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ORIGINAL ARTICLE

Association of Genetic Polymorphisms in the Beta-1 Adrenergic Receptor with Recovery of Left Ventricular Ejection Fraction in Patients with Heart Failure

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Abstract

Two common genetic polymorphisms in the beta-1 adrenergic receptor (*ADRB1* Ser49Gly [rs1801252] and Arg389Gly [rs1801253]) significantly affect receptor function in vitro. The objective of this study was to determine whether *ADRB1* Ser49Gly and Arg389Gly are associated with recovery of left ventricular ejection fraction (LVEF) in patients with heart failure. Patients with heart failure and baseline LVEF ≤ 40% were genotyped ($n = 98$), and retrospective chart review assessed the primary outcome of LVEF recovery to ≥ 40%. Un/adjusted logistic regression models revealed that Ser49Gly, but not Arg389Gly, was significantly associated with LVEF recovery in a dominant genetic model. The adjusted odds ratio for Ser49 was 8.2 (95% CI = 2.1–32.9; $p = 0.003$), and it was the strongest predictor of LVEF recovery among multiple clinical variables. In conclusion, patients with heart failure and reduced ejection fraction that are homozygous for *ADRB1* Ser49 were significantly more likely to experience LVEF recovery than Gly49 carriers.

Keywords Beta-1 adrenergic receptor · Genetics · Polymorphism · Beta-blocker · Heart failure · Left ventricular ejection fraction

Abbreviations

ADRB1	Gene for the beta-1 adrenergic receptor
AHA	American Heart Association
ACC	American College of Cardiology
Arg	Arginine
BMI	Body mass index
CEPH	Centre d'Etude du Polymorphism Humain lymphoblastoid cell lines

Dedication
In sorrow, we would like to dedicate this work to our friend and co-author, Joseph P. Kitzmiller. Dr. Kitzmiller passed away on October 3, 2018, shortly before our submission of this paper.

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CI	Confidence interval
FDA	United States Food and Drug Administration
Gly	Glycine
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
LVEF	Left ventricular ejection fraction
NYHA	New York Heart Association
OR	Odds ratio
SBP	Systolic blood pressure
Ser	Serine

Introduction

Heart failure (HF) is a prevalent health issue that currently affects approximately 6.5 million Americans, with that number expected to grow to over 8 million by 2030 [1]. While developments in medical therapy dramatically reduced heart failure mortality from 1979 to 2000, new evidence indicates that mortality improvements may be leveling off [1]. With one-year mortality still high at 29.6% and five-year mortality close to 50% [1], it is essential that improvements in HF outcomes do not stagnate. Left ventricular ejection fraction (LVEF) is a surrogate measure of clinical outcomes in patients with heart failure with reduced ejection fraction (HFrEF). Improvements in LVEF to near normal levels are associated with dramatically lower mortality rates than either persistent HFrEF or heart failure with preserved ejection fraction (HFpEF) (mortality 25% vs. 45% vs. 47%, respectively) [2]. Unfortunately, the percentage of HFrEF patients who achieve LVEF recovery is estimated to be only ≈ 20% [2, 3]. Increasing the percentage of patients who recover LVEF has potential to dramatically improve HFrEF outcomes, but to achieve that goal, we need a better understanding of the factors that predict LVEF recovery.

LVEF recovery can occur upon the removal of the inciting cardiac insult (e.g., hypertension or infection), but, more often, it occurs as the result of pharmacologic and non-pharmacologic therapies [4]. Effective non-pharmacologic therapy is cardiac resynchronization, and effective pharmacologic therapies are those that block neurohormonal activation (i.e., inhibitors of the sympathetic adrenergic and renin–angiotensin–aldosterone systems) [4]. While many mechanisms contribute to HFrEF, it has long been known that excessive adrenergic stimulation is cardiotoxic [5]. Consistent with those effects, inhibition of the beta-1 adrenergic receptor using beta-blocker therapy is strongly associated with left ventricular reverse remodeling and recovery in HFrEF patients [6, 7]. However, two common, non-synonymous genetic variants in the beta-1 adrenergic receptor (gene: *ADRB1*) have been characterized [8], and they dramatically affect beta-1 adrenergic receptor function in vitro; Ser49Gly (rs1801252) and Arg389Gly (rs1801253). The Gly49 allele increases agonist-promoted down-regulation of the receptor compared to the Ser49 allele [9], and the Gly389 allele decreases both basal- and agonist-promoted beta-1 adrenergic receptor activities compared to the Arg389 allele [10]. Given that both Gly alleles decrease beta-1 adrenergic receptor expression and activity, our hypothesis was that HFrEF patients that are homozygous for Ser49 or Arg389 would benefit most from beta-blockade and, thus, have improved LVEF recovery in response to optimal medical therapy. Therefore, the objective of this study was to determine whether *ADRB1* Ser49Gly and/or Arg389Gly is/are associated with the recovery of LVEF in HFrEF patients treated in a HF specialty clinic.

Methods

Patient Sample

This study was a retrospective chart review and genotyping of a convenience sample of 135 patients from the Heart Failure Clinic at the Ohio State University Wexner Medical Center in Columbus, Ohio, USA. Any patient with symptomatic American Heart Association/American College of Cardiology (AHA/ACC) stage C congestive heart failure [11] and willing to donate a blood sample for DNA analysis was enrolled into the overall study. The goal of the overall study was to investigate the effects of genetic variation on heart failure surrogate outcomes, regardless if the patient had HFrEF or HFpEF. Patients were enrolled from February 1999 to September 1999. Only patients with a documented baseline LVEF ≤ 40%, known *ADRB1* genotypes, and a documented follow-up LVEF at least 90 days since the baseline LVEF were included in this analysis. Previous work by our group identified five clinical variables significantly associated with higher rates of LVEF recovery, which are as follows: female sex, non-ischemic etiology, non-diabetics, increased systolic blood pressure, and decreased QRS duration [12]. Thus, we collected as much of the data as possible for those five variables, plus the patients' baseline age, self-reported race, body mass index (BMI), heart rate, serum creatinine, chronic kidney disease, atrial fibrillation, New York Heart Association (NYHA) functional class, left bundle branch block, and current vital status. If the patient did not have a diagnosis of chronic kidney disease anywhere in their electronic medical record at baseline, then it was recorded as stage 0. LVEFs were collected from echocardiograms performed as part of routine clinical care. The most extreme LVEFs for each patient were collected, i.e., the lowest LVEF in the electronic medical record was used as the baseline LVEF, and, then, the highest LVEF in the electronic medical record at least 90 days after the baseline LVEF was used as the follow-up LVEF. Clinical variables were obtained to the extent available through existing

electronic medical records. The investigators that performed the retrospective chart review were blinded to the patients' *ADRB1* genotypes. Given that the patients were enrolled nearly 20 years ago, outpatient medication records were not available as part of the retrospective chart review. However, the goal of the Heart Failure Clinic is to treat HFrEF patients with the optimal AHA/ACC guideline-recommended medical therapy, including beta-blockers and angiotensin-converting enzyme inhibitors (or angiotensin receptor blockers), titrated to target doses [11]. All patients signed informed consent for the collection of DNA via a blood sample and tabulation of clinical variables from electronic medical records. This study was performed in accordance with the Institutional Review Board of The Ohio State University, which reviewed and approved this protocol.

Genotyping

Whole blood samples were stored at − 80 °C until the DNA was extracted using standard techniques [13]. *ADRB1* Ser49Gly (rs1801252) genotype was determined using TaqMan® Genotyping Assay C___8898508_10 (ThermoFisher Scientific), and *ADRB1* Arg389Gly (rs1801253) genotype was determined using TaqMan® Genotyping Assay C___8898494_10 (ThermoFisher Scientific). Several quality control measures were used to ensure accurate genotyping. Negative and positive controls were run with each 96-well plate. Negative controls used molecular grade water instead of a DNA sample, and the positive controls were DNA samples derived from the Centre d'Etude du Polymorphism Humain (CEPH) lymphoblastoid cell lines from the Coriell Institute for Medical Research (Camden, New Jersey, USA). Two samples of each genotype (A/A, A/G, and G/G for Ser49Gly and C/C, C/G, and G/G for Arg389Gly) were used as positive controls. After initial genotyping, a randomly selected 10% of patient samples were re-genotyped to confirm concordance. Investigators performing the genotyping were blinded to the clinical data collected via retrospective chart review.

Statistical Analysis

Continuous baseline variables were described by the median ± interquartile range, and categorical baseline variables were described by counts and percentages. Baseline variables were described in all patients and stratified by the presence/absence of LVEF recovery, *ADRB1* Ser49Gly genotype, and *ADRB1* Arg389Gly genotype. LVEF recovery, the primary outcome, was defined as a documented follow-up LVEF ≥ 40% at least 90 days since the baseline LVEF. *ADRB1* genotypes were analyzed using the dominant genetic model (i.e., Ser49 homozygotes versus Gly49 carriers and Arg389 homozygotes versus Gly389 carriers). The Mann–Whitney *U* test was used to compare continuous baseline variables between all strata, and the chi-square test (or, when necessary, the Fisher's exact test) was used to compare the categorical baseline variables between all strata. Unadjusted and adjusted logistic regression models were used to test the association of each *ADRB1* genotype with the primary outcome of LVEF recovery. The primary model was adjusted for the five clinical variables previously identified by our group to be significantly associated with LVEF recovery [12]. Variables with > 15% missing values had the missing values imputed as the mean of the available values. Imputation was necessary for the following three clinical covariates: QRS duration, ischemic etiology, and NYHA functional class were missing in 33, 41, and 35% of the sample at baseline (imputed as 117, 0.172, and 2.8, respectively). Ischemic/not ischemic was imputed as a numerical variable in the model. Specifically, for the patients in which non-ischemic and ischemic status could be verified, they were assigned values of 0 and 1, respectively. From those patients with known values, the mean was 0.172 (corresponding with a mean overall frequency of ischemic etiology = 17.2%). Therefore, for the patients with the unverifiable non/ischemic status, they were assigned values of 0.172, which would be numerically similar as the probability of having ischemic etiology in this patient sample. To ensure that imputation did not confound the results, we ran similarly adjusted models omitting the imputed variables as covariates. Our most conservative model included the five previously identified clinical covariates plus the patients' age, self-reported race, and NYHA functional class. Hardy–Weinberg equilibrium was tested using the chi-square test stratified by self-reported race. Statistical significance was defined as $p < 0.05$. Given the analytical sample size, the minor allele frequencies of *ADRB1* Ser49Gly and Arg389Gly, and an a priori expected LVEF recovery rate of 25%, we estimated that we had 80% power to detect an odds ratio = 5 for Ser49Gly and an odds ratio = 4 for Arg389Gly for the primary outcome in univariate analysis. All statistical analyses were performed using SAS version 9.4 (Cary, NC).

Results

Of the 135 patients enrolled, 98 qualified for analysis. The most common reasons that patients were excluded from this analysis were that patients had a baseline LVEF > 40% (n = 23) or patients did not have a follow-up LVEF documented in their electronic medical record at least 90 days since the baseline LVEF (n = 13). Baseline characteristics overall and stratified by LVEF recovery are displayed in Table 1. A total of 36 (37%) patients had LVEF recovery to ≥ 40%. Overall, the patients were primarily self-reported whites (80%; 19% self-reported blacks) and male (71%) with a median and interquartile range BMI of 29 ± 9 kg/m^2 and heart rate of 80 ± 13 bpm.

Table 1 Baseline characteristics in all patients and stratified by LVEF recovery status

	Overall ($n = 98$)	LVEF recovered ($n = 36$) 36.7%	LVEF not recovered ($n = 62$) 63.3%	*p
Age (years)	49.5 ± 17.0	47.0 ± 13.0	50.0 ± 18.0	0.463
Female sex	28 (28.6%)	15 (41.7%)	13 (21.0%)	**0.029**
Self-reported whites	78 (79.6%)	29 (80.6%)	49 (79.0%)	1.000
Self-reported blacks	19 (19.4%)	7 (19.4%)	12 (19.4%)	
Ischemic etiology	10 (17.2%)	3 (13.6%)	7 (19.4%)	0.727
Diabetes	45 (46.4%)	19 (52.8%)	26 (42.6%)	0.333
Atrial fibrillation	35 (35.7%)	15 (41.7%)	20 (32.3%)	0.349
†Stage of chronic kidney disease	1.0 ± 1.5	1.0 ± 1.3	1.0 ± 1.6	0.873
Serum creatinine (mg/dL)	1.1 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	0.435
BMI (kg/m^2)	29.0 ± 9.2	29.7 ± 10.5	28.1 ± 10.3	0.511
SBP (mmHg)	118.0 ± 20.0	120.0 ± 22.0	116.6 ± 19.0	0.084
Heart rate (bpm)	80.0 ± 13.0	80.0 ± 13.3	80.0 ± 15.0	0.944
QRS duration (ms)	110.0 ± 48.0	98.0 ± 26.0	114.0 ± 46.0	0.139
†NYHA functional class	2.8 ± 0.9	2.5 ± 0.9	3.0 ± 0.8	0.063
Left bundle branch block	35 (36.5%)	11 (30.6%)	24 (40.0%)	0.352
Baseline LVEF (%)	20 ± 11	20 ± 9	20 ± 11	0.953
Follow-up LVEF (%)	25 ± 36	55 ± 8	20 ± 12	**< 0.0001**
Change in LVEF (%)	2 ± 34	35 ± 13	− 3 ± 9	**< 0.0001**
Time between baseline and follow-up LVEFs (years)	4.4 ± 7.2	4.1 ± 5.3	4.5 ± 8.0	0.565
ADRB1 Gly49 carriers	29 (29.6%)	4 (11.1%)	25 (40.3%)	**0.002**
ADRB1 Gly389 carriers	46 (48.9%)	21 (60.0%)	25 (42.4%)	0.098
Deaths	53 (54.1%)	20 (55.6%)	33 (53.2%)	0.823

ADRB1, gene for the beta-1 adrenergic receptor; BMI, body mass index; Gly, glycine; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional class; SBP, systolic blood pressure; Ser, serine

*p value for patients with LVEF recovery to ≥ 40% versus patients with follow-up EF < 40%. Bolded p values indicate statistical significance

†Reported as mean ± SD instead of median ± interquartile range because the medians were identical and, thus, did not reveal subtle differences in the values

The median age of the patients was 50 ± 17 years at baseline, with roughly half having diabetes (46%), 36% had atrial fibrillation, 37% had left bundle branch block, and 17% had ischemic etiology for their heart failure. The mean NYHA class was 2.8. The median baseline EF was 20 ± 11%, with the median and interquartile range for the change in LVEF being + 2% ± 34%. The median and interquartile range for the length of time between the baseline and follow-up LVEFs was 4.4 ± 7.2 years, and the median follow-up LVEF value was 25% ± 36%. The only statistically significant differences between the patients that did and did not have LVEF recovery were female sex ($p = 0.029$) and *ADRB1* Ser49Gly genotype ($p = 0.002$). Nearly twice as many women had LVEF recovery compared to men (54% vs. 30%, respectively). Approximately three times as many *ADRB1* Ser49 homozygotes had LVEF recovery compared to *ADRB1* Gly49 carriers (46% vs. 14%, respectively). Numerically more *ADRB1* Gly389 carriers in the group recovered LVEF (61% vs 45%), but the difference was not statistically significant ($p = 0.098$). Consistent with the previous findings by our group [12], systolic blood pressure was numerically higher and QRS duration was numerically lower in patients with LVEF recovery, but those differences were not statistically significant.

Genotyping results of the patient sample for *ADRB1* Ser49Gly (rs1801252) revealed 69 A/A homozygotes, 27 A/G heterozygotes, and 2 G/G homozygotes; hence, 29 (30%) were *ADRB1* Gly49 carriers. The genotyping call rate was 100%, and the genotype distributions were consistent with Hardy–Weinberg equilibrium within the self-reported white and black race groups ($p = 0.998$ and $p = 0.414$, respectively). The frequency of the Gly49 allele in this patient sample was 0.16 for the self-reported whites ($n = 78$) and 0.16 for the self-reported blacks ($n = 19$). These values are similar to previously reported allele frequencies for individuals with European (0.12) and African (0.17) ancestry residing in the United States [14]. Genotyping results of the patient sample for *ADRB1* Arg389Gly (rs1801253) revealed 48 A/A homozygotes, 37 A/G heterozygotes, and 9 G/G homozygotes; hence, $n = 46$ (49%) were *ADRB1* Gly389 carriers. The genotyping call rate for Arg389Gly was 96%, and the genotype

distributions were consistent with Hardy–Weinberg equilibrium within the self-reported white and black race groups ($p = 0.178$ and $p = 0.120$, respectively). The frequency of the Gly389 allele in this patient sample was 0.28 for the self-reported whites ($n = 74$) and 0.37 for the self-reported blacks ($n = 19$). These values are similar to previously reported allele frequencies for individuals with European (0.33) and African (0.37) ancestry residing in the United States [14]. There was 100% concordance between the observed and reported genotypes for the positive controls and the 10% of samples that were randomly re-genotyped for both *ADRB1* polymorphisms. These results support accurate genotyping.

Baseline characteristics overall and stratified by *ADRB1* Ser49Gly genotype are displayed in Supplementary Table 1. The only statistically significant differences between the two genotype groups were the follow-up LVEF (35% in the *ADRB1* Ser49 homozygotes vs. 20% in the *ADRB1* Gly49 carriers; $p = 0.002$), change in LVEF (+ 6% in the *ADRB1* Ser49 homozygotes vs. − 3% in the *ADRB1* Gly49 carriers; $p = 0.004$), and the percentage of patients with LVEF recovery to ≥ 40% (46% in the *ADRB1* Ser49 homozygotes vs. 14% in the *ADRB1* Gly49 carriers; $p = 0.002$). The difference in NYHA class at baseline was nearly significant between the Ser49 homozygotes and Gly49 carriers (2.7 vs. 3.1, respectively; $p = 0.051$). Notably, baseline LVEF, demographics, comorbidities, and vital signs were similar between *ADRB1* Ser49 homozygotes and *ADRB1* Gly49 carriers. Baseline characteristics overall and stratified by *ADRB1* Arg389Gly genotype are displayed in Supplementary Table 2. The only statistically significant difference between *ADRB1* Arg389 homozygotes and *ADRB1* Gly389 carriers was the duration of time between the baseline and follow-up LVEF measurements. The duration of time was significantly longer in the *ADRB1* Arg389 homozygotes than in the *ADRB1* Gly389 carriers (median 6.8 vs. 2.6 years, respectively; $p = 0.002$).

Unadjusted and adjusted logistic regression models for *ADRB1* Ser49Gly are displayed in Table 2. Univariate logistic regression found that patients who were homozygous for Ser49 were more than 5 times more likely to recover their LVEF to ≥ 40% than those who were carriers of Gly49 (odds ratio = 5.4; 95% confidence interval = [1.7–17.2]; $p = 0.004$). Regardless of the number or types of clinical covariates used to adjust the logistic regression models, Ser49Gly was significantly associated with LVEF recovery. The most conservative model was adjusted for the following eight baseline clinical characteristics: patients' age, self-reported race, sex, systolic blood pressure, QRS duration, diabetes, ischemic etiology, and NYHA class. Despite this rigorous covariate adjustment, *ADRB1* Ser49 homozygous genotype was an independent, statistically significant, and strong factor associated with LVEF recovery (adjusted odds ratio = 8.2; CI = [2.1, 32.9]; $p = 0.003$). The results were similar even when only patients with complete data for non/ischemic etiology were included (total $n = 54$). Table 3 shows the results for all variables in the most conservatively adjusted multivariable model. *ADRB1* Ser49 homozygous genotype and female sex remained statistically significant, and Ser49Gly was by far the strongest predictor of LVEF recovery. Supplemental Table 3 shows a similar model, but it only includes the patients that had complete data for non/ischemic etiology.

Unadjusted and adjusted logistic regression models for *ADRB1* Arg389Gly are displayed in Table 4. None of the models for *ADRB1* Arg389Gly were statistically significant for an association with LVEF recovery (all $p > 0.05$). The model only adjusted for patient's age, sex, and race trended toward statistical significance (odds ratio = 0.42; 95% confidence interval = 0.17–1.1; $p = 0.063$). However, the estimated odds ratio for that model and all other models for Arg389Gly were in the opposite direction than what would be expected based on the previous literature [10]. The distributions for the change in LVEF in patients stratified by *ADRB1* Ser49Gly and Arg389Gly genotypes are displayed in Figs. 1 and 2. The distribution for Ser49Gly more clearly shows a shift toward improved LVEF recovery for Ser49 homozygotes than the distribution for Arg389Gly.

Discussion

The aim of the present study was to investigate the association between *ADRB1* Ser49Gly and Arg389Gly with recovery of LVEF in patients with HFrEF treated in a HF specialty clinic. Homozygous *ADRB1* Ser49, but not Arg389, was significantly associated with LVEF recovery. The association of *ADRB1* Ser49Gly with LVEF recovery was independent of rigorous adjustment for multiple clinical variables, including those previously shown to be associated with recovery of ventricular function [12], and it was by far the strongest predictor of LVEF recovery among all variables assessed. Patients homozygous for the major allele Ser49 had significantly higher rates of LVEF recovery than Gly49 carriers. Our findings are consistent with prior in vitro data and studies assessing survival outcomes from beta-blocker treatment in patients with heart failure [15, 16].

Cell line data has shown that beta-1 adrenergic receptors with the Gly49 allele undergo increased down-regulation when exposed to long-term stimulation compared to receptors with Ser49 [9]. This result is supported by another cell line study by Levin et al. [17], which found increased catecholamine-induced desensitization in cells carrying the Gly49 allele. As excessive adrenergic stimulation has been associated with cardiotoxicity [5], down-regulation of the beta-1 adrenergic receptor may be cardioprotective. This supports the hypothesis that Gly49 is a beneficial adaptation in patients with HF, and patients without the Gly49 allele (Ser49 homozygotes) may benefit more from treatment with beta-

Table 2 Unadjusted and adjusted logistic regression models for the association of *ADRB1* Ser49 homozygous status with recovery of LVEF to ≥ 40%

Logistic regression model	*n*	OR (for Ser49 homozygotes)	95% CI	*p
Univariate	98	5.4	1.7–17.2	**0.004**
Adjusted for age, sex, and race	97	6.8	2.0–23.4	**0.002**
Adjusted for sex, SBP, diabetes	89	6.9	1.9–25.0	**0.003**
Adjusted for age, sex, SBP, diabetes, QRS duration[a], and ischemic etiology[a]	89	8.4	2.2–32.2	**0.002**
Adjusted for age, sex, race, SBP, and diabetes	89	6.9	1.9–25.2	**0.004**
Adjusted for age, race, sex, SBP, diabetes, QRS duration[a], NYHA class[a], and ischemic etiology[a]	89	8.2	2.1–32.9	**0.003**
Adjusted for age, race, sex, SBP, diabetes, QRS duration[a], NYHA class[a], and ischemic etiology[b]	54	10.9	1.3–89.5	**0.026**

ADRB1, gene for the beta-1 adrenergic receptor; CI, confidence interval; Gly, glycine; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; OR, odds ratio; SBP, systolic blood pressure; Ser, serine

[a] Mean values were used to impute missing data for $n = 32$ missing QRS duration, $n = 40$ missing ischemic etiology, and $n = 34$ missing NYHA functional class

[b] This model did not include patients that had non/ischemic etiology imputed. It only included patients in which non/ischemic etiology data was available

*p value for *ADRB1* Ser49 homozygotes versus Gly49 carriers within the specified model. Bolded p values indicate statistical significance

blockers; a treatment expected to have been implemented in a HF specialty clinic.

Four previous studies did not find a significant association of *ADRB1* Ser49Gly with ventricular remodeling responses to beta-blockers [18–21]. The major difference between our study and those four previous studies is the duration of follow-up. The median length of time between the baseline and follow-up LVEF measurements in our study was 4.4 years, whereas the longest length of follow-up in any of the previous studies was 1.6 years; with most of the studies having follow-up of several months. Despite using only a 90-day minimum between the baseline and follow-up LVEFs in our study, our results may still represent persistent LVEF recovery because of the long duration of time observed between the extreme LVEF measurements. Specifically, we selected the lowest documented LVEF as the baseline LVEF, and the highest documented LVEF at least 90 days since the baseline LVEF as the follow-up LVEF. By selecting the highest LVEF documented, we collected the maximum observed effect that resulted from a myriad of different therapeutic strategies used to treat HFrEF in a HF specialty clinic. Therefore, the effects of Ser49Gly on LVEF recovery may be long-term effects and have gone undetected in previous studies. This would explain why the results of our study are consistent with the results of survival outcome studies, which also had a few years of follow-up [15, 16].

Table 3 Full logistic regression model including all clinical covariates and *ADRB1* Ser49 homozygous status for the primary outcome of LVEF recovery to ≥ 40%

Variable	OR	95% CI	*p
ADRB1 Ser49 homozygotes	8.17	2.07–32.22	**0.003**
Age	1.00	0.96–1.04	0.991
Female sex	4.07	1.27–13.03	**0.018**
African-American race	1.01	0.29–3.51	0.983
Systolic blood pressure	1.03	0.99–1.06	0.074
Non-diabetic	0.84	0.28–2.51	0.757
[a]QRS duration	0.98	0.96–1.01	0.122
[a]Ischemic etiology	0.93	0.17–5.10	0.933
[a]NYHA functional class	0.69	0.34–1.39	0.296

ADRB1, gene for the beta-1 adrenergic receptor; CI, confidence interval; Gly, glycine; LVEF, left ventricular ejection fraction; OR, odds ratio; SBP, systolic blood pressure; Ser, serine

[a] Mean values were used to impute missing data for $n = 32$ missing QRS duration, $n = 40$ missing ischemic etiology, and $n = 34$ missing NYHA functional class

*p value is for type III analysis of effects; bolded values indicate statistical significance ($p < 0.05$)

Four previous studies found that *ADRB1* Arg389Gly was significantly associated with ventricular remodeling responses to beta-blockers [18, 21–23], but consistent with our study, three previous studies also did not find a significant association of *ADRB1* Arg389Gly [19, 24, 25]. Therefore, the true association of *ADRB1* Arg389Gly with left ventricular remodeling responses to beta-blockers is not clear. All of these studies, including our own, were small (each $n < 500$). Left ventricular remodeling is only a surrogate for clinical outcomes in patients with HFrEF, and, thus, the reasons for the discordant results between these ventricular remodeling studies and clinical outcome studies discussed in subsequent texts are not clear. The effects of these genetic polymorphisms on survival responses to beta-blockers could be through additional mechanisms than just ventricular remodeling. For example, the

Table 4 Unadjusted and adjusted logistic regression models for the association of *ADRB1* Arg389 homozygous status with recovery of LVEF to ≥ 40%

Logistic regression model	*n*	OR (for Arg389 homozygotes)	95% CI	*p
Univariate	94	0.49	0.21–1.2	0.101
Adjusted for age, sex, and race	93	0.42	0.17–1.1	0.063
Adjusted for sex, SBP, diabetes	86	0.49	0.19–1.3	0.142
Adjusted for age, sex, SBP, diabetes, QRS duration, and ischemic etiology[a]	86	0.55	0.20–1.5	0.230
Adjusted for age, sex, race, SBP, and diabetes	85	0.48	0.18–1.3	0.148
Adjusted for age, race, sex, SBP, diabetes, QRS duration, NYHA class, and ischemic etiology[a]	85	0.55	0.19–1.5	0.251
Adjusted for age, race, sex, SBP, diabetes, QRS duration[a], NYHA class[a], and ischemic etiology[b]	51	0.55	0.13–2.2	0.404

ADRB1, gene for the beta-1 adrenergic receptor; Arg, arginine; CI, confidence interval; Gly, glycine; LVEF, left ventricular ejection fraction; OR, odds ratio; SBP, systolic blood pressure

[a] Mean values were used to impute missing data for $n = 31$ missing QRS duration, $n = 39$ missing ischemic etiology, and $n = 34$ missing NYHA functional class

[b] This model did not include patients that had non/ischemic etiology imputed. It only included patients in which non/ischemic etiology data was available

*p value for *ADRB1* Arg389Gly genotype within the specified model. Bolded p values indicate statistical significance

beta-1 adrenergic receptor is not only expressed in the heart, but also in the kidney, where it affects the release of renin. The renin–angiotensin–aldosterone system is a critical system in the neurohormonal pathophysiology of heart failure.

Our findings are consistent with previous studies investigating clinical outcome responses to beta-blockers in patients with heart failure (i.e., survival benefit). In retrospective ($n = 184$) and prospective ($n = 190$) studies of Swedish patients with idiopathic dilated cardiomyopathy, patients homozygous for Ser49 gained more survival benefit from beta-blockers than patients carrying Gly49 [26, 27]. In a prospective, multicenter heart failure patient registry in the US (total $n = 715$), beta-blocker use was associated with reduced mortality in the overall study population (adjusted HR = 0.72, 95% CI 0.56–0.91, $p = 0.004$), and Ser49 homozygotes (adjusted HR = 0.56, 95% CI 0.42–0.75, $p < 0.001$), but not Gly49 carriers (adjusted HR = 1.28, 95% CI 0.80–2.04, $p = 0.31$) [16]. In a subsequent and independent prospective heart failure registry at a single center in the US ($n = 822$), only Ser49 homozygotes had a significant reduction in the risk for mortality from beta-blockers (adjusted HR = 0.41, $p = 0.022$ in Ser49 homozygotes; adjusted HR = 0.88, $p = 0.81$ in Gly49 carriers). All of the patients in these studies were treated with FDA-approved beta-blockers.

The survival data for *ADRB1* Arg389Gly also supports our findings, in that Arg389Gly may not have a significant effect on beta-blocker response, albeit for FDA-approved beta-blockers. Two prospective heart failure registries [28, 29]

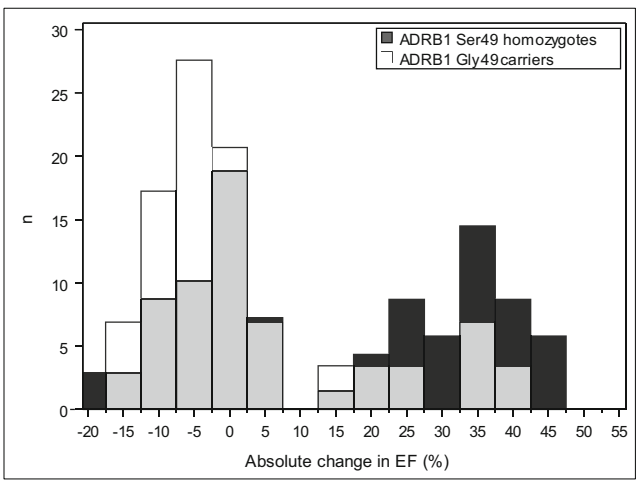

Fig. 1 Overlaid histograms of absolute change in ejection fraction (EF) for *ADRB1* Ser49 homozygotes (dark gray columns) vs Gly49 carriers (white columns). Light gray columns indicate overlapping data

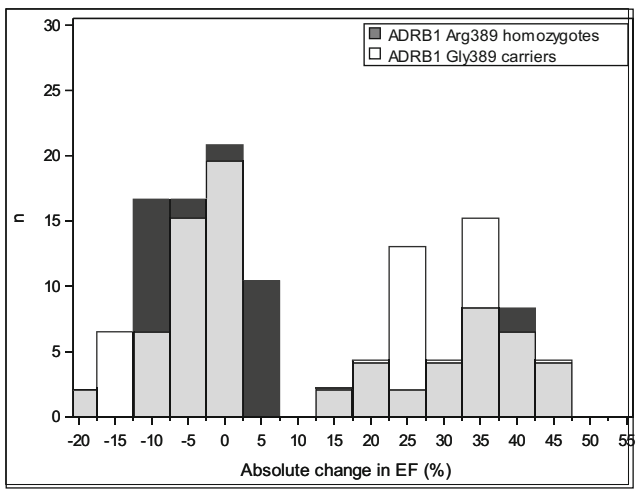

Fig. 2 Overlaid histograms of absolute change in ejection fraction (EF) for *ADRB1* Arg389 homozygotes (dark gray columns) vs Arg389 carriers (white columns). Light gray columns indicate overlapping data

and a genetic analysis of a landmark beta-blocker randomized controlled trial [30] did not find a significant association of Arg389Gly with survival benefit from FDA-approved beta-blockers. The strongest evidence to support an effect of Arg389Gly on beta-blocker survival benefit comes from a genetic analysis ($n = 1040$) of the Beta-Blocker Evaluation of Survival Trial (BEST), the landmark clinical trial assessing the effectiveness of bucindolol on mortality in patients with heart failure [10, 31]. Patients homozygous for Arg389 had a statistically significant improvement in survival compared with placebo (HR = 0.62; $p = 0.03$), whereas Gly389 carriers did not (HR = 0.90; $p = 0.57$). Bucindolol has pharmacologic effects that are unique compared to FDA-approved beta-blockers, in that it causes exaggerated sympatholysis (i.e., decreases in norepinephrine levels) [10]. Therefore, bucindolol may be affected by *ADRB1* Ser49Gly and Arg389Gly in ways that are unique from FDA-approved beta-blockers without sympatholytic effects.

This study has several limitations. Our study was small ($n = 98$ total). Only $n = 29$ patients were Gly49 carriers, and only $n = 4$ of the Gly49 carriers experienced the primary outcome of LVEF recovery. Therefore, the results could be due to chance. However, the low p value for LVEF recovery within the Gly49 carriers ($p = 0.003$) indicates that our results are unlikely due to chance and more likely represent a significantly lower LVEF recovery rate in the Gly49 carriers than in Ser49 homozygotes. Despite the small sample size, the long duration of follow-up between LVEF measurements (4.4 years) still provides a large number of patient-years, especially compared to most previous studies in which the length of follow-up was only several months [18–21]. Our study was underpowered to detect the significance of the many previously identified clinical predictors of LVEF recovery and mortality rates [12, 32]. However, it is worth noting that our most conservative model identified sex as being an independent predictor of LVEF recovery in addition to Ser49Gly. QRS duration and systolic blood pressure did not reach, but were trending toward, statistical significance (p values of 0.017, 0.125, and 0.070, respectively). Conversely, our study did not identify an association for diabetes, ischemic etiology, atrial fibrillation, or left bundle branch block. Subjects in this study were selected as a convenience sample from a single site, creating the possibility of selection bias and non-generalizability of results to outside sites. In addition, DNA collection occurred nearly 20 years ago, complicating data collection from electronic medical records. This was particularly evident with ischemic etiology, QRS interval duration data, and NYHA class, which were missing many values. Thus, our models either excluded those variables or relied on imputation of missing data, which could incorporate bias. Variables of potential importance that we were unable to retrieve from the electronic medical records were glomerular filtration rate, right ventricular ejection fraction, and duration of heart failure.

Outpatient medication records were not available within the time frame of the study, and, thus, we were not able to verify that patients were treated with optimal medical therapy. We can only assume that because the patients were managed in a heart failure specialty clinic at a major academic medical center that they were treated with (or at least attempted to be treated with) beta-blockers effective for HFrEF and at target doses. However, given that the patients were enrolled in 1999, and the landmark beta-blocker clinical trials were published around that same time, we cannot assume widespread beta-blocker use had occurred yet. However, it may be reasonable to assume that beta-blocker treatment was similar between the Ser49 homozygotes and the Gly49 carriers. First, the providers were unaware of the patients' Ser49Gly genotypes. Second, patient characteristics that would limit beta-blocker dose titration were not significantly different between the genotype groups at baseline. Specifically, mean systolic blood pressure, heart rate, age, and BMI were all similar, if not identical, between the Ser49 homozygotes and Gly49 carriers.

Conclusion

ADRB1 Ser49Gly, but not Arg389Gly, was significantly associated with recovery of LVEF in patients with HFrEF. HFrEF patients that were homozygous for Ser49 had significantly more LVEF recovery than Gly49 carriers. *ADRB1* Ser49 homozygous status was by far the strongest predictor of LVEF recovery among several clinical variables assessed. These data suggest that genetic factors may be the strongest determinant of recovery of ventricular performance beyond recognized clinical variables. The findings point to the need for further research into genetic determinants of recovery of ventricular function, which may constitute therapeutic targets that will promote normalization of ventricular function in an increasing proportion of patients with HFrEF.

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Compliance with Ethical Standards

Disclosures None.

Human Subjects/Informed Consent Statement All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Animal Studies No animal studies were carried out by the authors for this article.

Clinical Relevance These findings from a small study at a single site need to be replicated in a larger study of multiple sites to be clinically relevant. The potential clinical relevance is that a patient's *ADRB1* Ser49Gly (rs1801252) genotype may be a predictor of whether or not the patient with HFrEF will have recovery of their LVEF to ≥ 40%. This genotype was the strongest predictor of LVEF recovery, even compared to multiple clinical variables. Thus, if validated, HFrEF patients that are *ADRB1* Gly49 carriers may need additional monitoring or therapies compared to Ser49 homozygotes.

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