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APPROVAL SHEET

Are Novel Biomarkers of Metabolomics and Oxidative Stress Associated with Racial Differences in Heart Failure Outcomes?

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ABSTRACT COVER PAGE

Are Novel Biomarkers of Metabolomics and Oxidative Stress Associated with Racial Differences in Heart Failure Outcomes?

by

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An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Clinical Research

2016

ABSTRACT

Background: Prior studies have shown clinical outcomes for heart failure with reduced ejection fraction (HFrEF) are worse for Black Americans, even after adjusting for confounders. We sought to determine if unique small molecule metabolites contribute to racial differences in HF outcomes.

Methods: We performed a metabolome wide association study to identify metabolites differentially expressed between 225 Black and White patients (46.5% Black) with HFrEF enrolled in the Atlanta Cardiomyopathy Consortium. Kaplan-Meier analysis and Cox proportional hazards regression were used to estimate the association of race and small molecule metabolites with a composite primary endpoint of death and HF hospitalization.

Results: Compared to Whites, Blacks were younger, and were more likely to have nonischemic HF etiology, hypertension, and chronic kidney disease. During the study period, (median follow-up 1114 days, IQR 710 – 1422 days), the composite primary endpoint occurred in 176 (78.2%) patients, including 34 (15.1%) deaths and 174 (77.3%) hospitalizations. After adjustment for covariates, Black race was associated with a higher risk for the primary endpoint (HR 1.59, 95% CI 1.03 – 2.46; P=0.03). At false discovery rate=0.2, 86 metabolites were identified to be differentially expressed between Blacks and Whites after adjustment for the covariates. The highest risk for the primary endpoint was in Blacks in the highest salsolinol quartile, while the lowest risk for the primary endpoint was in Whites in the lowest salsolinol quartile (P=0.06 for race*salsolinol interaction). In race stratified Cox models, elevated salsolinol levels were associated with increased risk for the primary endpoint in Whites (quartile 4 vs. 1: adjusted HR 3.07, 95% CI 1.18 – 7.96; P=0.02) and Blacks (quartile 4 vs. 1: adjusted HR 2.24, 95% CI 0.93 – 5.40; P=0.07).

Conclusion: In a cohort of patients with HFrEF, we have confirmed 86 metabolites differentially expressed between Blacks and Whites. Moreover, the metabolite salsolinol was associated with a higher risk for death and HF hospitalizations. Further investigations are warranted to confirm these findings in larger cohorts.

Keywords: race/ethnicity, racial disparities, heart failure, metabolomics, oxidative stress

COVER PAGE

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INTRODUCTION

Heart failure (HF) with reduced ejection fraction (HFrEF) affects over 2.5 million Americans, with >50% mortality within 5 years of diagnosis.(1) Important racial disparities in the epidemiology of HFrEF have been identified, with Black patients having a higher incidence of HF, as well as higher rates of hospitalization and death compared to other racial groups.(1, 2) Although differences in access to care and traditional risk factors for cardiovascular (CV) disease may impact racial disparities, data also suggest that the pathophysiology of HF may be different in Blacks than Whites. Small studies have shown increased oxidative stress (OS) and lower nitric oxide (NO) bioavailability in Blacks, pathways which are both involved in the pathogenesis of vascular and myocardial dysfunction. Increased OS disrupts NO signaling, inducing endothelial dysfunction and increased vascular stiffness that augments the workload for the failing left ventricle (LV).(3) Prior data from our laboratory in non-HF patients demonstrate greater OS, lower NO bioavailability, and impaired vascular function in Blacks as compared to Whites.(4-6) Although unfavorable balance of OS and NO, and impaired vascular function may contribute to the excess HFrEF observed in Blacks, this has not been proven in clinical studies. Currently, there are limited data examining biomarkers of OS in relation to myocardial function, or clinical events such as death or hospitalization in HFrEF patients.

Metabolomic analysis is an emerging field with the potential for discovery of novel pathways associated with disease. An assay of >20,000 detectable metabolites in human plasma provides a comprehensive view of human physiology and metabolism.(7) Utilizing high-resolution metabolomics assays, in addition to traditional biomarkers, is a

novel, hypothesis-generating approach that can be used to discover new pathways that distinguish more severe HF phenotypes, including pathways that may be uniquely altered in Blacks. Prior data in limited cohorts has identified upregulation of metabolites associated with pathways of OS.(8, 9) However, to date, there are limited reports of metabolites associated with racial differences in HF severity.

Utilizing a retrospective cohort study design, the purpose of this analysis was to 1) examine racial differences in clinical HF outcomes including death and hospitalization, 2) to identify novel metabolites that are differentially expressed between Blacks and Whites, and can be used to further characterize racial differences in HF phenotypes through unique metabolic pathways.

BACKGROUND

It is estimated that 5.8 million Americans have HF, and roughly half have HF with reduced ejection fraction (HFrEF).(1) However, important racial disparities exist in HF, with Blacks having the highest risk for HF compared to other race/ethnic groups. Black patients are more likely to develop HF at younger age (10), have a greater prevalence of nonischemic HF (11, 12), and experience higher rates of hospitalization and a higher risk of death.(2, 10, 13-15) It must be considered whether these worrisome trends are the result of important differences in the underlying pathophysiology of HF that may be different in Blacks as compared to Whites.

Epidemiologic data document a possible genetic predisposition for dilated cardiomyopathy in Blacks(16), as well as higher rates of traditional cardiovascular (CV) risk factors, including hypertension, obesity and diabetes. Moreover, Blacks are more likely to be of lower socioeconomic status, which has been associated with higher risk for adverse CV outcomes.(17-19) However, the morbidity and mortality from HF in Blacks exceeds what would be expected solely based on differences in traditional CV risk factor burden and SES.(15) In order to improve prognosis and racial disparities in HF related clinical outcomes, future investigations must be targeted towards elucidating alternate pathophysiologic pathways in order to clarify racial disparities in HF etiology as well as differences in response to pharmacologic therapy for HF**.**

The balance between oxidative stress (OS) and nitric oxide (NO) plays a central role in the regulation of ventricular function and vascular tone, which are major determinants of hemodynamic status in HF. NO is a potent vasodilator, and the

vasodilatory effects of NO critically depend on the equilibrium between NO and OS. A central pathophysiological effect of OS is the disruption of NO signaling, which promotes vascular inflammation, endothelial dysfunction, and a higher afterload for the failing LV.(20) OS can also directly impair LV contractile function through modification of proteins central to excitation-contraction coupling.(21) Thus, the increases in OS associated with neurohormonal activation in worsening HF unfavorably shifts the balance away from the beneficial vascular effects of NO (22), adding to the vasoconstriction and depressed myocardial function that are characteristic of worsening HF.

Racial differences in systemic OS may be a key factor underlying racial disparities in HF. Basic science and translational studies suggest that the endothelial cells of Blacks generate more OS leading to enhanced NO inactivation.(5) Our prior studies confirm higher levels of OS in Blacks compared to Whites, even after adjusting for traditional CVD risk factors and inflammation.(4) Currently there is little clinical data to show that racial differences in OS specifically affect HF outcomes. However, the African American HF trial (A-HeFT) also suggests that imbalance of OS and NO impacts treatment response in Blacks with HF. This randomized controlled trial showed a significant benefit in mortality and HF hospitalizations in Blacks treated with fixed dose hydralazine plus isosorbide dinitrate compared to standard HF therapy.(23) It has been postulated that the improvement in outcomes in Blacks treated with this regimen is due to the biologic underpinnings of this drug combination; isosorbide dinitrate is an organic nitrate that stimulates NO signaling and improves NO bioavailability, and hydralazine is a vasodilator and antioxidant that inhibits the enzymatic formation of reactive oxygen species and ameliorates excess OS.

Since the metabolome represents the final downstream products of genetics, epigenetics, proteomics, and environmental influences affecting disease outcomes (7), metabolomic analysis could provide a complete profile of the small-molecule metabolites that characterize worsening HF. Moreover, metabolomics analysis has the potential to elucidate novel molecular pathways involved in the pathogenesis of the phenotype, as well as the identification of novel diagnostic markers and therapeutic targets. Recent studies have identified metabolites related to pathways of OS, that are associated with clinical outcomes in patients with HF. Levels of trimethylamine-N-oxide (TMAO), an intestinal microbiota-dependent metabolite formed from dietary trimethylamine-containing nutrients, was associated with higher mortality in a cohort of stable HF patients.(24)

To date, there are few studies examining whether biomarkers of OS and metabolomics profiles are associated with racial differences in HF outcomes. This gap in the medical literature forms the rationale for my thesis proposal.

METHODS

Research goals. The purpose of this analysis was to 1) examine racial differences in clinical HF outcomes including death and hospitalization, and 2) identify novel metabolites associated with pathways of OS that can be used to further characterize racial differences in HF phenotypes through unique metabolic pathways. For these study aims, we hypothesized that 1) Black patients will have higher rates of death and HF hospitalization, even after adjustment for demographic, socioeconomic and clinical variables, and 2) Black patients will have unique metabolomic and oxidative profiles that are associated with higher rates of death and HF hospitalization.

Study population. We utilized data from the Atlanta Cardiomopathy Consortium (TACC), which was a prospective cohort study that enrolled outpatients with HF from 3 Emory University-affiliated hospitals in the greater metropolitan Atlanta area from 2007-2011. Participants were recruited according to previously published methods.(25) Inclusion criteria included age older than 18 years, ability to understand and sign written informed consent and participate, and a diagnosis of HF with either reduced or preserved ejection fraction (EF). Exclusion criteria included congenital heart disease, previous heart or other solid organ transplant or awaiting transplant, known cardiac infiltrative disease (eg, amyloidosis), end-stage HF requiring outpatient continuous inotrope infusion, or the presence of any medical condition other than HF that are likely to alter the participant's status over the 6 months after enrollment. After informed consent and enrollment, interviews and medical records were used to collect pertinent data, including

demographics, medical history, medication list, laboratory results, and medical procedure results. In addition, biospecimens were obtained and stored in the TACC Biobank.

Study design. For the purpose of my thesis, we utilized a retrospective cohort design to examine data only for TACC participants who had heart failure with reduced ejection fraction (HFrEF), defined by EF $\leq$ 40% by echocardiogram at the time of enrollment.

Study covariates. Information on demographic, socioeconomic, and clinical covariates were collected at the baseline TACC study visit. The primary exposure of interest was defined as self-reported Black or White race. Covariates of interest included the following variables: age, gender, HF etiology (ischemic vs. non-ischemic), history of hypertension, history of diabetes mellitus (DM), history of chronic kidney disease (CKD), history of hyperlipidemia, level of education, marital status, insurance status, living alone, presence of device therapy, New York Heart Association (NYHA) symptoms, blood pressure (BP), body mass index (BMI), serum creatinine, left ventricular end-diastolic diameter (LVIDd), and B-type natriuretic peptide (BNP).

The proportion of missing data was examined for each covariate. There was no missing data for age or gender. Missing data was present for the following variables, expressed as N(%): HF etiology 2 (0.9), device therapy 2 (0.9), marital status 3 (1.3), history of CKD 4 (1.8), history of hypertension 5 (2.2), level of education 5 (2.2), history of hyperlipidemia 5 (2.2), history of DM 6 (2.7), BP 2 (0.9), NYHA symptoms 6 (2.7), BMI 6 (2.7), serum creatinine 10 (4.4), LVIDd 44 (19.6), and BNP 45 (20.0). For continuous variables, imputation to the mean was used to replace missing data. For categorical variables, use of an indicator variable was employed to allow these participants to contribute their available risk factors to the multivariable models.

Study outcomes. Data on clinical outcomes including death, hospitalizations (all-cause and HF specific), emergency department (ED) visits, and HF clinic visits were prospectively collected at 6-month intervals and adjudicated by an independent review committee. Mortality data were collected through medical record review, information from family members, and Social Security Death Index query. Data on hospitalizations, ED and HF clinic visits were obtained from electronic health records review, outpatient notes from any specialty encounter for any admission to an outside hospital, and direct patient inquiry during follow-up.

The primary endpoint for this analysis was defined as the composite of time to first HF hospitalization and/or death. Secondary endpoints included the individual endpoints of death, HF hospitalization, ED visits, and HF clinic visits. Censoring occurred at the time of loss to follow-up, receipt of advanced HF therapies (i.e. left ventricular assist device or heart transplant), or last date of follow-up on April 9, 2012.

High-resolution metabolomic profiling. Plasma specimens were collected at the baseline TACC study visit, processed according to standard methodology outlined in the TACC Manual of Operating Procedures, and stored on a designated rack and shelf at -80°C. Samples were extracted and analyzed as previously described.(26, 27) Briefly, extractions were performed with acetonitrile containing a mixture of internal standards and maintained in an autosampler maintained at 4°C until injection. Samples were analyzed in triplicate by liquid chromatography–Fourier transform mass spectrometry (Accela-LTQ Velos Orbitrap; *m/z* range from 85 to 850) with 10 uL injection volume using a dual chromatography setup (anion exchange and C18) and a formic acid/acetonitrile gradient. Electrospray ionization was used in the positive ion mode. Data

were extracted using apLCMS (28) with modifications by xMSanalyzer as *m/z* features (29), where an *m/z* feature is defined by *m/z* (mass-to-charge ratio), RT (retention time) and ion intensity (integrated ion intensity for the chromatographic peak). Identities of many of the *m/z* features are known from previous research using ion dissociation patterns by tandem mass spectrometry (MS/MS), coelution with authentic standards and cross-platform validation. Possible identities of other *m/z* features were obtained using the Metlin Mass Spectrometry Database.(30) Where feasible, metabolite identities were confirmed via MS/MS and matching fragmentation patterns to those of known standards.

Statistical analysis. Data are presented as mean ± standard deviation (SD), median (interquartile range [IQR]), or N (%) of patients. Baseline characteristics were compared between patients according to race using the Student t-test for normally distributed continuous variables, the Wilcoxon rank-sum test for non-normally distributed continuous variables, and the χ^2 test for categorical variables. Kaplan-Meier analysis with Cox proportional hazards regression was used to estimate the association of race with the primary endpoint. The proportional hazards assumption was tested and verified for all risk factors using Schoenfeld residual correlation analysis. All variables in Table 1 were considered for inclusion in the multivariable models. Variables that differed by racial group, and/or that were associated with the primary endpoint (based on our data or well known in the literature) were included in the multivariable models. LVIDd and BNP were not considered for inclusion in the multivariable models due to the amount of missing data. Multivariable adjustments were made for the following risk factors in Model 1: age, gender, HF etiology (ischemic vs. non-ischemic), history of HTN, history of CKD, history of hyperlipidemia, BP, BMI, and serum creatinine. Additional multivariable

adjustments were made for the following risk factors in Model 2: Model 1 + history of DM, level of education, marital status, NYHA symptoms, and presence of device therapy. Data were analyzed with the use of SAS statistical software version 9.4 (SAS Institute Inc, Cary, NC).

Bioinformatics analyses. Feature and sample filtering retained m/z features that had a median coefficient of variation (CV) <50%, a Pearson correlation >0.7 among technical replicates, and <30% missing values. We identified 7,208 m/z features with mean CV 14.2%. Multiple linear regression was performed to adjust for the covariates identified in Model 1. The Benjamini and Hochberg false discovery rate (FDR) method was used to correct for multiple comparisons.(31) Because this study was developed to discover potentially important associations with race, we used $q = 0.2$ (where the q value is the FDR adjusted p value) as a reference cut-off to minimize type 2 statistical errors (i.e., failure to reject a false null hypothesis). At $q = 0.2$, 80 % of values are expected to be correct and 20 % are expected to be false discovery. Features were annotated by searching Metlin with m/z tolerance of 10 ppm. Correlation analysis was performed using Pearson's correlation method. Hierarchical clustering was performed using the built-in hclust() function in R that uses the complete-linkage method for clustering. Data were analyzed with the use of R statistical software. Metabolites differentially expressed between Blacks and Whites were added to the risk factors in Model 1 using Cox proportional hazards regression.

RESULTS

Study population. During the study period, 333 participants enrolled in the TACC trial. Of these, 108 had HF with preserved ejection fraction, so were excluded from the current analysis. The remaining 225 participants formed the study cohort.

Baseline characteristics. The characteristics at enrollment of the 225 participants who formed our analytic cohort are shown in **Table 1**. Compared to White patients, Blacks were younger and less likely to be married. Blacks were more likely to have a history of nonischemic HF etiology, hypertension, and CKD, and less likely to have a history of dyslipidemia. Blacks had higher BP, and higher serum values for creatinine and BNP. There were no differences in optimal HF medical therapy, except for higher use of hydralazine and nitrates in Blacks which is expected based on current guidelines.(32) There was a trend towards a lower proportion of Blacks having device therapy.

Clinical factors associated with the primary endpoint. During the study period, (median follow-up 1114 days, IQR 710 – 1422 days), the composite primary endpoint occurred in 176 (78.2%) patients, including 34 (15.1%) deaths and 174 (77.3%) hospitalizations. Median time to the primary endpoint was 392 days, IQR 316 – 504 days. The frequency of clinical outcomes is shown in **Table 2**. Blacks had a higher frequency of hospitalizations, HF clinic visits, and emergency department visits.

Figure 1 shows the Kaplan-Meier analysis of the primary endpoint stratified by race. Black race was associated with an increased risk for the primary endpoint (HR 1.76, 95% CI 1.31 – 2.38; P=0.0002). Other factors univariately associated with the primary endpoint included history of hypertension (HR 1.52, 95% CI 1.09 – 2.11; P=0.01),

history of DM (HR 1.43, 95% CI 1.05 – 1.95; P=0.02), history of CKD (HR 1.90, 95% CI 1.39 – 2.61; P<0.0001), college education (reference = high school, HR 0.72, 95% CI 0.52 – 0.99; P=0.04), creatinine (per 1 mg/dL increase, HR 1.45, 95% CI 1.18 – 1.78; P=0.0003), NYHA class (per 1-unit increase, HR 1.44, 95% CI 1.04 – 1.99; P=0.03), BNP (per 1-SD increase, HR 1.20, 95% CI 1.03 – 1.39; P=0.02), and LVIDd (per 1-cm increase, HR 1.02, 95% CI 1.01 – 1.04; P=0.01). After adjustment for the risk factors in Models 1 and 2, Black race remained independently associated with a higher risk for the primary endpoint (HR 1.59, 95% CI 1.03 – 2.46; P=0.03).

Racial differences in metabolites. Raw *p* values were used in a Manhattan plot (−log2 *p* vs metabolic feature) to visualize the calculated significance for individual metabolite correlations with the FDR 0.05 and 0.2 thresholds identified as horizontal lines (**Figure 2**). At FDR 0.05, 38 metabolites were identified to be differentially expressed between Blacks and Whites after adjustment for the covariates in Model 1, and 86 metabolites were identified to be differentially expressed at FDR 0.2. Two way hierarchical clustering analysis revealed the 86 metabolites grouped into 33 clusters for Black/White samples (**Figure 3**). **Table 3** shows the metabolites identified that are higher in Blacks compared to White, their known functions, as well as the associated biologic pathways.

Metabolites associated with the primary endpoint. Cox modeling was used to examine the association of each metabolite shown in **Table 4** with the primary endpoint. Although levels of betaine/TMAO were higher in Whites, there was no association of betaine/TMAO with the primary endpoint in our cohort. **Figure 3** represents the Kaplan-Meier analysis of the primary endpoint stratified by race and quartiles of salsolinol, showing the highest risk for the primary endpoint in Blacks in the highest salsolinol

quartile, while the lowest risk for the primary endpoint was in Whites in the lowest salsolinol quartile (P=0.06 for race*salsolinol interaction). In race stratified Cox models adjusted for the covariates in Model 1, elevated salsolinol levels were associated with increased risk for the primary endpoint in Whites (quartile 4 vs. 1: HR 3.07, 95% CI 1.18 – 7.96; P=0.02) and Blacks (quartile 4 vs. 1: HR 2.24, 95% CI 0.93 – 5.40; P=0.07).

DISCUSSION

In a well phenotyped cohort of patients with HFrEF, we have confirmed higher risk for death and HF hospitalizations among Black subjects even after adjustment for multiple demographic, socioeconomic and clinical variables. Moreover, we have identified a number of small molecule metabolites which were differentially expressed between Blacks and Whites with HFrEF. Finally, salsolinol, a metabolite related to pathways of OS, was associated with a higher risk for death and HF hospitalizations in both Blacks and Whites in this cohort. These findings are novel because they represent the first comparison of small molecule metabolites between Blacks and Whites with HF, in addition to our confirmation of a novel molecule that increases the risk for adverse clinical events.

Multiple prior studies have documented a higher risk for death and HF hospitalizations in Black patients with HF compared to other racial groups. In these studies, the authors hypothesized that lower socioeconomic status (18, 33), limited access to care (33, 34), poorer social support and self-care practices (35, 36), and/or decreased use of HF medical therapy(37) may have contributed to the increased risk for adverse events observed in Black patients. Our study is unique because our population was well educated, had access to and utilized outpatient specialty HF care, and was on optimal HF medical therapy, and yet we were still able to confirm racial differences in the risk for adverse clinical events. For example, there were no differences between Blacks and Whites in our population with respect to level of education or insurance. Although Blacks in our cohort were less likely to be married, there was no difference between races in the

likelihood of living alone, suggesting that Black patients had intact social support despite differences in marital status. The patterns of health care resource utilization also suggest differences. Prior studies have documented increased use of emergency services, and lower use of outpatient services in Blacks with HF.(35) Although Blacks in our cohort had more frequent ED visits, they also had more frequent HF clinic visits, suggesting that the increased risk for the primary endpoint in Blacks was not driven by a lack of access to or compliance with primary or specialty outpatient medical care. Although we do not have data on compliance with medical therapy, we did not see significant differences in the prescription of optimal medical therapy for HF in our cohort.

Prior cohort studies have documented metabolites associated with incident HF as well as clinical HF outcomes. Investigators from the Atherosclerosis Risk in Communities (ARIC) study identified two metabolites, dihydroxy docosatrienoic acid and hydroxyleucine/hydroxyisoleucine, associated with incident HF in Blacks, independent of traditional CV risk factors and renal function.(9) However, the authors did not examine metabolite profiles in White subjects in the ARIC cohort as a comparator group, and the analysis did not differentiate metabolites in patients with HFrEF versus patients with HF and preserved EF. Tang et al. examined the relationship between fasting plasma TMAO and all-cause mortality in 720 patients with stable HF.(24) Over a 5-year follow-up period, higher plasma TMAO levels were associated with a 3.4-fold increased risk for mortality. Compared to this cohort, our population had less ischemic HF etiology; Tang et al. did not report the racial distribution of patients in their cohort, so we are unable to compare the racial distribution of our population to theirs.

The findings from our metabolome wide association study are the first report of racial differences in small molecule metabolites. We did confirm higher levels of betaine/TMAO in Whites in our population, presumably related to the higher proportion of Whites who had an ischemic HF etiology. However, betaine/TMAO was not related to clinical HF outcomes in our cohort. We did find higher levels of metabolites including L-carnitine, hypoxanthine, serotonin, and serine in Blacks in our cohort. As demonstrated in Table 4, many of the metabolites that are upregulated in Blacks appeared to be related to pathways associated with vasoconstriction, cardiac fibrosis, and left ventricular hypertrophy. This would lend credence to the hypothesis that the higher incidence of nonischemic HF and hypertensive heart disease in Black patients is related to a vascular diathesis in Blacks.(38)

We did confirm an association of the metabolite salsolinol with an increased risk of death and HF hospitalizations in Blacks and Whites in our cohort. Salsolinol is an endogenously synthesized catechol isoquinoline that has been detected in the brain tissue of rats and humans. Salsolinol can be synthesized from dopamine, and has been detected in many areas of the brain that are rich in dopaminergic neurons.(39) Much of the data on salsolinol is related to its possible role in the pathogenesis of Parkinson's disease. However, there is evidence for the role of dopamine in the periphery, and peripheral conversion of dopamine to salsolinol may influence its effects on cardiac myocytes. Animal studies have confirmed that salsolinol produces a dose-dependent inotropic effect on cardiac tissue; lower concentrations of salsolinol between 10^{-7} and 10^{-4} M caused a slight increase in the inotropic effect of rat left atria, but a negative inotropic effect was observed at higher concentrations of 3×10^{-4}–3×10^{-3} M.(40) There are no reports of

salsolinol as a biomarker associated with incident CVD or HF. Our findings need to be confirmed in larger datasets to determine if salsolinol is just a marker of activation of the sympathetic nervous system in worsening HF, or whether it acts along the causal pathway in HF pathogenesis.

Our study has several important limitations. Although we were able to adjust for a number of socioeconomic variables, we lacked information on other factors such as household income and neighborhood that have been shown to affect HF outcomes.(17, 41) Our metabolomic analysis is also limited by small sample size, and so these data will need to be validated in a larger cohort. Similarly, our chosen FDR of 0.2 means that 20% of our findings may be false positive associations. However, the raw p-value of 0.0000055 for salsolinol in Whites certainly suggests that this association is likely not due solely to chance, and deserves to be confirmed in follow-up studies.

In conclusion, in a cohort of Black and White patients with HFrEF, we have confirmed a higher risk of death and HF hospitalization in Black patients even after adjustment for a number of demographic, socioeconomic and clinical variables. Our analysis has also confirmed a number of small molecule metabolites that are differentially expressed between Blacks and Whites, even after adjustment for covariates. It is plausible that some of these metabolites may be related to the pathogenesis of HFrEF, or could serve as biomarkers that could be used for risk stratification. Further investigations are warranted to confirm whether racial differences in these and other metabolites influence racial disparities in HF outcomes.

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TABLES AND FIGURES

	Black N=114	White N=111	P-value
Age, years	53.6±11.2	59.3±12.2	0.0004
Female	41 (36.0)	33 (29.7)	0.3
Education •High school •College •Graduate school	 50 (44.3) 50 (44.3) 13 (11.4)	 37 (34.6) 50 (46.7) 20 (18.7)	0.2
Marital status •Never married •Married •Divorced/Separated •Widowed	 28 (24.8) 48 (42.5) 27 (23.9) 10 (8.9)	 11 (10.1) 66 (60.6) 26 (23.8) 6 (5.5)	0.003
Insurance	102 (91.9)	98 (92.5)	0.9
Lives alone	21 (18.9)	25 (23.6)	0.4
Ischemic HF etiology	38 (33.6)	55 (50.0)	0.01
Prior CABG	16 (14.3)	27 (25.0)	0.04
Hypertension	87 (78.4)	62 (56.9)	0.0006
Diabetes Mellitus	36 (31.9)	35 (33.0)	0.9
Atrial fibrillation	32 (28.1)	39 (35.1)	0.3
Dyslipidemia	51 (46.0)	65 (59.6)	0.04
Chronic kidney disease	49 (44.1)	28 (25.5)	0.004
Current smoker	13 (11.7)	15 (14.2)	0.6
Ejection fraction, %	21.0±7.8	22.6±8.6	0.1
LVIDd*, cm	6.3±1.2	6.1±0.8	0.3
NYHA class	2.2±0.6	2.2±0.6	0.6
Systolic BP, mm Hg	114.6±21.7	109.5±15.8	0.04
Diastolic BP, mm Hg	74.6±12.9	71.4±10.3	0.04
BMI, kg/m^2	32.1±8.0	30.4±6.2	0.07
Creatinine (mg/dL)	1.4±0.7	1.2±0.4	0.02
BNP* (pg/mL)	535 (116.0-950.0)	202.0 (78.0-570.0)	0.003
Medications •ACEi •ARB •Beta-blocker •MCA •Hydralazine	 95 (83.3) 28 (24.6) 109 (95.6) 58 (50.9) 42 (36.8)	 88 (80.0) 27 (24.6) 106 (96.4) 50 (45.5) 9 (8.2)	 0.5 0.9 0.8 0.4 <0.0001

•Nitrates •Diuretics	37 (32.5) 101 (88.6)	23 (20.9) 91 (82.7)	0.05 0.2
ICD/CRT-D	77 (68.1)	87 (79.1)	0.06

Table 1. Baseline characteristics of the 225 HFrEF patients in the TACC cohort. Data are mean ± standard deviation, median (interquartile range), or N (%). ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, b-type natriuretic peptide; CABG, coronary artery bypass grafting; CRT-D, cardiac resynchronization therapy-defibrillator; ICD, implantable cardioverter defibrillator; MCA, mineralocorticoid receptor antagonist. LVIDd, left ventricular end-diastolic diameter; NYHA, New York Heart Association.

*Data are missing for >10%.

	Black N=114	White N=111	P-value
Death	19 (16.7)	15 (13.5)	0.5
HF Hospitalizations			
•Total	95 (83.3)	79 (71.2)	0.03
•Per patient	1.6±2.7	0.9±1.8	0.04
HF clinic visits (per patient)	0.5±1.2	0.2±0.5	0.01
Emergency Department visits (per patient)	2.2±3.7	0.9±1.4	0.0003

Table 2. Frequency of clinical events among the 225 HFrEF patients in the TACC cohort. Data are mean ± standard deviation, or N (%)

Metabolite	Function	Pathway	P-value
Upregulated in Blacks			
L-Carnitine	AA derivative, constituent of striated muscle and liver → transports long-chain fatty acids into the mitochondrial matrix → anti-apoptotic activity, prevents doxorubucin induced apoptosis of cardiac myocytes	Saturated fatty acids beta-oxidation; Lysine metabolism; Carnitine shuttle; Fatty Acid Metabolism	0.0009
Hypoxanthine	An intermediate product of uric acid synthesis	Purine metabolism	0.0008
Indole-3-acetaldehyde	Intermediate in degradation of tryptophan to serotonin → Left ventricular hypertrophy, cardiac fibrosis, vasoconstriction to stop bleeding	Tryptophan metabolism	0.0079
N-Hydroxy-1-aminonaphthalene	Drug metabolism, typically via cytochrome P450 pathway	Xenobiotics metabolism	0.0079
2-Aminoacrylate (Dehydroalanine)	Alpha AA derived from post-transcriptional modification of serine and cysteine → Compensation for MetS → found in food proteins, alkylates lysine to form lysinoalanine which is thought to cause renal failure in rats	Glycine, serine, alanine and threonine metabolism; Tyrosine metabolism; Methionine and cysteine metabolism	0.0075
Serotonin	Monoamine neurotransmitter derived from tryptophan → growth promoting effect on cardiac myocytes and stored in platelets → Left ventricular hypertrophy, cardiac fibrosis, vasoconstriction to stop bleeding, pulmonary hypertension	Tryptophan metabolism	0.0036
Serine	Alpha AA that participates in biosynthesis of purines and pyrimidines → Compensation for MetS → serine is increased in expression in myocytes of spontaneously hypertensive HF rats	Glycosphingolipid metabolism; Vitamin B9 (folate) metabolism; Sialic acid metabolism; Glycine, serine, alanine and threonine metabolism; Methionine and cysteine metabolism; Glycerophospholipid metabolism; Selenoamino acid metabolism	0.0151

Upregulated inWhites			
Noradrenochrome	Oxidation product (free radical) derived from norepinephrine → contribute to redox cycling, toxicity, and apoptosis, as well as endothelial damage	Tyrosine metabolism	0.0000089
Salsolinol	Metabolite of ethanol produced by the condensation of dopamine with acetaldehyde → potential neurotoxin that increases production of ROS and decreased glutathione levels, possibly related to alcohol consumption, negative inotropic effect on rat left atria	Tyrosine metabolism	0.0000055
3-Methoxytyramine	Metabolite of dopamine → regulates neurotransmission of norepinephrine and serotonin → upregulation of sympathetic nervous system	Tyrosine metabolism	0.0000043
Tetradecanoyl carnitine	Involved in β-oxidation of long-chain fatty acids, comes from dietary sources including red meat → accumulation of acyl-carnitines indicates deregulated β-oxidation and mitochondrial dysfunction	Carnitine shuttle	0.0054
L-Alanine	Non-essential AA involved in sugar metabolism, provides energy for muscle tissue → alanine levels are higher ventricles of CAD hearts compared to aortic valve disease	Alanine and Aspartate Metabolism; Glycine, serine, alanine and threonine metabolism; Glutathione Metabolism; Tryptophan metabolism;	0.0053
β-Alanine	Rate-limiting precursor for synthesis of the dipeptide carnosine which is produced within and stored in high concentrations in cardiac muscle → Compensation for MetS → carnosine is cardioprotective from ischaemia-reperfusion damage, and doxorubicin-induced cardiomyopathy, procontractile	Pyrimidine metabolism; Beta-Alanine metabolism; Alanine and Aspartate Metabolism; Histidine metabolism	0.0053
Pantothenate	Protects cells against peroxidative damage by increasing the level of glutathione	CoA Catabolism; Vitamin B5 - CoA biosynthesis from	0.0028

		pantothenate	
Betaine; Trimethylaminoacetate (Glycine betaine; N,N,N-Trimethylglycine; Trimethylammonioacetate)	Betaine participates as a methyl donor in the methionine cycle in the liver → alteration in liver metabolism contributes to plasma dyslipidemia and CAD	Glycine, serine, alanine and threonine metabolism	0.0018
Kynurenate	endogenous antagonist of the glutamate ionotropic excitatory amino acid receptors (NMDA, etc.), Neuroprotective and anticonvulsive activities demonstrated in animal models of neurodegenerative diseases	Tryptophan metabolism	0.0011

Table 3. m/z features differentially expressed between Blacks and Whites in the TACC cohort. AA, amino acid; CAD, coronary artery disease; MetS, metabolic syndrome; NMDA, N-methyl-D-aspartate; ROS, reactive oxygen species.

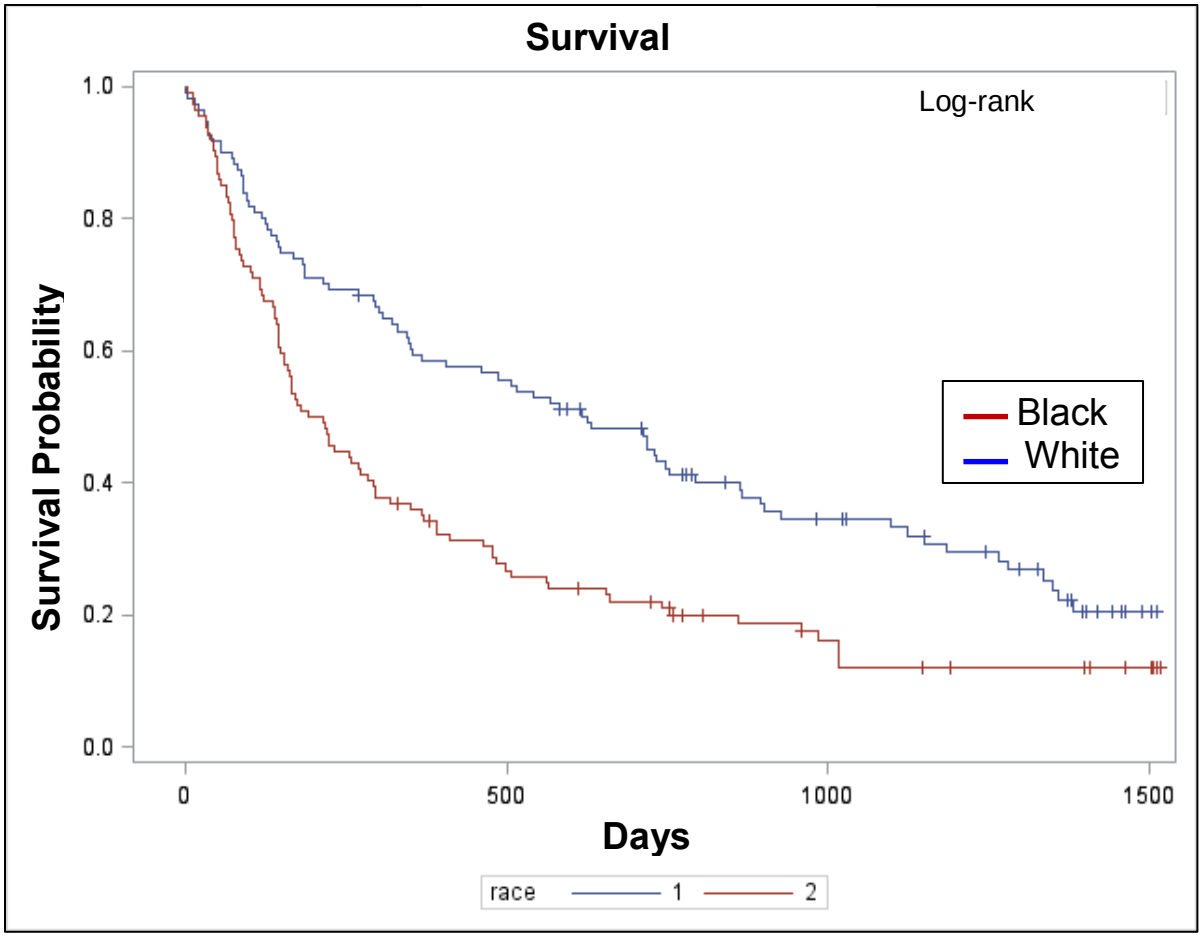

Figure 1. Kaplan-Meier estimates of the composite primary endpoint stratified according to race.

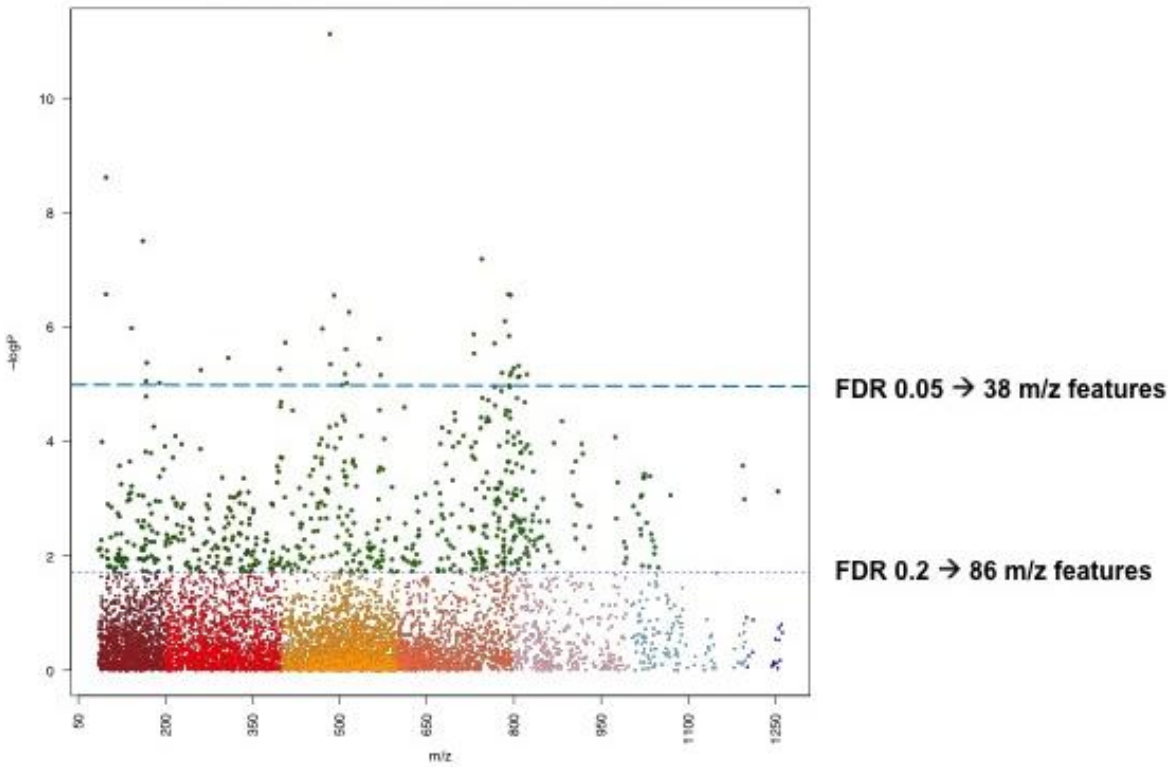

Figure 2. Type 1 Manhattan plot of the m/z features differentially expressed between Blacks and Whites. Data are adjusted for age, gender, heart failure etiology, history of hypertension, diabetes, chronic kidney disease, blood pressure, body mass index, and serum creatinine.

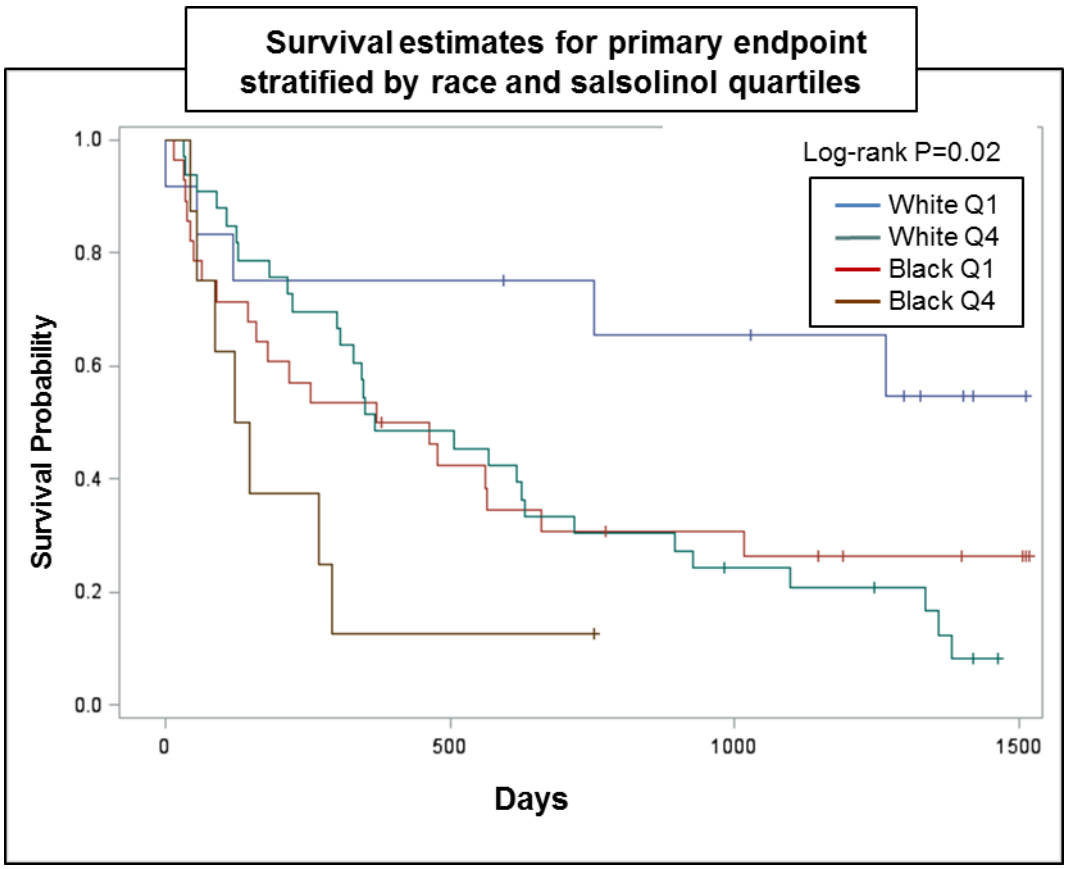

Figure 3. Kaplan-Meier estimates of the composite primary endpoint stratified according to race and quartiles of salsolinol. Q1: lowest quartile of salsolinol, Q4: highest quartile of salsolinol.