Association of Aldosterone Synthase Polymorphism (CYP11B2 -344T>C) and Genetic Ancestry with Atrial Fibrillation and Serum Aldosterone in African Americans with Heart Failure

Adam Bress1, Jin Han1, Shitalben R. Patel1, Ankit A. Desai2,3, Ibrahim Mansour2, Vicki Groo1,2, Larisa H. Cavallari1*

1 Department of Pharmacy Practice, University of Illinois at Chicago, Chicago, Illinois, United States of America, 2 Department of Medicine, Section of Cardiology, University of Illinois at Chicago, Chicago, Illinois, United States of America, 3 Department of Medicine, Institute for Personalized Respiratory Medicine, University of Illinois at Chicago, Chicago, Illinois, United States of America, 4 Department of Medicine, Section of Pulmonary, Critical Care, Sleep and Allergy, University of Illinois at Chicago, Chicago, Illinois, United States of America, 5 Division of Health Policy and Administration, University of Illinois at Chicago, Chicago, Illinois, United States of America, 6 Department of Medicine, Section of Thoracic Surgery, University of Illinois at Chicago, Chicago, Illinois, United States of America

Abstract

The objective of this study was to examine the extent to which aldosterone synthase genotype (CYP11B2) and genetic ancestry correlate with atrial fibrillation (AF) and serum aldosterone in African Americans with heart failure. Clinical data, echocardiographic measurements, and a genetic sample for determination of CYP11B2 -344T>C genotype and genetic ancestry were collected from 194 self-reported African Americans with chronic, ambulatory heart failure. Genetic ancestry was determined using 105 autosomal ancestry informative markers. In a sub-set of patients (n = 126), serum was also collected for determination of circulating aldosterone. The CYP11B2 -344C allele frequency was 18% among the study population, and 19% of patients had AF. Multiple logistic regression revealed that the CYP11B2 -344CC genotype was a significant independent predictor of AF (OR 12.7, 95% CI 1.60–98.4, p = 0.0150, empirical p = 0.011) while holding multiple clinical factors, left atrial size, and percent European ancestry constant. Serum aldosterone was significantly higher among patients with AF (p = 0.036), whereas increased West African ancestry was inversely correlated with serum aldosterone (r = -0.19, p = 0.037). The CYP11B2 -344CC genotype was also overrepresented among patients with extreme aldosterone elevation (>90th percentile, p = 0.0145). In this cohort of African Americans with chronic ambulatory heart failure, the CYP11B2 -344T>C genotype was a significant independent predictor of AF while holding clinical, echocardiographic predictors, and genetic ancestry constant. In addition, increased West African ancestry was associated with decreased serum aldosterone levels, potentially providing an explanation for the lower risk for AF observed among African Americans.

Citation: Bress A, Han J, Patel SR, Desai AA, Mansour I, et al. (2013) Association of Aldosterone Synthase Polymorphism (CYP11B2 -344T>C) and Genetic Ancestry with Atrial Fibrillation and Serum Aldosterone in African Americans with Heart Failure. PLoS ONE 8(7): e71268. doi:10.1371/journal.pone.0071268

Editor: Hassan Ashktorab, Howard University, United States of America

Received March 18, 2013; Accepted June 26, 2013; Published July 30, 2013

Copyright: © 2013 Bress et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by the National Institutes of Health, National Institute on Aging (AG033381-01, LHC), National Center for Research Resources/National Center for Advancing Translational Sciences (UL1RR029879, AAD), and the Office of the Vice Chancellor for Research, University of Illinois at Chicago. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: humma@uic.edu

Introduction

Heart failure is a common and costly phenotype, with apparent differences in prevalence between African Americans and non-African Americans [1,2]. African Americans display differences in heart failure pathogenesis, drug response, and treatment outcomes compared to other ethnic groups, with significant genetic contribution [3,4,5,6,7]. African Americans also carry a paradoxically low risk for atrial fibrillation (AF), despite having a higher prevalence of well-established AF risk factors such as heart failure, hypertension, diabetes and larger body size [8,9,10,11,12]. Interestingly, higher European ancestry among African Americans was predictive of incident AF in a previous study [13]. However, African Americans remain under-represented in genetic studies in heart failure, particularly as they relate to risk of AF development.

Activation of the renin-angiotensin-aldosterone system (RAAS) plays a critical role in the pathogenesis of both heart failure and AF [14,15,16,17]. Specifically, aldosterone binding to the mineralocorticoid receptor stimulates myocyte apoptosis, leading to cardiac fibrosis, which is central to both heart failure and AF pathophysiology [18,19]. Mineralocorticoid receptors are up-regulated in atrial myocytes in the setting of AF, potentially amplifying the effects of aldosterone binding [20]. As further evidence of the importance of aldosterone and mineralocorticoid receptors in the pathogenesis of AF, mineralocorticoid receptor antagonists (MRAs) have been shown to reduce the risk of new-
onset AF in predominantly European populations with systolic heart failure [21,22,23].

Aldosterone synthase is the enzyme that catalyzes the final reaction to generate aldosterone. The aldosterone synthase gene (CYP11B2) consists of 9 exons and is localized to chromosome 8q22 [24]. A common SNP in the promoter region of the CYP11B2 gene, c.2344T>C (rs1799998) occurs in approximately 30% of African Americans and 46% of Europeans [25], with 4-times greater affinity for the steroidogenic transcription factor 1 (SF-1) and increased aldosterone excretion reported with the 2344C allele [26,27]. In clinical studies, the 2344C allele has been linked to increased left ventricular size in Europeans and both systemic hypertension and ischemic stroke in Asians [28,29]. The 2344CC genotype was also predictive of risk for AF in an Israeli population with heart failure and predictive of both worsening left ventricular remodeling and increased risk of death and hospitalization in African Americans with heart failure [30,31]. In contrast, a study of 67 African Americans with chronic systolic HF linked the −344C allele to lesser cardiac remodeling [32].

Despite the central role of aldosterone in heart failure progression and risk for AF in heart failure, the relationship between the CYP11B2 −344C allele and AF in African Americans remains unknown. We, therefore, evaluated the association between the 2344T>C SNP and AF in an ambulatory, African American, chronic heart failure population. Given the potential role for genetic heterogeneity and ancestry in heart failure outcomes, genetic ancestry was also analyzed.

Methods

Ethics statement

The study was approved by the Institutional Review Board at the University of Illinois at Chicago. Written, informed consent was obtained from all patients prior to study enrollment.

| Characteristic | Total Cohort (n = 194) | TT (n = 131) | TC (n = 56) | CC (n = 7) |
|----------------|------------------------|-------------|------------|------------|
| Age (yrs)      | 55±14                  | 56±14       | 51±15      | 60±7       |
| Male sex       | 93 (48)                | 71 (54)     | 18 (32)    | 4 (57)     |
| BMI, kg/m²     | 34±11                  | 34±11       | 34±9.9     | 35±14      |
| NYHA Class     | 2.5 (2–3)              | 2.5 (2–3)   | 3 (2–3)    | 2 (1–3)    |
| Ischemic Etiology | 44 (23)       | 32 (24)     | 11 (20)    | 1 (14)     |
| Diabetes       | 63 (32)                | 47 (36)     | 13 (23)    | 3 (43)     |
| Systemic Hypertension | 160 (82)   | 110 (84)    | 44 (79)    | 6 (86)     |
| Atrial Fibrillation | 37 (19)     | 22 (17)     | 10 (18)    | 5 (71)*    |
| CrCl (mL/min)  | 65±26                  | 63±25       | 71±29      | 45±13**    |
| B-Blocker use  | 188 (97)               | 126 (96)    | 56 (100)   | 6 (86)     |
| ACE inhibitor or ARB use | 192 (99)   | 130 (99)    | 55 (98)    | 7 (100)    |
| MRA use        | 20 (10)                | 17 (13)     | 3 (5)      | 0          |
| Loop Diuretic Use | 112 (58)   | 76 (58)     | 30 (53)    | 6 (86)     |
| Thiazide Diuretic Use | 7 (4)      | 5 (4)       | 2 (4)      | 0          |
| Digoxin Use    | 59 (30)                | 37 (28)     | 18 (32)    | 4 (57)     |

| Echocardiograph measurements | Total Cohort (n = 194) | TT (n = 131) | TC (n = 56) | CC (n = 7) |
|------------------------------|------------------------|-------------|------------|------------|
| LA Size (mm)                 | 44±8                   | 44±7.9      | 43±8.1     | 47±13      |
| LVEDD (cm)                   | 5.9±1.0                | 5.9±0.9     | 5.8±1.1    | 5.7±1.4    |
| Ejection Fraction (%)        | 30±14                  | 30±14.1     | 28±14      | 35±17      |
| Mod-Severe or Severe MR      | 32 (16)                | 20 (15)     | 12 (21)    | 0          |
| Ejection Fraction <40%       | 145 (75)               | 96 (73)     | 45 (80)    | 4 (57)     |
| 40–50%                       | 32 (16)                | 26 (20)     | 4 (7)      | 2 (29)     |
| >50%                         | 17 (9)                 | 9 (7)       | 7 (13)     | 1 (14)     |
| Genetic ancestry (%)         |                        |             |            |            |
| European                     | 18±11                  | 17±11       | 18±12      | 22±12      |
| West African                 | 75±13                  | 76±13       | 74±14      | 70±13      |
| Native American              | 7±6                    | 7±5         | 8±6        | 8±6        |

Count (%), mean ± SD, or median (interquartile range).

*p = 0.007 value for comparison between genotype groups.

**p = 0.03 value for comparison between genotype groups.

ACE, angiotensin converting enzyme; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI, body mass index; CrCl, creatinine clearance; MR, mitral regurgitation; MRA, mineralocorticoid receptor antagonist; LA, left atrium; LVEDD, left ventricular end diastolic diameter; NYHA, New York Heart Association.

doi:10.1371/journal.pone.0071268.t001
Study population

African Americans (by self-report) at least 18 years of age, with a diagnosis of heart failure (either with reduced or preserved ejection fraction) for at least 3 months were included. Additional inclusion criteria were treatment with an ACE inhibitor or angiotensin receptor blocker (or if contraindicated, the combination of hydralazine and nitrates) for at least 6 months with no change in doses of these medications for at least 2 months. Patients with a history of liver disease were excluded.

Study procedures

After obtaining written, informed consent, a buccal cell or venous blood sample was collected for determination of genotype and, in a subset of patients, additional blood was collected for determination of serum aldosterone concentration. Since serum aldosterone exhibits diurnal variation and may be influenced by body position, all blood samples were drawn between 8 am and 1 pm while patients were seated after being upright for at least 2 hours. [33] Samples were stored at -80°C until further analysis.

Demographic, clinical, and social data were collected at the time of enrollment. AF was defined by a University of Illinois Hospital and Health Science System (UI-Health) cardiologist at study enrollment and diagnosed by documentation in either the electronic medical record or evidence on 12-lead electrocardiography (ECG) and/or Holter monitoring, as previously published [34]. Atrial fibrillation cases associated with a recent surgery or hyperthyroidism were excluded.

Echocardiography

Transtracheal echocardiographic studies were performed within 12 months of enrollment using an Acuson SC2000™ ultrasound system. Echocardiographic measurements of left ventricular end diastolic diameter (LVEDD) were performed using standard 2D and M-Mode methods. Left ventricular ejection fraction was assessed using 2D methods and the Simpson method of discs, and left atrial size was determined using linear measurements as outlined by the American Society of Echocardiography [35]. Severity of mitral regurgitation was determined using color Doppler and the PISA method (when appropriate) as outlined by the American Society of Echocardiography [36].

Aldosterone assay

Samples for aldosterone were assayed using a commercially available kit containing I-125-labeled aldosterone (Beckman Coulter, Brea, CA), as previously described [37]. All samples were assayed in duplicate. Intra-assay and inter-assay coefficients of variation for this assay were 1.5% and 1.9% respectively [37].

Genotyping

Genomic DNA was isolated from buccal cells or whole blood using a Puregene® kit (Qiagen, Valencia, CA). Genotyping for the

| Table 2. Patient characteristics according atrial fibrillation status. |
|---------------------------|-----------------|-----------------|-----------------|
| Characteristic           | AF (n = 37)     | No AF (n = 157) | p Value         |
| Age (yrs)                | 61±13           | 54±14           | 0.007           |
| Male sex                 | 21 (57)         | 72 (46)         | 0.23            |
| BMI, kg/m²               | 34±11           | 34±11           | 0.92            |
| NYHA Class               | 2 (1–3)         | 3 (2–3)         | 0.06            |
| Ischemic Etiology        | 6 (16)          | 38 (24)         | 0.29            |
| Diabetes                 | 9 (24)          | 54 (34)         | 0.23            |
| Systemic Hypertension    | 32 (86)         | 128 (82)        | 0.47            |
| CrCl (mL/min)            | 50±22           | 68±26           | <0.001          |
| B-Blocker                | 36 (97)         | 152 (97)        | 0.99            |
| ACE inhibitor or ARB     | 37 (100)        | 155 (99)        | 0.99            |
| MRA Use                  | 3 (8)           | 17 (11)         | 0.76            |
| Loop Diuretic Use        | 21 (57)         | 91 (58)         | 0.99            |
| Thiazide Diuretic Use    | 1 (3)           | 6 (4)           | 0.99            |
| Digoxin Use              | 11 (30)         | 48 (31)         | 0.92            |

Echocardiographic Measurements

- LA Size (mm): 50±7.6 vs 43±7.7 (p <0.001)
- LVEDD (cm): 5.9±1.1 vs 5.9±1.0 (p = 0.93)
- Ejection Fraction (%): 30±12 vs 30±15 (p = 0.97)
- Mod-Sev or Severe MR: 10 (27) vs 22 (14) (p = 0.055)
- Genetic ancestry (%): European 18% vs 12% (p = 0.77), West African 74% vs 75% (p = 0.65), Native American 8% vs 67% (p = 0.65)

| Table 3. CYP11B2 – 344T>C genotype and allele frequencies. |
|--------------------|-----------------|-----------------|-----------------|
| Genotype frequency | Total Cohort (n = 194) | AF (n = 37) | No AF (n = 157) | p Value |
| Genotype frequency, n (%) | 131 (68) | 22 (59) | 109 (69) | 0.002* |
| Genotype frequency, n (%) | 56 (29) | 10 (27) | 46 (29) |
| Genotype frequency, n (%) | 7 (3.6) | 5 (14) | 2 (1.3) |
| Allele Frequency, n (%) | 318 (82) | 54 (73) | 264 (84) | 0.014** |
| Allele Frequency, n (%) | 70 (18) | 20 (27) | 50 (16) |

*by χ² analysis for differences in frequencies between genotype groups
**by Cochran-Armitage trend test for differences in allele frequencies between AF groups
CYP11B2 in Atrial Fibrillation
CYP11B2 -344T>C (rs1799998) polymorphism was done via PCR and capillary sequencing, with primers and annealing temperatures shown in Table S1. Genotype results were verified using a different primer set. Each genotype was scored by two independent investigators blinded to AF status. Individual genetic ancestry was determined for each person using 105 autosomal DNA ancestry informative markers for West African, Native American, and European genetic ancestry using published methods [38,39]. Each participant was then scored from 0% to 100% for individual estimates of West African, Native American and European ancestry.

Data analysis
Creatinine clearance was calculated using the equation of Cockcroft and Gault and ideal body weight [40]. Hardy-Weinberg equilibrium was tested by $\chi^2$ analysis. Normally distributed continuous data are presented as mean ± SD and were compared by unpaired t-tests and analysis of variance. Continuous data that were non-normally distributed are presented as median (IQR) and were compared with Mann Whitney U and Kruskal Wallis tests. The $\chi^2$ or Fischer's exact test was used to compare categorical data, and the Cochran-Armitage trend test was used to compare allele frequencies between groups. Multiple logistic regression permitted tests of association (odds ratio) between CYP11B2 -344T>C and presence of AF while holding clinical factors, echocardiographic measurements, and genetic ancestry constant. Dominant, additive, recessive and genotypic effects models were all used to test the association of CYP11B2 -344T>C genotype and presence of AF. Based on previous data, risk factors for AF included as covariates in the multiple logistic regression models were age, sex, body size, systemic hypertension, diabetes, coronary artery disease, creatinine clearance, left atrial size, mitral regurgitation and genetic ancestry [41,42,43,44,45,46]. Marginal standardization was used for the final logistic regression model to estimate adjusted prevalence differences between genotype groups [47]. Bootstrapping was used to quantify the confidence intervals of the prevalence difference generated from the marginal standardization [48]. Given the low prevalence of the CC homozygous genotype, we used permutation to generate a distribution genotypic effects under the assumption of a true null hypothesis, which creates an empirical p value for the association of the CYP11B2 −344T>C recessive effects model and AF [49].

For the exploratory analysis of serum aldosterone, linear regression (ordinary least squares) was used to examine the association between genetic ancestry and aldosterone levels. Serum aldosterone was natural-log transformed to produce a more normal distribution of regression residuals, as done previously [50]. Mean log serum aldosterone was compared between genotype and AF groups by the unpaired t-test. We also examined the association between genotype and extreme elevation of log aldosterone despite standard heart failure therapy, which was defined as a serum log aldosterone level at or above the 90th percentile for the study population, using Fisher’s exact test.

A two-sided p value of less than 0.05 was considered as statistical significance. Statistical analyses were performed with the SAS software package, version 9.2 (SAS Institute, Cary, NC, USA), and

### Table 4. Predictors of atrial fibrillation in multiple logistic regression analysis.

| Variable                      | Adjusted OR | 95% CI   | P value | $P_{emp}$ value* |
|-------------------------------|-------------|----------|---------|-----------------|
| **Model 1 (Allelic Effects Model)** |             |          |         |                 |
| CYP11B2 −344C, (0,1,2)        | 2.14        | 1.05–4.32| 0.035   |                 |
| LA Size (per 5mm)             | 1.65        | 1.21–2.25| 0.001   |                 |
| CrCl (per 10 ml/min increase) | 0.74        | 0.56–0.96| 0.024   |                 |
| **Model 2 (Genotypic Effects Model)** |             |          |         |                 |
| CYP11B2 −344 CC               | 13.4        | 1.70–105 | 0.014   |                 |
| CYP11B2 −344 CT               | 1.24        | 0.47–3.3 | 0.662   |                 |
| LA Size (per 5mm)             | 1.68        | 1.23–2.31| 0.001   |                 |
| CrCl (per 10 ml/min increase) | 0.75        | 0.57–0.99| 0.040   |                 |
| **Model 3 (Recessive Effects Model)** |             |          |         |                 |
| CYP11B2 −344 CC               | 12.7        | 1.60–98.4| 0.015   | 0.011           |
| LA Size (per 5mm)             | 1.69        | 1.23–2.31| 0.001   |                 |
| CrCl (per 10 ml/min increase) | 0.75        | 0.57–0.99| 0.041   |                 |
| **Model 4 (Dominant Effects Model)** |             |          |         |                 |
| CYP11B2 −344 CC or CT         | 1.867       | 0.77–4.52| 0.166   |                 |
| LA Size (per 5mm)             | 1.643       | 1.21–2.23| 0.001   |                 |
| CrCl (per 10 ml/min increase) | 0.726       | 0.56–0.95| 0.018   |                 |

LA, left atrial; CrCl, creatinine clearance.

Adjusted for age, sex, body size (BMI), mitral regurgitation, systemic hypertension, coronary artery disease, diabetes mellitus, left atrial size, creatinine clearance, and percent European ancestry.

*Empirical p value generated by permuting the CC genotype term in the logistic model (10,000 reps). The p values represents the proportion of permutations that led to a coefficient on the CC term at least as large as the one observed in the actual sample.
Results

A total of 194 African Americans were enrolled and successfully genotyped. Table 1 shows the clinical characteristics, echocardiographic measurements, and genetic ancestry of the entire study cohort and by CYP11B2 −344T>C genotype. Patients were well treated, with nearly all receiving an ACE inhibitor or angiotensin II receptor antagonist and 97% taking a β-blocker. All 20 patients taking an MRA had the 2344CT or TT genotype. Clinical and echocardiographic characteristics were similar between CYP11B2 −344T>C genotype groups, with the exception of AF, which was more prevalent with the 2344CC versus TC or TT genotype (71 versus 17%, p = 0.003), and creatinine clearance, which was lower with the −344CC genotype (45±13 versus 65±26 mL/min, p = 0.04).

Table 2 displays the clinical characteristics, echocardiographic measurements, and genetic ancestry by AF status. AF was present in 37 (19%) participants. As expected based on past reports [1,13,17,41], patients with AF were older, had worse renal function and larger left atrial size, and were more likely to have moderately-severe or severe mitral regurgitation compared to those without AF.

Table 3 shows the genotype and allele frequencies in the total cohort and by AF status. Genotype distribution did not deviate from Hardy-Weinberg equilibrium ($\chi^2$ expected versus observed, p = 0.74). The −344C allele frequency in the total cohort was consistent with previous reports in Africans and African Americans and was significantly higher in those with AF [31].

Figure 1 displays the distribution of genetic ancestry. On average, the cohort had 75% West African, 18% European and

Figure 2. Unadjusted and adjusted (marginal standardization) proportions of individuals among CYP11B2 −344T>C genotype groups with AF.
doi:10.1371/journal.pone.0071268.g002

Figure 3. Serum aldosterone level by AF status and CYP11B2 genotype.
doi:10.1371/journal.pone.0071268.g003
7% Native American ancestry. Ancestry was not associated with AF by either bivariate or multivariate analyses.

**CYP11B2 – 344 T>C and atrial fibrillation**

Table 4 shows the results of the multiple logistic regression models testing the association of the CYP11B2 –344 T>C polymorphism with AF while adjusting for clinical and echocardiographic covariates and genetic ancestry. Covariates were selected for inclusion in the model based on previous data [41,42,43,44,45,46] and included age, sex, body size (BMI), creatinine clearance, systemic hypertension, diabetes, coronary artery disease, left atrial size, mitral regurgitation and European ancestry. Including percent European ancestry allowed for control for population admixture and also for potential confounding as European ancestry was previously associated with AF in African Americans [51]. We specified the CYP11B2 –344 T>C genotype four different ways: Model 1, allelic effects (0, 1, 2 for number of C alleles carried); Model 2, genotypic effects (indicator variables for CC, CT and TT as the reference group); Model 3, recessive effects (0 for CT or TT, 1 for CC); and Model 4, dominant effects (0 for CC or CT, 1 for TT). Both Models 1 and 3 demonstrated an association between the CYP11B2 –344T>C genotype and AF, with the greatest effects observed with the 344CC genotype. Also in Model 1, each 5 millimeter increase in left atrial size was associated with a 69% increase in the odds of AF. The size of association between left atrial size and AF was similar between genotype model specifications. Creatinine clearance was associated with AF in all models, with each 10 ml/min increase in clearance decreasing odds of AF by approximately 25%. None of the other clinical and echocardiographic covariates were significant (p<0.05) predictors of AF.

**Marginal standardization and adjusted prevalence difference estimates**

We used the recessive effects model, Model 3, to generate adjusted prevalence differences using marginal standardization. Figure 2, shows the unadjusted association of CYP11B2 –344CC genotype with AF status (prevalence difference of 0.54, 95% CI 0.20 to 0.88, p = 0.003) on the left. Figure 2 on the right displays marginal standardization, which is the average prevalence difference between genotype groups adjusted for age, sex, body size, creatinine clearance, systemic hypertension, diabetes, coronary artery disease, left atrial size, mitral regurgitation, and European ancestry. The CYP11B2 CC genotype was associated with an adjusted AF prevalence difference of 40 percentage points (95% CI 9 to 67).

To enhance the strength of our association estimate for Model 3, we generated empirical p values using permutation. This test assumes that the null hypothesis is true (no association), and shows the probability of obtaining our observed association estimate simply by chance. The p value on our observed coefficient for CYP11B2 –344 CC genotype was 0.011 for the recessive effects model. This corresponds to the proportion of permutations that led to a coefficient at least as large as the one observed in the actual sample. This suggests it is unlikely that the association observed in our data is simply by chance.

**CYP11B2 association with aldosterone levels**

To explore the possible causal pathway underlying the CYP11B2 association with AF, we measured serum aldosterone in a subset of 126 patients who provided samples for this analysis. None of these patients were on an MRA. The median (range) aldosterone concentration in the study population was 90 (18 to
392) pg/ml. Thirteen patients (10%) had aldosterone levels in the upper 90th percentile (>196 pg/ml). Figure 3 shows the distribution of log serum aldosterone by CYP11B2 genotype and AF status. Log aldosterone concentration was significantly higher in those with versus without AF (p = 0.036). There was no significant association between log aldosterone concentration examined as a continuous variable and CYP11B2 −344T>C genotype (p = 0.13). However, 50% of patients with the CYP11B2 −344CC genotype versus 8% with the TC or TT genotype had log aldosterone levels in the upper 90th percentile for the study sample (OR 11, 95% CI 2.0 to 62, p = 0.015).

Genetic ancestry and aldosterone levels

Figure 4 displays the correlation between West African ancestry and log serum aldosterone levels in 126 subjects. In our cohort of self-reported African Americans, West African ancestry was associated with lower log serum aldosterone levels (R2 = 0.035, p = 0.057), while European ancestry was associated with higher levels (R2 = 0.031, p = 0.048). There was no association between Native American ancestry and serum aldosterone (p = 0.30).

Discussion

Our study has two main findings. First, the CYP11B2 −344T>C genotype was associated with a significant increase in odds of AF in an African American, ambulatory heart failure population, after controlling for a rich set of clinical and echocardiographic covariates as well as controlling for population admixture using genetic ancestry. This observation is consistent with previous findings in Asian and Middle Eastern populations [30,52]. Specifically, the CYP11B2 −344CC genotype was associated with a 2.4-fold increase in the odds of AF among an Israeli cohort with systolic HF [30]. Similarly, a meta-analysis of six studies including over 2,700 Asian patients with essential hypertension, hypertensive heart disease, or heart failure showed a 2-fold increase in odds of AF with the CC genotype [52]. Consistent with previous studies, our data also support a recessive effects model for the CYP11B2 −344T>C genotype link to AF [30]. While the odds ratio from the recessive effects model in our study is greater in size than that previously reported, this might be reflective of lower precision due to smaller sample size in the current study, or alternatively, could reflect differences in the patient populations under study.

Consistent with previous studies of patients with hypertension or dilated cardiomyopathy, we also found that the CYP11B2 −344CC genotype was over-represented among individuals with extreme elevation of aldosterone, defined as the 90th percentile for the study population [53,54]. This finding sheds light on the mechanism potentially explaining increased risk for AF with the CYP11B2 −344CC genotype. Further, evidence that the CYP11B2 −344CC genotype is associated with extremes in serum aldosterone levels may help to explain the previously observed associations between the −344CC genotype and greater left ventricular mass and decreased event-free survival among African Americans with heart failure [29,31,33]. Specifically, higher aldosterone levels with the −344CC genotype could lead to greater cardiac fibrosis and remodeling, which are important contributors to heart failure-related morbidity, mortality and risk for developing AF [19,55].

Our second major finding from our study is the novel association between genetic ancestry and serum aldosterone levels. Specifically, we found that among African Americans with heart failure, greater European ancestry was correlated with higher serum aldosterone concentrations, and greater West African ancestry was correlated with lower aldosterone levels. These findings are consistent with previous observations of lower plasma aldosterone in African Americans compared to Europeans with various stages of hypertension [56,57]. Moreover, the link between European ancestry and elevated aldosterone may provide insight into the mechanism underlying the association between European ancestry and increased risk of AF in African Americans in the Atherosclerosis Risk in Communities (ARIC) Study [51]. In particular, our data support the hypothesis that the genetic ancestry effect on incident AF could be due, in part, to differences in aldosterone levels by ancestry. However, we did not observe a significant association between ancestry and AF in our study, which is in contrast to data from the ARIC study. It is possible that our study was underpowered to detect such an association, especially with the modest effect size (HR 1.17) observed in ARIC between European ancestry and incident AF [51].

The implication of our findings that CYP11B2 genotype is associated with both AF risk and elevated aldosterone is that CYP11B2 may be a useful biomarker to identify heart failure patients at risk for AF in whom aldosterone antagonism may attenuate such risk. In addition to increasing the risk for stroke, AF can also exacerbate heart failure symptoms and reduce exercise capacity [58,59]. As such, the ability to predict patients at greater risk for AF, in whom modalities could be instituted to ameliorate this risk, could potentially lessen heart failure-related morbidity.

There is strong evidence that aldosterone antagonism with an MRA in systolic heart failure improves survival and reduces heart failure-related morbidity, including AF [21,22,23]. There are also data that the MRA spironolactone attenuates the deleterious effects of aldosterone in AF at the level of atrial tissue, where mineralocorticoid receptors appear to be up-regulated, lending support to the idea that an MRA may attenuate the risk for AF in heart failure patients with a −344CC genotype [20]. However, the role of MRAs in heart failure patients with the CYP11B2 −344CC genotype-associated AF risk has yet to be ascertained.

It is important to note that there have been discrepant results with the CYP11B2 −344T>C variant and its association with aldosterone secretion and the presence of cardiovascular disease [26]. Along with type-I error due to sample size, one of several explanations is confounding association due to population admixture, which we have addressed by adjusting for genetic ancestry [60]. Allele and genotype frequencies in our study are similar to those from the African American Heart Failure Trial (A-HeFT) [31]. However, there are significant differences in CYP11B2 −344T>C allele and genotype frequencies among ethnic groups. African Americans have a lower frequency of the CC genotype (~3–10%) than both Europeans (~20–30%) and Asians (~7–12%), which may explain the intra-ancestral discrepancy in certain association studies [25,30,31,52,61]. Some of the previous studies focused on patient populations with cardiovascular diseases other than heart failure, and failed to find an association between −344CC genotype and AF [61,62]. It is possible that aldosterone regulation plays a greater role in the pathogenesis of AF in heart failure patients than patients with other diseases considering the significant aldosterone involvement in the ventricular remodeling and cardiac fibrosis [14,16,19].

There are several limitations to our study. First, the incidence of AF is complex, multi-factorial, and subject to many confounders. Although we controlled for a rich set of important clinical and echocardiographic covariates in our analysis, other potential variables such as alcohol consumption and family history were not explored. Similar to other pharmacogenetic studies, this study comprised a small sample size limiting the precision of association estimates. In addition, the −344CC genotype occurs at low
frequency in African Americans, and thus, our association estimates with this genotype are exploratory and require confirmation. However, the permutation analysis of the recessive effects model suggests that these findings are unlikely to be simply due to chance (p value = 0.019). Further, this study population was of African descent and thus caution is warranted in drawing conclusions for other racial groups. Finally, contribution by variants not interrogated in this study is also possible, as there may be other, unobserved, polymorphisms in or near CYP11B2 that may be functionally important [54,63,64].

Conclusion

In summary, in a cohort of African Americans with chronic ambulatory heart failure, the CYP11B2 344CC genotype was a significant and independent predictor of AF beyond conventional clinical and echocardiographic predictors of AF and genetic ancestry. The CYP11B2 344CC genotype was also associated with extreme elevation of serum aldosterone, providing insight into the mechanism underlying AF risk with the CYP11B2 genotype. Also, among self-reported African Americans, increasing West African ancestry was associated with decreased serum aldosterone levels. Whether strategies, such as aldosterone antagonism with an MRA, might impact the risk for AF conferred by the −344CC genotype remains to be determined.

Supporting Information

Table S1 PCR primers.

Author Contributions

Conceived and designed the experiments: AB, JH, LC, RK, SP, TS, CW. Performed the experiments: AB, SP, RK ES TS KG. Analyzed the data: AB, LC, RK, ES, JH, IM, KG. Wrote the paper: AB, JH, SP, AD, IM, KG, TS. Contributed reagents/materials/analysis tools: AB, TS, KG, SP, ES, JG, SP. Obtained funding: AB, JH, LC, RK, SP, TS, CW. Enrolled patients: VG, KG, SP, AD, JH. Data analysis tools: AB, TS, LC, RK, SP. Wrote the paper: AB, JH, SP, AD.

References

1. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, et al. (2013) Heart disease and stroke statistics-2013 update: a report from the American Heart Association. Circulation 127: e6-e245.
2. Wang G, Zhang Z, Ayala C, Wall HK, Fang J (2010) Costs of heart failure-related hospitalizations in patients aged 18 to 61 years. Am J Manag Care 16: 769–776.
3. Yancy CW (2003) Does race matter in heart failure? Curr Opin Cardiol 18: 351–357.
4. Exner DV, Dries DL, Domanski MJ, Cohn JN (2001) Lesser response to angiotensin-converting-enzyme inhibitor therapy in black as compared with white patients with left ventricular dysfunction. N Engl J Med 344: 1351–1357.
5. Yancy CW (2000) Heart failure in African Americans: a cardiovascular enigma. J Card Fail 6: 103–106.
6. Carson P, Ziesche S, Johnson G, Cohn JN (1999) Racial differences in response to therapy for heart failure: analysis of the vasodilator-heart failure trials. Valsodilator-Heart Failure Trial Study Group. J Card Fail 5: 178–187.
7. Dries DL, Exner DV, Gersh BJ, Cooper HA, Carson PE, et al. (1999) Racial differences in the outcome of left ventricular dysfunction. N Engl J Med 340: 699–616.
8. Centers for Disease Control and Prevention (CDC) (2009) Differences in prevalence of obesity among black, white, and Hispanic adults–United States, 2006-2008. MMWR Morb Mortal Wkly Rep 58: 680–684.
9. Bibbins-Domingo K, Pletcher MJ, Lin F, Vittinghoff E, Gardin JM, et al. (2009) Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. JAMA 295: 2079–2085.
10. Krum H, Veldhuisen DJ, Schwammenthal E, Tighiouart H, de Lemos JA, et al. (2011) Eplerenone in patients with systolic heart failure and mild symptoms. N Engl J Med 364: 11–21.
11. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, et al. (1999) The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 341: 709–717.
12. Bassett MH, White PC, Rainey WE (2004) The regulation of aldosterone synthesis. Mol Cell Endocrinol 217: 67–74.
13. Database of Single Nucleotide Polymorphisms (dbSNP), Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. dbSNP accession: rs1799998 (dbSNP Build ID: (135)). Available: http://www.ncbi.nlm.nih.gov/SNP/.
14. White PC, Rainey WE (2005) Editorial: polymorphisms in CYP11B2 genes and 11β-hydroxylase activity. J Clin Endocrinol Metab 90: 1252–1253.
15. White PC, Slusker L (1995) Haplootype analysis of CYP11B2. Endocr Rev 26: 437–442.
16. Delles C, Erdmann J, Jacob J, Håger KW, Fleck E, et al. (2001) Aldosterone synthase (CYP11B2) gene polymorphism as a determinant of atrial fibrillation in patients with heart failure. Am J Cardiol 102: 326–329.
17. McNamara DM, Tam SW, Sobelwinski ML, Toleman P, Janosko K, et al. (2006) Aldosterone synthase promoter polymorphism predicts outcome in African Americans with heart failure: results from the A-HeFT Trial. J Am Coll Cardiol 48: 1277–1292.
18. Bello A, Chao T, Dhurany TA, Kohlyar E, Allenworth-Davies D, et al. (2007) Usefulness of the aldosterone synthase gene polymorphism C-344T to predict cardiac remodeling in African-Americans versus non-African-Americans with chronic systolic heart failure. Am J Cardiol 100: 285–290.
19. Hurvitz S, Cohen RJ, Williams GH (2004) Atrial remodeling and plasma renin activity: timing relation to melanin and cortisol and consistency after prolonged bed rest. J Appl Physiol 96: 1406–1414.
20. Delaney JT, Jeff JM, Brown NJ, Prentorius M, Okahre HE, et al. (2012) Characterization of genome-wide association-identified variants for atrial fibrillation in African Americans. PLoS One 7: e23381.
21. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foschi A, et al. (2005) Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 18: 1440–1463.
22. Zoghbi WA, Enriquez-Sarano M, Foster E, Grayburn PA, Kraft JD, et al. (2003) Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. J Am Soc Echocardiogr 16: 777–802.
23. Cavallari LH, Gross VL, Viana MA, Dey I, Patel SR, et al. (2010) Association of aldosterone concentration and mineralocorticoid receptor genotype with potassium response to spironolactone in patients with heart failure. Pharmacother 30: 1–9.
38. Giri VN, Epleston R, Ruth K, Uzzo RG, Chen DY, et al. (2009) Race, genetic West African ancestry, and prostate cancer prediction by prostate-specific antigen in prospectively screened high-risk men. Cancer Prev Res (Phila) 2: 244–250.

39. Tian C, Hinds DA, Shigeta R, Kirdies R, Ballinger DG, et al. (2006) A genomewide single-nucleotide-polymorphism panel with high ancestry information for African American admixture mapping. Am J Hum Genet 79: 640–649.

40. Cockcroft DW, Gaulth MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16: 31–41.

41. Huxley RR, Lopez FL, Folsom AR, Agarwal SK, Loehr LR, et al. (2011) Absolute and attributable risks of atrial fibrillation in relation to optimal and borderline risk factors: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 123: 1501–1508.

42. Chamberlain AM, Agarwal SK, Folsom AR, Soliman EZ, Chambless LE, et al. (2011) A clinical risk score for atrial fibrillation in a biracial prospective cohort (from the Atherosclerosis Risk in Communities [ARIC] study). Am J Cardiol 107: 85–91.

43. Schnabel RB, Asplund T, Li G, Sullivan LM, Suchy-Dicey A, et al. (2010) Validation of an atrial fibrillation risk algorithm in whites and African Americans. Arch Intern Med 170: 1909–1917.

44. Watanabe H, Watanabe T, Sasaki S, Nagai K, Roden DM, et al. (2009) Close bidirectional relationship between chronic kidney disease and atrial fibrillation: the Niigata preventive medicine study. Am Heart J 158: 629–636.

45. Schnabel RB, Sullivan LM, Levy D, Pencina MJ, Massaro JM, et al. (2009) Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. Circulation 125: 739–745.

46. Krahn AD, Manfreda J, Tate RB, Mathewson FA, Cuddy TE. (1995) The natural history of atrial fibrillation: incidence, risk factors, and prognosis in the Manitoba Follow-Up Study. Am J Med 98: 476–484.

47. Localio AR, Margolis DJ, Berlin JA (2007) Relative risks and confidence intervals were easily computed indirectly from multivariable logistic regression. J Clin Epidemiol 60: 874–882.

48. Elfron B, Tibshirani R (1993) An introduction to the bootstrap. New York: Chapman & Hall. p. 436.

49. Rosenbaum PR (2002) Observational studies. New York: Springer. p. 375.

50. Efron B, Tibshirani R (1993) An introduction to the bootstrap. New York: Chapman & Hall. p. 436.

51. Marcus GM, Alonso A, Peralta CA, Lettre G, Vittinghoff E, et al. (2010) Patterns of left ventricular remodeling in chronic heart failure: prevalence and prognostic implications. Am Heart J 161: 1088–1095.

52. Li YY, Zhou CW, Xu J, Qian Y, Wang B (2012) CYP11B2 T-344C gene polymorphism and atrial fibrillation: a meta-analysis of 2,738 subjects. PLoS One 7: e50910.

53. Takai E, Akira H, Kanazawa K, Shiga N, Terahima M, et al. (2002) Association between aldosterone synthase (CYP11B2) gene polymorphism and left ventricular volume in patients with dilated cardiomyopathy. Heart 88: 649–650.

54. Popoga L, Gazier S, Blanc H, Guyene TT, Poirier O, et al. (1998) Genetic determination of plasma aldosterone levels in essential hypertension. Am J Hypertens 11: 856–860.

55. Dini FL, Capozza P, Donati F, Simionescu A, Corciu AI, et al. (2011) Patterns of left ventricular remodeling in chronic heart failure: prevalence and prognostic implications. Am Heart J 161: 1088–1095.

56. Jones J, Park JH, Dowling T, Phares D, Park JY, et al. (2006) Role of potassium excretion and percent body fat on ethnic differences in plasma aldosterone levels. Edin Din 16: 54–10-14.

57. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P (2002) Hyperaldosteronism among black and white subjects with resistant hypertension. Hypertension 40: 892-896.

58. Agostoni P, Emidin M, Corra U, Veglia F, Magri D, et al. (2008) Permanent atrial fibrillation affects exercise capacity in chronic heart failure patients. Eur Heart J 29: 2367–2372.

59. Dries DL, Euner DV, Gersh BJ, Domanski MJ, Wactlawie MA, et al. (1998) Atrial fibrillation is associated with an increased risk for mortality and heart failure progression in patients with asymptomatic and symptomatic left ventricular systolic dysfunction: a retrospective analysis of the SOLVD trials. Studies of Left Ventricular Dysfunction. J Am Coll Cardiol 32: 695-703.

60. Kirdies RA, Chen W, Pangubuli RK, Abaghotu G, Jackson A, et al. (2002) CYP11A1-V and prostate cancer in African Americans: causal or confounding association because of population stratification? Hum Genet 110: 533–560.

61. Sun X, Yang J, Hou X, Li J, Shi Y, et al. (2011) Relationship between CYP11B2 polymorphism in the aldosterone synthase gene and atrial fibrillation in patients with essential hypertension. J Renin Angiotensin Aldosterone Syst 12: 557–563.

62. Huang M, Gai X, Yang X, Hou J, Lan X, et al. (2009) Functional polymorphisms in ACE and CYP11B2 genes and atrial fibrillation in patients with hypertensive heart disease. Clin Chem Lab Med 47: 32–37.

63. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, et al. (2012) Meta-analysis identifies six new susceptibility loci for atrial fibrillation. Nat Genet 44: 670-675.

64. Schnabel RB, Kerr KF, Lubitz SA, Akylbekova EL, Marcus GM, et al. (2011) Large-scale candidate gene analysis in whites and African Americans identifies IL6R polymorphism in relation to atrial fibrillation: the National Heart, Lung, and Blood Institute’s Candidate Gene Association Resource (CARE) project. Circ Cardiovasc Genet 4: 557–564.