Effect of specific *ADRB1/ADRB2/AGT* genotype combinations on the association between survival and carvedilol treatment in chronic heart failure: a substudy of the ECHOS trial

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Objectives **The aim of the present study was to determine whether carvedilol-treated chronic heart failure patients have a different prognosis when stratified for a specific combination of a gain-of-function genotype of the adrenergic β-1 receptor gene (*ADRB1*) (Arg389-homozygous), two gain-of-function genotypes of the angiotensinogen gene (*AGT*) (Thr174-homozygous and Thr235-homozygous), and a downregulated genotype of the adrenergic β-2 receptor gene (*ADRB2*) (Gln27-carrier).**

Methods **Genotyping of 618 patients was carried out using the Sequenoms MassARRAY genotyping system. Outcome was all-cause mortality and statistics were calculated using a multivariable Cox proportional hazards model. Internal validation was performed using the bootstrap procedure.**

Results **Eighty-seven of the 618 patients included in the study were treated with carvedilol. There was a significant interaction between the outcome of carvedilol treatment and the combination of the gain-of-function *ADRB1* genotype (Arg389-homozygous) and the gain-of-function *AGT* genotype (Thr174-homozygous) ($P_{interaction}=0.003$; hazard ratio 2.19, 95% confidence interval 1.26–3.78, $P=0.005$). There was also a significant interaction when the downregulated *ADRB2* genotype (Gln27-carrier) was added to the *ADRB1/AGT* combination ($P_{interaction}=0.0005$; hazard ratio 2.67, 95% confidence interval 1.51–4.72, $P=0.0007$). Two hundred and four patients were treated with metoprolol. There was no interaction between metoprolol treatment and the specific genotype combinations as there was no difference in the overall survival. The validity of the results was supported by the bootstrap procedure.**

Conclusion **We found a doubling of the hazard of mortality in carvedilol-treated patients with the combination of the gain-of-function *ADRB1* genotype (Arg389-homozygous), the gain-of-function *AGT* genotype (Thr174-homozygous), and the downregulated *ADRB2* genotype (Gln27-carrier). This might be valuable when stratifying chronic heart failure patients to the right β-blocker therapy. *Pharmacogenetics and Genomics* 00:000–000** © **2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Chronic heart failure (HF) is recognized as a disease of the entire hemodynamic system, that is involving the heart, blood vessels, and kidneys [1]. The pharmacological treatment of HF is targeted toward the adrenergic system and the renin–angiotensin–aldosterone system (RAAS) [2]. When investigating the response to β-blockers, pharmacogenetic studies have so far focused on the adrenergic system [3]. Accordingly, we showed that poor responders to carvedilol treatment might be identified by a specific combination of the gain-of-function Arg389-homozygous genotype of the adrenergic β-1 receptor gene (*ADRB1*) and the downregulated Gln27-carrier genotype of the adrenergic β-2 receptor gene (*ADRB2*) [4].

However, several studies have shown that the adrenergic system and RAAS communicate in several ways [5–7] and that β-blockers and angiotensin-converting enzyme inhibitors

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(ACEi)/angiotensin II receptor blockers (ARBs) are more closely related than previously believed [7]. In the present study, we applied a candidate gene approach to examine a possible synergistic interaction between single nucleotide polymorphisms (SNPs) from the adrenergic system and RAAS.

The findings from our previous study indicate that the gain-of-function *ADRB1* Arg389-homozygous genotype and the downregulated *ADRB2* Gln27-carrier genotype might be risk genotypes with respect to carvedilol treatment in HF. The gain-of-function *ADRB1* genotype has specifically been associated with increased plasma renin activity (PRA) [8] – the enzyme activating RAAS by converting angiotensinogen into angiotensin I. With this study, we hypothesized that two gain-of-function genotypes from the angiotensinogen gene (*AGT*), Thr174-homozygous and Thr235-homozygous, might also be risk genotypes with respect to carvedilol treatment. These two genotypes have been associated with increased levels of angiotensinogen. We hypothesized that the simultaneous presence of increased PRA (*ADRB1* Arg389-homozygous) and its substrate angiotensinogen (*AGT* Thr174-homozygous and Thr235-homozygous) would be associated with a worsened outcome in patients treated with carvedilol. As hypothesized earlier [4], this could be because of hypersensitized adrenergic β-1 receptors indirectly caused by the failure of carvedilol to upregulate these receptors [9]. Any activation and hypersensitization of the adrenergic system is associated with worsened outcome in HF [10,11].

Using the same cohort as that in our previous study, we combined the four risk genotypes in various ways to determine whether this would further define a subgroup of HF patients with a poor prognosis when treated with carvedilol. As none of the SNPs investigated in this study have consistently been associated with HF as disease modifiers, we anticipated the same allele frequencies in this HF cohort as in the background Caucasian population.

Materials and methods

Participants

This is a retrospective cohort study of 618 Danish out of 1000 HF patients from the EchoCardiography and Heart Outcome Study (ECHOS) [12]. All hypotheses were proposed before conducting the analyses. Patients were enrolled from 2001 to 2002. Survival was assessed through the Danish Civil Registration System [13], and the last follow-up was performed in November 2008. ECHOS was a prospective, double-blind, randomized, placebo-controlled, multicenter trial, investigating the clinical effect of nolomirole in HF patients. Nolomirole is a presynaptic stimulator of DA2-dopaminergic and α-2 adrenergic receptors in peripheral sympathetic nerve endings [12]. Blood samples were collected from the Danish participants, hence only 618 patients of a total of 1000 patients from ECHOS were included in the present study. Participants were recruited from among patients who were 18 years of age or older and who had been admitted with a diagnosis of HF. Patients were required to have had symptoms of HF (dyspnea, fatigue) corresponding to New York Heart Association (NYHA)-class III–IV in the month before enrollment. At the time of enrollment, patients were required to be treated with a diuretic and had to be in NYHA class II–IV. Patients had to be recruited within 7 days of hospitalization, and in this period, an echocardiograph had to show a left ventricular wall motion index (WMI) score of less than or equal to 1.2, corresponding to a left ventricular ejection fraction of 35% or less (using a reverse-scoring method [14]).

The inclusion and exclusion criteria have been described earlier [12].

ECHOS was approved by the authorities in the participating countries and the local ethics committees. It was conducted in accordance with the Declaration of Helsinki III and the Guidelines for Good Clinical Practice in the European Union. All participating patients provided their informed consent for their blood samples to be used for the analyses.

Genetic analysis

Genotyping was carried out using the Sequenom MassARRAY Genotyping system (Sequenom, San Diego, California, USA). Primers for PCR and extension probes were designed using the MassARRAY Assay Design 3.1 software (Sequenom).

The primer sequences used are listed in Appendix 1 (Supplementary digital content 1, *http://links.lww.com/FPC/A486*).

A detailed description of the genetic analyses has been provided elsewhere [4].

Quality control

The call rate was 97% for Arg389Gly, 99% for Thr174Met, 99% for Met235Thr, and 97% for Gln27Glu. Therefore, none of the risk stratifications represent all 618 patients from the cohort.

Combinations of risk genotypes

As we defined four different risk genotypes with respect to carvedilol treatment, there were 11 possible combinations (of which one has been described earlier [4]) (Table 1). For each combination (risk combination 1–risk combination 11), the survival for this specific subgroup was compared with the rest of the cohort (reference group) – within 87 patients in carvedilol treatment, within 204 patients in metoprolol treatment, and within the entire cohort of 618 patients.

Statistical analysis and end-point

The end-point was defined as time to death from any cause. Hazard ratios (HRs) with 95% confidence intervals

Table 1 Distribution of chronic heart failure patients according to risk groups and their corresponding reference groups

Stratification	Genotypes risk group	Risk group/reference group		
		Carvedilol (N = 87)	Metoprolol (N = 204)	All patients (N = 618)
1	A + B	45/37	92/105	269/317
2	A + C	34/49	98/103	276/319
3	A + D	5/80	20/182	49/549
4	B + C	54/28	123/76	371/226
5	B + D	11/73	24/176	64/536
6	C + D	4/81	15/189	40/569
7	A + B + C	33/47	73/122	215/366
8	B + C + D	4/78	11/188	33/562
9	A + B + D	4/78	14/182	34/550
10	A + C + D	1/82	11/190	21/573
11	A + B + C + D	1/79	7/188	16/564

The total number of patients (risk group + reference group) differs from the total number of patients (carvedilol, metoprolol, and all patients) because call rates for the four single nucleotide polymorphisms were not 100%.
Genotype A: *ADRB1* Arg389-homozygous.
Genotype B: *ADRB2* Gln27-carrier.
Genotype C: *AGT* Thr174-homozygous.
Genotype D: *AGT* Thr235-homozygous.
ADRB1, adrenergic β-1 receptor gene; *ADRB2*, adrenergic β-2 receptor gene; *AGT*, angiotensinogen gene.

(95% CIs) were calculated using the Cox proportional hazards model. These HR estimates were carried out separately, only including one risk group (risk group vs. reference group 1–11, Table 1) per analysis. Using the Cox model, an interaction analysis was carried out for each risk group (1–11), including carvedilol treatment (yes vs. no), risk group (1–11), and the interaction between these two variables (risk group carvedilol treatment).

At the time of inclusion in ECHOS, we had information on the following variables: age, sex, ethnicity, diabetes mellitus, chronic obstructive pulmonary disease (COPD), ischemic heart disease, atrial fibrillation, previous percutaneous coronary intervention or coronary artery bypass graft surgery, ACEi, ARB, spironolactone, insulin, diuretics, nolomirole, WMI, NYHA class, and smoker status. Significant HRs were adjusted for covariables associated with mortality at a significance level of P less than 0.10 (backward selection); these were sex, age, COPD, ischemic heart disease, insulin, spironolactone, ACEi, NYHA, and WMI.

A post-hoc bootstrap procedure was used to verify any significant associations [15–20]. We used the bootstrap procedure to construct 2000 replicate cohorts. For each of the 2000 replicates, HRs with 95% CIs were calculated using the Cox proportional hazards model. The 2000 replicates were constructed from the original data set, where the bootstrap procedure randomly replaced one or more samples from the risk combination group and the reference group in question with another from the same group to rule out any hidden confounders. Thus, the size of the replicates remained the same as in the original cohort, but the composition differed. If 65% of the 2000 HRs made in the bootstrap model were significant, this was considered to be confirmatory of the original association. The final HR obtained from the bootstrap procedure was a mean of the 2000 parameter estimates and the 95% CI was calculated from the distribution of the 2000 HRs.

Results

Allele distribution

No deviation from Hardy–Weinberg equilibrium was observed in the genotype distribution of *ADRB1* Arg389-Gly (rs1801253) ($P = 0.987$), *AGT* Thr174Met (rs4762) ($P = 0.875$), *AGT* Met235Thr (rs699) ($P = 0.806$), or *ADRB2* Gln27Glu (rs1042714) ($P = 0.315$). Allele frequencies are presented in Appendix 2 (Supplementary digital content 2, *http://links.lww.com/FPC/A487*).

Study population characteristics

A total of 389 of 618 patients (63%) died during a median follow-up period of 6.7 years (range 5.2–7.8 years). Fifty-two percent of the total of 618 HF patients were treated with β-blockers at the time of enrollment (33% with metoprolol, 14% with carvedilol, 3% with bisoprolol, and 2% with other β-blockers) and 88% were treated with RAAS inhibitors (ACEi or ARB). Within the subgroup of carvedilol-treated patients, 55 of 87 patients (63%) died, and within the subgroup of metoprolol-treated patients, 114 of 204 patients (56%) died. Compared with metoprolol-treated patients, at baseline, patients treated with carvedilol were characterized by a poorer NYHA-class (2.47 vs. 2.19), younger age (65.7 vs. 69.7 years), greater incidences of COPD (18 vs. 7%), were more frequently smokers (34 vs. 23%) and men (88 vs. 68%), and more likely to being treated with spironolactone (73 vs. 54%) (data not shown in table).

Patients were distributed into risk combination groups and their reference groups according to Table 1.

Survival analyses

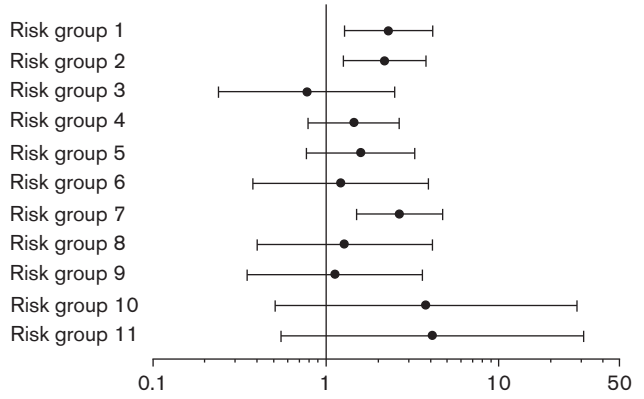
The unadjusted HRs with 95% CIs are presented in Table 2. None of the 11 risk combinations was associated with increased mortality in the entire cohort of 618 HF patients or in the subpopulation of 204 metoprolol-treated patients. In the subpopulation of carvedilol-treated

Table 2 Hazard ratio estimates for risk groups versus reference groups

	Carvedilol-treated patients ($N=87$)			Metoprolol-treated patients ($N=204$)			All patients ($N=618$)		
Stratification	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
1	2.30	1.28–4.14	0.0053	0.98	0.68–1.43	0.93	0.96	0.78–1.18	0.69
2	2.19	1.26–3.78	0.0052	1.14	0.79–1.65	0.48	0.99	0.81–1.22	0.95
3	0.78	0.24–2.50	0.67	0.87	0.45–1.66	0.67	1.25	0.84–1.84	0.27
4	1.45	0.79–2.65	0.23	0.90	0.62–1.31	0.60	0.99	0.81–1.22	0.93
5	1.59	0.77–3.26	0.21	0.61	0.32–1.67	0.14	1.17	0.83–1.64	0.36
6	1.22	0.38–3.91	0.74	0.79	0.37–1.69	0.54	1.08	0.72–1.64	0.71
7	2.67	1.51–4.74	0.0007	0.97	0.66–1.42	0.86	0.96	0.78–1.18	0.69
8	1.28	0.40–4.12	0.68	0.54	0.20–1.45	0.22	1.26	0.79–2.03	0.33
9	1.13	0.35–3.62	0.84	0.67	0.29–1.52	0.33	1.31	0.82–2.12	0.26
10	3.79	0.51–28.34	0.19	1.04	0.46–2.36	0.93	1.13	0.63–2.00	0.69
11	4.13	0.55–31.01	0.17	0.69	0.22–2.18	0.53	1.37	0.68–2.76	0.38

CI, confidence interval; HR, hazard ratio.

Fig. 1



Forest plot of the hazard ratios for the risk groups (1–11) versus the reference groups (1–11) within the subgroup of carvedilol-treated patients.

patients, three risk combinations were associated with increased mortality when compared with their respective reference groups (Fig. 1). These where the combination of the gain-of-function *ADRB1* Arg389-homozgyous genotype and the downregulated *ADRB2* Gln27-carrier genotype (risk combination 1: HR 2.30, 95% CI 1.28–4.14, $P = 0.005$, as reported previously [4]), the combination of the gain-of-function *ADRB1* genotype and the gain-of-function *AGT* Thr174-homozygous genotype (risk combination 2: HR 2.19, 95% CI 1.26–3.78, $P = 0.005$), and the combination of all three risk genotypes (risk combination 7: HR 2.67, 95% CI 1.51–4.72, $P = 0.0007$). There was a significant interaction between carvedilol treatment and these three risk combinations (risk combination 1: $P_{\text{interaction}} = 0.003$; risk combination 2: $P_{\text{interaction}} = 0.003$; risk combination 7: $P = 0.0005$). The adjusted HR for risk combination 1 was 2.40 (95% CI 1.22–4.71, $P = 0.011$) as reported previously [4], for risk combination 2, the adjusted HR was 2.05 (95% CI 1.10–3.84, $P = 0.024$), and for risk combination 7, the adjusted HR was 2.45 (95% CI 1.26–4.77, $P = 0.008$).

Other analyses

Bootstrap procedure

For risk stratification 2, 80% of the HRs constructed (1591/2000) were significantly increased. The mean HR (2.21) and 95% CI (1.22–3.69) supported the primary result from this stratification in carvedilol-treated patients.

For risk stratification 7, 93% of the HRs constructed (1857/2000) were significantly increased (mean HR 2.64, 95% CI 1.52–3.59), and for risk stratification 1, 84% of the HRs constructed (1677/2000) were significantly increased (mean HR 2.32, 95% CI 1.35–3.56).

Survival according to genotypes

There was no association between the overall mortality and Arg389Gly genotypes ($P = 0.773$), Gln27Glu genotypes ($P = 0.957$), Thr174Met genotypes ($P = 0.457$), and Met235Thr genotypes ($P = 0.445$). There was no association between survival and genotypes either in carvedilol-treated patients (Arg389Gly: $P = 0.132$, Gln27Glu: $P = 0.324$, Thr174Met: $P = 0.134$, Met235Thr: $P = 0.369$) or in metoprolol-treated patients (Arg389Gly: $P = 0.526$, Gln27Glu: $P = 0.317$, Thr174Met: $P = 0.541$, Met235Thr: $P = 0.300$).

Interaction between risk group 2 and nolomirole and renin–angiotensin–aldosterone system inhibitors, respectively

There was no interaction between nolomirole and risk group 2 ($P = 0.885$), and nolomirole treatment did not affect survival ($P = 0.168$). There was no interaction between risk group 2 and ACEi ($P = 0.702$) or ARB ($P = 0.102$) treatments. There was no interaction between *AGT* Thr174Met and ACEi ($P = 0.362$) or ARB ($P = 0.762$).

Discussion

In this study, we found that the combination of the gain-of-function *ADRB1* genotype (Arg389-homozygous) and a gain-of-function *AGT* genotype (Thr174-carrier) was associated with a poor prognosis among carvedilol-treated HF patients. This interaction with carvedilol was also

significant on combining these two genotypes with the downregulated *ADRB2* genotype (Gln27-carrier). We found a doubling of the hazard of mortality in patients with these genotype combinations compared with all other patients. The individual functionalities of the three SNPs in question and the pharmacodynamic properties of carvedilol might explain this interaction, and yet, any causal explanation is merely speculative.

Stimulation of the adrenergic β-1 receptor is known to increase renin secretion [5], and the *ADRB1* Arg389-homozygous genotype has been associated with a more potent increase in PRA than the Gly389-homozygous genotype [8]. Furthermore, the *ADRB1* Arg389-homozygous genotype has been associated with a hyperactive state of the adrenergic β-1 receptor, as indicated by increased adenylyl cyclase activity [21]. The *AGT* Thr174-homozygous genotype when combined with the gain-of-function *AGT* Thr235-homozygous genotype has been associated with elevated angiotensinogen levels in men and women and hypertension in women [22]. Renin converts angiotensinogen into angiotensin I, which is the precursor of RAAS activation. It is therefore reasonable to assume that increased PRA (as with the *ADRB1* Arg389-homozygous genotype) together with increased levels of angiotensinogen (as with the *AGT* Thr174-homozygous genotype) might synergistically activate RAAS, this being unfavorable in HF patients.

We hypothesized that these gain-of-function genotypes of *ADRB1* and *AGT* might be risk genotypes with respect to carvedilol treatment. We found that when present simultaneously, this hyperactive state of RAAS specifically interacted with carvedilol treatment and was associated with a decreased survival response when compared with all other HF patients. This study does not allow any conclusions on causality, but we find that some specific pharmacodynamic properties of carvedilol might explain why we did not find the same interaction with metoprolol.

A drug-specific interaction has been shown *in vivo* between the *AGT* Thr174Met polymorphism and irbesartan (ARB) that was not found for atenolol, that is an adrenergic β-1 selective β-blocker such as metoprolol [23]. To our knowledge, no such study has been carried out with carvedilol. However, carvedilol might resemble irbesartan in some ways. It has been shown that by blocking the adrenergic α-1 receptor, carvedilol blocks angiotensin II-mediated receptor sensitization [7]. This angiotensin II antagonist effect of carvedilol is probably crucial in the treatment of HF and is comparable to irbesartan [7]. We hypothesize that increasing levels of RAAS activators reach a threshold at which carvedilol, through adrenergic α-1 receptor inhibition and NO stimulation, no longer counteracts the deteriorating effects of angiotensin II. In this situation, angiotensin II may exert its effect through direct vasoconstriction [7]. In patients with increased PRA (*ADRB1* Arg389-homozygous) and increased angiotensinogen levels (*AGT* Thr174-homozygous), RAAS might be activated to a degree beyond the angiotensin II-blocking effect of carvedilol. In our study, no interaction was found between the *AGT* Thr174Met and ACEi treatment outcome or ARB treatment outcome.

Furthermore, we hypothesize that carvedilol indirectly potentiates the effects of catecholamines on the noninhibited adrenergic β-1 receptors [9] and thereby the stimulation of RAAS. Unlike metoprolol, carvedilol does not restore the catecholamine-induced receptor downregulation [9]. Through this mechanism, but also irrespective of receptor downregulation [24], carvedilol sensitizes the remaining adrenergic β-1 receptors to agonist stimulation [24]. This is probably favorable unless the adrenergic β-1 receptor is hyperactive as in the case of the Arg389-homozygous phenotype. It is not unlikely that carvedilol might exert this potentiating effect on the noninhibited receptors, as sympathetic denervation in the heart has been associated with implantable cardioverter defibrillator discharges and mortality in HF patients [10,11].

In summary, we hypothesize that carvedilol fails to block RAAS in the presence of increased angiotensinogen levels (*AGT* Thr174-homozygous) and activates the adrenergic system and RAAS in the presence of a hyperactive adrenergic β-1 receptor (*ADRB1* Arg389-homozygous). If carvedilol only interfered with these pathways, it would be detrimental to patients with HF. However, carvedilol has a wide pharmacodynamic profile and undoubtedly affects other pathways that are still beneficial in HF [25–27]. Therefore, we do not expect carvedilol to be harmful in patients with the specific combination of the *ADRB1* Arg389-homozygous genotype and the *AGT* Thr174-homozygous genotype.

The addition of the *ADRB2* Gln27-carrier genotype to the *ADRB1*/*AGT* combination (risk combination 7) might improve the interaction with carvedilol, but this could not be concluded from the present study. It is not unlikely, however, that the *ADRB1* SNP and the *AGT* SNP may interact with the Gln27Glu polymorphism, as other studies have proposed synergistic properties for both Arg389Gly and Thr174Met when combined with SNPs from other genes [28,29].

We do not believe that the present findings conflict with the COMET study [30], which found an overall benefit of carvedilol over metoprolol. As for most drugs, there might be a subgroup of poor responders to carvedilol representing 25–50% of HF patients [31]. A major limitation with the present study is that ECHOS was not designed to compare survival between treatment groups (carvedilol vs. metoprolol and no-β-blocker). The group of carvedilol-treated patients was confounded in several ways compared with both metoprolol-treated patients and

patients without β-blockers, as shown in the result section. Retrospective studies such as the present are at risk of being biased, although it is reasonable to assume an equal distribution of covariables between genotype groups. We have attempted to account for this disadvantage by using the bootstrap procedure. This test supports the reproducibility of our findings, and we believe that our method is consistent with the criteria for genetic association studies [32]. To examine whether metoprolol is superior to carvedilol in the subgroup of potential poor carvedilol responders, a randomized clinical trial of HF patients with combinations of the specific *ADRB1/ADRB2/AGT* genotypes is required. The small sample size is an obvious limitation of the present study, which may have caused type II errors, especially with respect to some of the risk groups within the carvedilol-treated patients (Fig. 1).

Furthermore, this study is limited by the lack of supplementary end-points and would have been strengthened by having information available about the medication taken following enrollment in the study. In this respect, doses of carvedilol and RAAS inhibitors would be valuable, although it is reasonable to assume that patients from the two genotype groups are dosed equally, that is no attending physicians were aware of the patients' genetic constitution.

Conclusion

This study shows twice the mortality hazard in carvedilol-treated patients with the combination of the gain-of-function *ADRB1* Arg389-homozygous genotype and the gain-of-function *AGT* Thr174-homozygous genotype compared with all other patients (any other genotype/phenotype combination). In addition to its value in generating hypotheses, this pharmacogenetic study supports the merit of combining genotypes of different genes that, from a pathophysiological point of view, are linked [33]. With respect to carvedilol treatment, our study suggests that HF patients may benefit from a risk stratification of a specific *ADRB1/ADRB2/AGT* combination. It is important to emphasize that this is a hypothesis-generating study, and our findings should be replicated in a larger cohort of HF patients.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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