)	81 (56)	63 (53)		54 (49.5)	80 (53)	29 (48)		58 (49)	78 (54)	32 (52)	
Number who did not reach target (%)	4 (7)	18 (11)	5 (4)		6 (6)	18 (12)	2 (3)		10 (8)	12 (8)	4 (6)	
Number of men (%)	36 (62)	100 (64)	72 (61)		58 (53)	98 (65)	43 (72)		61 (51)	98 (68)	44 (71)	
Age at randomization (years) (± SD)	55 (11)	53 (12)	53 (10)	NS	55 (11)	52 (11)	54 (10)	0.03	56 (11)	52 (10)	53 (10)	0.02
Baseline MAP (mmHg) (± SD)	110 (15)	116 (16)	114 (16)	NS	109 (14)	117 (17)	114 (15)	0.002**	111 (14)	117 (16)	114 (15)	0.02
Body mass index (kg/m ²) (± SD)	31 (6)	31 (6)	30 (6)	NS	32 (6)	31 (6)	30 (5)	NS	32 (6)	31 (6)	29 (5)	0.008
Serum creatinine (mg/dl) (± SD)	1.93 (0.64)	2.11 (0.76)	1.95 (0.73)	NS	1.97 (0.72)	2.08 (73)	2.01 (0.78)	NS	1.93 (0.64)	2.06 (0.78)	2.10 (0.79)	NS
Glomerular filtration rate (mg/ml per 1.73 m ²) (± SD)	47 (12)	45 (13)	47 (12)	NS	46 (13)	45 (13)	49 (12)	NS	47 (13)	45 (13)	47 (13)	NS
Total cholesterol (mg/dl) (± SD)	216 (45)	212 (45)	213 (42)	NS	214 (45)	212 (42)	209 (40)	NS	213 (47)	214 (42)	207 (38)	NS
Average number of daily medications (± SD)	3.38 (1.21)	3.80 (1.27)	3.74 (1.27)	0.02	3.31 (1.22)	3.87 (1.23)	3.66 (1.25)	0.002**	3.38 (1.21)	3.80 (1.27)	3.74 (1.27)	0.02
Average MAP in first year after randomization (± SD)	102 (8)	101 (8)	100 (6)	NS	100 (7)	101 (8)	100 (8)	NS	101 (8)	101 (7)	100 (7)	NS
Median days to target MAP (interquartile range)	33 (10 to 108)	35 (7 to 122)	33 (11 to 82)	NS	32 (9 to 83)	40 (10 to 134)	27 (7 to 58)	0.02	35 (11 to 108)	34 (10 to 115)	24 (6 to 64)	NS

* Comparison between genotype, ANOVA except for median days to target mean arterial pressure (MAP) (Log-rank test).

** $P \leq 0.005$.

Table 4

Characteristics and preliminary outcomes, stratified by mean arterial pressure goal and attainment of a target mean arterial pressure (≤ 107 mmHg)

	All (n = 347)	Low MAP (≤ 92 mmHg), (n = 179)	Usual MAP ($\leq$ 107 mmHg), (n = 168)	P*	Reached target MAP ($\leq$ 107 mmHg), (n = 319)	Did not reach target MAP (≤ 107 mmHg), (n = 28)	P**
Number of men (%)	217 (63%)	118 (66%)	99 (59%)		200 (63%)	17 (61%)	
Age at randomization, years ($\pm$ SD)	53 (11)	54 (11)	54 (11)	NS	54 (11)	48 (10)	0.003***
Baseline MAP (mmHg) ($\pm$ SD)	114 (16)	115 (16)	114 (16)	NS	113 (15)	124 (20)	0.001***
Body mass index (kg/m^2) ($\pm$ SD)	31 (6)	31 (6)	31 (6)	NS	31 (6)	33 (8)	0.04
Serum creatinine (mg/dl) ($\pm$ SD)	2.02 (0.73)	2.01 (0.75)	2.03 (0.72)	NS	2.00 (0.73)	2.23 (0.75)	NS
Glomerular filtration rate (mg/ml per 1.73 m^2) ($\pm$ SD)	46 (13)	47 (13)	46 (15)	NS	46 (13)	44 (14)	NS
Total cholesterol (mg/dl) ($\pm$ SD)	213 (43)	215 (45)	211 (41)	NS	213 (43)	210 (47)	NS
Average number of daily medications ($\pm$ SD)	3.63 (1.25)	3.85 (1.19)	3.40 (1.28)	<0.001***	3.56 (1.25)	4.42 (1.01)	<0.001***
Average MAP in first year after randomization ($\pm$ SD)	100 (7)	96 (6)	104 (5)	<0.001***	99 (6)	116 (7)	<0.001***
Median days to target MAP (interquartile range)	34 (9 to 101)	31 (8 to 86)	38 (9 to 125)	0.05	NA	NA	NA

* Comparison between low and usual mean arterial pressure (MAP), ANOVA except for days to target MAP (Log- rank test).

** Comparison between those who did and did not reach target MAP, ANOVA.

*** $P \leq 0.005$.

Table 5

Cox proportional hazards model for participants randomized to usual mean arterial pressure (≤ 107 mmHg): unadjusted and adjusted a hazard ratios for *ACE* genotypes and final haplotype model

Model	Hazard ratio	95% Confidence interval	<i>P</i> , overall model (χ^2)	<i>P</i> , genotype or haplotype β -coefficient
G12269A: AA and GG, compared to AG (<i>n</i> = 162)				
Unadjusted	1.42	1.02 to 1.98	0.03	0.03
Adjusted	1.86	1.32 to 3.23	<0.001 [*]	< 0.001 [*]
C17888T: CC and TT, compared to CT (<i>n</i> = 156)				
Unadjusted	1.65	1.18 to 2.33	0.004 [*]	0.004 [*]
Adjusted	1.49	1.01 to 2.13	<0.001 [*]	0.02
G20037A: AA and GG, compared to AG (<i>n</i> = 157)				
Unadjusted	1.18	0.84 to 1.64	0.35	0.35
Adjusted	1.39	0.81 to 1.56	<0.001 [*]	0.57
Final haplotype model, two copies of the same Haplotype (ACA, GTG or GCA), compared to one copy of a haplotype (<i>n</i> = 168)				
Unadjusted	1.44	1.10 to 1.81	0.006	0.001 [*]
Adjusted	1.40	1.13 to 1.75	<0.001 [*]	0.003 [*]

^a Adjusted for baseline MAP and average number of medications used in first year.

^{*} $P \leq 0.005$.