EXPEDITED REVIEW

Aldosterone Synthase Promoter Polymorphism Predicts Outcome in African Americans With Heart Failure
Results From the A-HeFT Trial

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OBJECTIVES

We sought to evaluate the effect of the aldosterone synthase promoter polymorphism on heart failure outcomes for subjects in the African American Heart Failure Trial (A-HeFT).

BACKGROUND

Genetic heterogeneity modulates clinical outcomes in subjects with heart failure (HF); however, little data exist in African American populations. A common polymorphism exists in the promoter region of the aldosterone synthase gene (CYP11B2) at position −344 (T/C). The −344C allele, associated with higher aldosterone synthase activity, has been linked to hypertension; however, its impact on outcomes in HF is unknown.

METHODS

A total of 354 subjects from A-HeFT participated in the GRAHF (Genetic Risk Assessment of Heart Failure in African Americans) substudy and were genotyped for the aldosterone synthase polymorphism. Patients were followed prospectively, and event-free survival (freedom from death and HF hospitalization) compared by CYP11B2 genotype.

RESULTS

Of the cohort, 218 patients were TT, 114 CT, and 22 patients were CC. Baseline etiology, blood pressure, and functional class were not significantly different among the 3 cohorts. The C allele was associated with significantly poorer HF hospitalization-free survival with the best survival among TT subjects, intermediate for heterozygotes, and the poorest for CC homozygotes (p = 0.018), and a higher rate of death (% death TT/TC/CC = 1.8/3.5/18.2, p = 0.001). The TT genotype, more prevalent in blacks, was associated with greater impact of fixed combination of isosorbide dinitrate and hydralazine on the primary composite end point (p = 0.01).

CONCLUSIONS

The aldosterone synthase promoter −344C allele linked to higher aldosterone levels is associated with poorer event-free survival in blacks with HF. The role of aldosterone receptor antagonists in diminishing this apparent genetic risk remains to be explored. (J Am Coll Cardiol 2006;48:1277–82) © 2006 by the American College of Cardiology Foundation

Activation of aldosterone may play an important role in the progression of heart failure. Stimulation of myocardial aldosterone receptors increases apoptosis, resulting in fibrosis and ventricular remodeling (1). Blockade of the aldosterone receptor has been shown to improve heart failure outcomes. In the RALES (Randomized Aldactone Evaluation Study), the addition of the aldosterone receptor antagonist spironolactone improved survival in subjects with severe heart failure (2). Aldosterone antagonists also reduce left ventricular remodeling, and the addition of the selective antagonist eplerenone post-myocardial infarction improves survival (3).

Significant clinical heterogeneity exists in heart failure outcomes, and much of this variability is genetically based. Aldosterone synthase (CYP11B2) is a 9-exon gene occurring on chromosome 8q22 (4). A common single nucleotide polymorphism, C to T transition for position −344, occurs within the promoter region of CYP11B2 (5). The −344C allele binds the steroidogenic transcription factor 1 (SF-1) 4 times more than the T allele (6), and has been linked to increased aldosterone production (7,8). The CYP11B2 promoter polymorphism has been linked to hypertension (9–12) and the −344C allele in particular to the risk of coronary disease (13,14). Despite the central role of aldosterone in heart failure progression, the impact of the −344C allele on clinical outcomes is unknown.

The heart failure phenotype differs in African American and white cohorts (15). Hypertension is a more frequent etiology in blacks, while despite significant risk factors, coronary disease is less common. Angiotensin-converting
enzyme inhibitors are less effective in African Americans (16); however, racial differences in the impact of aldosterone antagonists have not been investigated. In contrast with angiotensin-converting enzyme inhibitors, the nitric oxide (NO) donor combination, isosorbide dinitrate and hydralazine (I/H), appears to be more efficacious in blacks. In the A-HeFT (African-American Heart Failure Trial), I/H in fixed combination markedly improved survival in a cohort with systolic dysfunction (17).

While the impact of genetic heterogeneity on heart failure outcomes has been extensively studied in predominantly white cohorts, few studies have investigated the impact of genomic variation in blacks. The GRAHF study (Genetic Risk of Heart Failure in African Americans) was a genetic substudy of the A-HeFT study. This substudy was initiated to explore the impact of functional genomic variation of heart failure mediators in an African-American cohort. We investigated the impact of the −344 T/C polymorphism of the aldosterone synthase promoter in the GRAHF cohort.

METHOD

Study population. A total of 354 subjects in the A-HeFT study were enrolled in a genetic substudy, the GRAHF substudy. Inclusion criteria for the A-HeFT study (17) include self designation as African Americans, heart failure due to systolic dysfunction, and standard background therapy for heart failure with neurohormonal blockade, including angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists, and beta-blockers. Subjects were randomized to either I/H in fixed combination or placebo in addition to standard therapy. The white heart failure cohort from the GRACE (Genetic Risk Assessment of Cardiac Events) trial, a single-center investigation based at the heart failure clinic at the University of Pittsburgh (18), was utilized for comparisons of allele frequencies by race.

Genotyping. Subjects were enrolled in the GRAHF study at the A-HeFT study 6-month visit. Deoxribonucleic acid was isolated from peripheral blood by leukocyte centrifugation and cell lysis (PureGene, Gentra Systems, Minneapolis, Minnesota). The aldosterone synthase (CYP11B2) promoter −344 T/C polymorphism was assessed using a TaqMan SNP Genotyping Assay with tagged primers (ABI, Norwalk, Connecticut), and products were read using the Applied Biosystems 7000 (Applied Biosystems, Foster City, California).

Outcomes analysis. Subjects were followed to an end point of death or heart failure hospitalization. Quality-of-life (QoL) assessment was performed by the Minnesota Living with Heart Failure Questionnaire (MLHFQ) at baseline and the 6-month visit. Left ventricular function was assessed by transthoracic echocardiography at baseline and 6 months in a subset (n = 273 at baseline, n = 268 at 6 months) of the GRAHF study subjects. The primary end point for the A-HeFT study was a composite weighted score with 3 components: mortality, heart failure hospitalization, and change in QoL at 6 months (19). Hardy-Weinberg equilibrium was evaluated by chi-square analysis. Event-free survival was compared by genotype class by Kaplan-Meier log rank analysis. The impact of the −344C allele on aldosterone levels is predicted to be additive, with homozygous CC subjects having the highest levels, TT subjects the lowest, with heterozygous subjects being intermediate. Therefore, a priori a linear model was utilized that predicts an intermediate phenotype for heterozygotes. Continuous variables such as left ventricular ejection fraction (LVEF) and composite score were compared by genotype class by linear analysis of variance (ANOVA). For the interaction of aldosterone genotype and the impact of therapy, ANOVA was used to compare outcomes (composite score and QoL score) by treatment subset (I/H vs. placebo) within genotype subsets. For this pharmacogenetic analysis, subjects with the C allele (CC and TC genotypes) were pooled and compared with the TT genotype subset given the limited number of CC subjects.

RESULTS

The GRAHF study population was 60% men, 25% ischemic, and 98% New York Heart Association functional class III, with a mean age of 57. Over the course of follow-up, there were 60 (17%) heart failure hospitalizations and 12 deaths (3.4%). In terms of the CYP11B2 −344 T/C promoter polymorphism, 218 subjects (62%) were TT, 114 CT (32%), and 22 patients were CC (6%). The observed distribution was consistent with Hardy-Weinberg equilibrium (allele frequency T/C = 0.78/0.22, expected genotype frequencies % TT/TC/CC = 61%/34%/5%, chi-square expected vs. observed, p = 0.63). Comparisons of baseline etiology, medical therapy, blood pressure, and functional class were not significantly different among the 3 genotype subsets (Table 1). The allele frequencies differed markedly by race, as the T allele was much more prevalent in the black cohort in the A-HeFT study when compared with the white cohort from the GRACE study (p < 0.001) (Fig. 1).
Table 1. Baseline Characteristics by CYP11B2 −344 Genotype*

<table>
<thead>
<tr>
<th></th>
<th>CC (n = 22)</th>
<th>TC (n = 114)</th>
<th>TT (n = 218)</th>
<th>All Patients (n = 354)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>56.6 ± 12.2</td>
<td>57.9 ± 11.8</td>
<td>57.2 ± 13.5</td>
<td>57.4 ± 12.8</td>
</tr>
<tr>
<td>Female (%)</td>
<td>31.8</td>
<td>34.2</td>
<td>44.5</td>
<td>40.4</td>
</tr>
<tr>
<td>NYHA functional class, (%) (III/IV)</td>
<td>95/5/4.5</td>
<td>96.5/3.5</td>
<td>96.8/3.2</td>
<td>96/6.3/4.4</td>
</tr>
<tr>
<td>Ischemic (%)</td>
<td>22.7</td>
<td>26.3</td>
<td>25.2</td>
<td>25.4</td>
</tr>
<tr>
<td>LVEF core entry (n = 270)</td>
<td>0.31 ± 0.07</td>
<td>0.35 ± 0.08</td>
<td>0.35 ± 0.09</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>125 ± 20</td>
<td>128 ± 17</td>
<td>127 ± 17</td>
<td>127 ± 17</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>79 ± 14</td>
<td>78 ± 11</td>
<td>76 ± 10</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor (%)</td>
<td>72.7</td>
<td>74.6</td>
<td>77.1</td>
<td>76.0</td>
</tr>
<tr>
<td>Aldosterone receptor antagonist</td>
<td>45.5</td>
<td>33.3</td>
<td>36.5</td>
<td>36.1</td>
</tr>
<tr>
<td>Beta-blocker (%)</td>
<td>77.3</td>
<td>86.8</td>
<td>82.6</td>
<td>83.6</td>
</tr>
</tbody>
</table>

*No significant differences in characteristics by CYP11B2 genotype.

Aldosterone synthesis polymorphism in heart failure. The event-free survival of overall subjects in the GRAHF study at 90, 180, and 360 days was 94%, 91%, and 81%, respectively. The C allele was associated with significantly poorer hospitalization-free survival (Fig. 2) (p = 0.018) with the best survival among TT subjects (% event-free survival at 90/180/360 days = 94/93/85), intermediate for heterozygotes (% event-free = 93/90/77), and the poorest for CC homozygotes (% event-free = 96/81/63). Mortality in the overall GRAHF study cohort was significantly greater in subjects with the C allele (% deaths TT/TC/CC = 1.8%, 3.5%, 18.2%; p = 0.001).

Aldosterone genotype and left ventricular remodeling. Baseline ejection fraction did not differ at baseline among groups; however, there was a trend toward lower LVEF at 6 months for subjects with the −344C allele (LVEF % for genotype subsets: TT/TC/CC = 38/37/33, p = 0.10) (Table 2). Aldosterone receptor antagonists did not limit the impact of the C allele on 6-month LVEF, as in fact the impact was more pronounced for subjects on antagonists (−344C allele linked to lower LVEF, subjects on aldosterone receptor antagonists: TT/TC/CC = 39/36/32, p = 0.03). In contrast with the impact of aldosterone antagonists, treatment with I/H appeared to eliminate the impact of the C allele on remodeling, as the impact on LVEF was evident among subjects treated with placebo (LVEF: TT/TC/CC = 37/36/32, p = 0.05) but not for subjects on I/H (LVEF: TT/TC/CC = 38/38/40, p = 0.79). Consistent with the impact of genotype on LVEF, subjects on placebo

![Figure 1. Genotype frequencies for the aldosterone synthase (CYP11B2) −344 T/C polymorphism in the white heart failure cohort in the GRACE trial and the African American heart failure cohort from GRAHF substudy. The prevalence of the T allele is significantly higher (p < 0.001) in African Americans.](Image)

![Figure 2. Event-free survival by CYP11B2 −344 genotype subsets. Freedom from heart failure hospitalization was significantly poorer (p = 0.018) in subjects with the C allele, with the worst outcomes in CC homozygotes, best outcomes in TT subjects, and intermediate in heterozygotes.](Image)
with the C allele had a greater left ventricular end-diastolic diameter (LVDD) at 6 months (LVDD [cm] TT/TC/CC = 6.0/6.3/6.8, p = 0.01) (Table 2).

**DISCUSSION**

In the GRAHF study, the CYP11B2 promoter −344C allele linked to higher expression of aldosterone synthase was associated with an increased risk of death and hospitalization for African American subjects with heart failure. Analysis by treatment subset suggests the C allele also worsens left ventricular remodeling. Of interest, the impact of the NO donor strategy I/H was greatest in subjects with the TT genotype, a genotype predominant in African Americans and previously linked to low-renin hypertension. The results of this investigation suggest that genetic variation in aldosterone production plays an important role in left ventricular remodeling and disease progression in African Americans with heart failure, a population underrepresented in previous genetics outcomes investigations.

While the −344C allele in vitro has increased binding of SF-1 (6), the impact on transcriptional activity and aldosterone levels in vivo remains controversial. In clinical studies in essential hypertension, the C allele was associated with higher circulating levels of aldosterone in a gene-ordered fashion with the highest levels in the CC genotype subset, intermediate in heterozygotes, and lowest in TT homozygotes (20). However, the linkage of the −344 genotype with aldosterone levels has been inconsistent as several reports actually associate the −344T allele with higher levels (21,22). An analysis from the Framingham study suggests the variance in aldosterone levels in populations is primarily due to non-genetic factors (23). The impact of the CYP11B2 genotype on aldosterone levels may be dependent on a subject’s overall level of neurohormonal activation. While these previous studies have been done in normal subjects or in those with hypertension, little data exist on the interaction of the −344 T/C polymorphism with aldosterone levels in heart failure cohorts.

As demonstrated in the GRAHF study, the −344T allele is consistently more prevalent in black cohorts (21,24). The heart failure phenotype differs in African American and white cohorts (15), with a much greater prevalence of hypertensive cardiomyopathy. Low-renin hypertension, in which the aldosterone/renin ratio is elevated, is particularly more prevalent in African Americans (25,26) and has been linked to the T allele (21,27). In a hypertension study comparing the aldosterone antagonist eplerenone to angiotensin receptor antagonists, eplerenone was more effective than angiotensin receptor antagonists in black cohorts.

**Table 2.** Left Ventricular Ejection Fraction (LVEF) and Left Ventricular Diastolic Diameter (LVDD) at 6 Months by −344 Genotype and Treatment

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>TT (%)</th>
<th>TC (%)</th>
<th>CC (%)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>262</td>
<td>38 ± 9</td>
<td>37 ± 9</td>
<td>33 ± 8</td>
<td>NS (0.10)</td>
</tr>
<tr>
<td>LVDD (cm)</td>
<td>268</td>
<td>6.1 ± 1.3</td>
<td>6.2 ± 1.3</td>
<td>6.5 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF on spironolactone (%)</td>
<td>99</td>
<td>39 ± 10</td>
<td>36 ± 9</td>
<td>32 ± 6</td>
<td>0.03</td>
</tr>
<tr>
<td>LVDD (cm) on spironolactone</td>
<td>101</td>
<td>6.2 ± 1.4</td>
<td>6.3 ± 1.5</td>
<td>6.5 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF on placebo (%)</td>
<td>136</td>
<td>37 ± 10</td>
<td>36 ± 10</td>
<td>32 ± 7</td>
<td>0.05</td>
</tr>
<tr>
<td>LVDD (cm) on placebo</td>
<td>137</td>
<td>6.0 ± 1.3</td>
<td>6.3 ± 1.2</td>
<td>6.8 ± 0.9</td>
<td>0.01</td>
</tr>
<tr>
<td>LVEF on I/H (%)</td>
<td>126</td>
<td>38 ± 9</td>
<td>38 ± 8</td>
<td>40 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>LVDD (cm) on I/H</td>
<td>131</td>
<td>6.2 ± 1.4</td>
<td>6.0 ± 1.4</td>
<td>5.6 ± 1.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Comparisons of means by linear ANOVA. I/H = isosorbide dinitrate and hydralazine in fixed combination; other abbreviations as in Table 1.
Whether aldosterone antagonists are more effective as heart failure therapy in African Americans will require further investigation.

Stimulation of the myocardium by aldosterone induces left ventricular remodeling, hypertrophy, and fibrosis (1). In a Finnish cohort free of cardiac disease, the −344C allele was associated with increased left ventricular size and mass (30). In a study of 995 members of 229 families, the CYP11B2 haplotype was linked to left ventricular cavity size and wall thickness (31). In the current GRAHF study cohort, the C allele was associated with a trend towards lower LVEF at 6 months. This was particularly significant for the subset on placebo and was not evident for subjects randomized to I/H. Of note, the impact of the C allele was also more pronounced for subjects on aldosterone receptor antagonists. Aldosterone receptor antagonists were not randomized in the GRAHF study, and their use may represent a marker for higher-risk subjects rather than a pharmacogenetic interaction. Overall, the current study is consistent with previous reports of an increased risk of remodeling with the −344C allele, and this may be the mechanism of its adverse effect on heart failure outcomes.

Low-renin hypertension is associated with endothelial dysfunction (32) and has been linked to the −344TT genotype (33). Aldosterone excess in low-renin states is associated with impaired NO-mediated vasodilation (34). Treatment with aldosterone antagonists enhance endothelial nitric oxide synthase (NOS3) expression (35), which may help to restore endothelial function and contribute to their therapeutic effects in subjects with heart failure. In V-HeFT I, the therapeutic impact of treatment with I/H was greater in the African American cohort (36), and this finding was confirmed by the marked benefits in heart failure survival with treatment in the A-HeFT study (17). In the GRAHF study, the impact of I/H was primarily in subjects with the −344TT genotype predominant in African Americans. Whether NO donor strategies are more effective in low-renin states remains to be determined.

While this study suggests the TT genotype subjects received greater benefit from I/H, this finding was driven by improvements in the QoL score and reevaluated in a prospective trial of pharmacogenetic targeting. Indeed, though treatment with I/H did not affect composite score in subjects with the C allele, therapy did appear to limit the adverse impact of the C allele on 6-month LVEF, particularly among CC subjects (LVEF 32% placebo vs. 40% on I/H). However, this impact of treatment on left ventricular remodeling must be viewed with caution given the small number of CC subjects.

The design of the A-HeFT and the GRAHF studies imposed several limitations on this analysis. Circulating mediators were not evaluated as part of the GRAHF study, so the impact of the −344 T/C polymorphism on aldosterone level was not investigated. The mortality rate for subjects in GRAHF (3.4%) was lower than in the A-HeFT study itself, and therefore the ability to evaluate the impact of genotype on survival as a single end point was limited. Treatment with aldosterone receptor antagonists was not randomized and was utilized in a minority of subjects (39% in A-HeFT and 36% in the GRAHF cohort), so the impact of CYP11B2 genotype on treatment designed to block aldosterone could not be evaluated. Future studies should evaluate the influence of CYP11B3 genotype on treatment response to both aldosterone receptor antagonists.

The current investigation demonstrates that the −344 T/C promoter polymorphism of CYP11B2 influences clinical outcomes in an African American cohort with heart failure, and provides evidence for the importance of aldosterone in heart failure progression. The heart failure phenotype for African Americans differs from whites, and the allele frequencies of this functional polymorphism differ markedly in black and white cohorts. These results from the GRAHF study suggest that genetic variation in aldosterone production may contribute to these phenotypic differences. In determining optimal heart failure treatment for an individual, race is likely a surrogate marker for differences in genetic background. Future investigations will determine the role of genomic markers for tailoring therapy for individuals with heart failure.

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REFERENCES