BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2010.03868.x

Association of beta-adrenergic receptor polymorphisms and mortality in carvedilol-treated chronic heart-failure patients

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Keywords
carvedilol, drug–gene specific interaction, functional genotype group, heart failure, metoprolol, mortality

Received
7 July 2010

Accepted
21 October 2010

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Chronic heart failure (HF) is a syndrome with increasing prevalence. Though mortality is still high, the introduction of β-adrenoceptor blockers for its treatment has improved survival considerably.
- As is the case for all medical treatment, not all patients benefit from β-adrenoceptor blocker treatment, and stratifying patients to different β-adrenoceptor blockers by the use of pharmacogenomics might be of great value in improving HF therapy.
- Previous studies have shown that the two single nucleotide polymorphisms (SNPs) *ADRB1* Arg389Gly and *ADRB2* Gln27Glu interact with the β-adrenoceptor blockers metoprolol and carvedilol, respectively. These interactions have led to stratified responses with regard to surrogate parameters, e.g. left ventricular ejection fraction (LVEF), pulse and blood pressure.
- Several studies have failed to show a stratified survival response when stratifying for *ADRB1* Arg389Gly and *ADRB2* Gln27Glu.

WHAT THIS STUDY ADDS

- With the present study we tested a specific combination of *ADRB1* Arg389Gly and *ADRB2* Gln27Glu and showed that, when stratifying HF patients according to this genotype combination, a stratified carvedilol response was seen with respect to survival over a median follow-up period of 6.7 years.
- This genotype combination did not show a stratified metoprolol response.

AIM

Pharmacogenetics can be used as a tool for stratified pharmacological therapy in cardiovascular medicine. We investigated whether a predefined combination of the Arg389Gly polymorphism in the adrenergic β_1-receptor gene (*ADRB1*) and the Gln27Glu polymorphism in the adrenergic β_2-receptor gene (*ADRB2*) could predict survival in carvedilol- and metoprolol-treated chronic heart failure (HF) patients.

METHODS

Five hundred and eighty-six HF patients (carvedilol $n = 82$, metoprolol $n = 195$) were genotyped for *ADRB1* Arg389Gly (rs1801253) and *ADRB2* Gln27Glu (rs1042714). The end-point was all-cause mortality, and median follow-up time was 6.7 years. Patients were classified into two functional genotype groups: group 1 combination of Arg389-homozygous and Gln27-carrier (46%) and group 2 any other genotype combination (54%). Results were fitted in two multivariate Cox models.

RESULTS

There was a significant interaction between functional genotype group and carvedilol treatment (adjusted$_1$ $P = 0.033$, adjusted$_2$ $P = 0.040$). Patients treated with carvedilol had shorter survival in functional genotype group 1 ($P = 0.004$; adjusted$_1$ hazard ratio (HR) 2.67, 95% CI 1.27, 5.59, $P = 0.010$; adjusted$_2$ HR 2.05, 95% CI 1.06, 3.95, $P = 0.033$). There was no interaction between genotype group and metoprolol treatment ($P = 0.61$), and there was no difference in overall survival between genotype groups ($P = 0.69$).

CONCLUSIONS

A combination of *ADRB1* Arg389-homozygous and *ADRB2* Gln27-carrier in HF patients treated with carvedilol was associated with a two-fold increase in mortality relative to all other genotype combinations. There was no difference in survival in metoprolol-treated HF patients between genotype groups. Patients in genotype group 1 may benefit more from metoprolol than carvedilol treatment.

Table 1
Combinations of Arg389Gly (*ADRB1*) and Gln27Glu (*ADRB2*)

Genotypes	Functionality	*A priori* stratification Functional genotype group (*n* = 586)	*Post hoc* stratification Functional genotype group (*n* = 586)
Arg389-homozygous*/Gln27-carrier†	↑ adenylyl cyclase activity and coupling to G_s-protein‡ and receptor down regulation§.	A *n* = 269 (46 %) N_{events} = 172	1 (identical with A) *n* = 269 (46 %) N_{events} = 172
Arg389-homozygous*/Glu27-homozygous† **or** **Gly389-carrier*/Gln27-carrier†**	↑ β_1-receptor activity and resistance to β_2-receptor down regulation or ↓ β_1-receptor activity and β_2-receptor down regulation.	B *n* = 265 (45 %) N_{events} = 166	2 *n* = 317 (54%) N_{events} = 201
Gly389-carrier*/Glu27-homozygous†	↓ β_1-receptor activity and resistance to β_2-receptor down regulation.	C *n* = 52 (9 %) N_{events} = 35	

**ADRB1*; †*ADRB2*; ‡β_1-adrenergic receptor; §β_2-adrenergic receptor.

Introduction

Cardiovascular disease may be a leading area in personalized/stratified medicine in the near future [1]. The immense benefit of the β-adrenoceptor blockers, carvedilol and metoprolol, on survival in chronic heart failure (HF) patients and the diverse pharmacodynamic profile of these β-adrenoceptor blockers open up the possibility of pharmacogenetic testing as a tool for stratified therapy [2–4]. Targeting the use of the two β-adrenoceptor blockers towards individual pathophysiological traits might advance pharmacological therapy of HF and expand the group of responders.

Generally, there are two different approaches to pharmacogenetic studies. One approach is to test hypotheses based on genome-wide-association studies (GWAS) in a more specific setting. Another approach is to reproduce or build new hypotheses on previously conducted studies investigating the influence of one or few polymorphisms or single nucleotide polymorphisms (SNPs) on different outcomes. This, also known as the candidate gene approach, mostly refers to non-synonymous SNPs, i.e. causing amino acid substitution in the corresponding protein. With the present study we applied a candidate gene approach in which we combined the two best characterized SNPs from the two best characterized genes regarding stratified metoprolol and carvedilol response in HF patients.

The hypotheses addressed in this study were based on the following premises:

1 The Arg389-homozygous genotype of the adrenergic β_1-receptor gene (*ADRB1*) is associated with increased agonist-stimulated intracellular activity compared with its counterpart the Gly389-carrier state [5], and the Arg389-homozygous genotype has been associated with improved left ventricular ejection fraction (LVEF), heart rate (HR) and blood pressure in response to metoprolol treatment [6–8]. Inhibition of the Arg389-homozygous genotype is theoretically of value in HF.

2 The Gln27 allele of the Gln27Glu polymorphism in the adrenergic β_2-receptor gene (*ADRB2*) is associated with receptor down regulation [9], and a greater increase in LVEF has been associated with the Glu27-homozygous genotype in response to carvedilol treatment [10, 11].

3 Carvedilol has its effect mainly on the adrenergic β_2-receptors and induces adrenergic β_1- receptor down regulation [12]. Indirectly, this might sensitize the remaining functional adrenergic β_1-receptors to agonist stimulation [13], which is unfavourable in the context of an active Arg389-homozygous phenotype.

4 Metoprolol selectively blocks the adrenergic β_1-receptor and is associated with adrenergic β_1-receptor up regulation [4, 12, 14, 15].

5 The *ADRB1* Arg389Gly and *ADRB2* Gln27Glu polymorphisms have not consistently been associated with mortality/morbidity [16–19] and a combination of genotypes has been proposed as a more valuable strategy in stratified therapy with β-adrenoceptor blockers [20, 21].

In this retrospective cohort study of 586 HF patients, of whom 277 were treated with β-adrenoceptor blockers at discharge (carvedilol *n* = 82, metoprolol *n* = 195), we proposed the *a priori* hypotheses that carvedilol and metoprolol interact in different ways with specific genotype combinations of *ADRB1* and *ADRB2*. A combination of the two genotypes, *ADRB1* Arg389-homozygous and *ADRB2* Gln27-carrier (genotype group A, Table 1), compared with any other genotype combination would be associated with increased mortality in carvedilol-treated HF patients but decreased mortality in metoprolol-treated patients. A combination of the reciprocal genotypes, *ADRB1* Gly389-carrier and *ADRB2* Glu27-homozygous (genotype group C, Table 1), compared with any other genotype combination would be associated with increased mortality in metoprolol-treated patients but decreased mortality in carvedilol-treated patients.

Methods

Subjects

In this retrospective cohort study of the EchoCardiography and Heart Outcome Study (ECHOS) [22], patients were followed prospectively. Hypotheses were formulated before performing the analysis. Patients were enrolled in the period from 2001 to 2002. The last follow-up was in November 2008.

Criteria for inclusion in the present study were participation in the ECHOS study, being Danish citizens and the availability of a blood sample for DNA analysis. Of the 1000 patients in the ECHOS cohort, blood samples were collected from 629 HF patients and their DNA purified. The remaining 371 patients were either non-Danish patients or were excluded from the present study because sample material was not available.

To be eligible for inclusion in ECHOS, patients were required to have HF from any cause, and had to have been in New York Heart Association (NYHA) class III–IV during the month before enrolment, and in NYHA class II–IV at the time of randomization to the placebo or nolomirole (DA_2-dopaminergic receptor agonist and α_2-adrenergic receptor agonist in peripheral sympathetic nerve endings) groups [22]. Patients were required to have a wall motion index (WMI) $\leq$1.2 (estimated by echocardiography using a reverse scoring system [23]) corresponding to a left ventricular ejection fraction $\leq$35%. Patients with clinically significant hepatic or renal disease or any illness or disorder other than HF that could preclude participation or severely limit survival were excluded from ECHOS. Further inclusion and exclusion criteria have been described elsewhere [22].

Information was available about the use of the following types of medication: β-adrenoceptor blockers, inhibitors of the renin-angiotensin system, aldosterone antagonists, diuretics and insulin. Furthermore, information was collected at enrolment about gender, NYHA class, age, ethnic origin, diabetes mellitus (DM), atrial fibrillation, ischaemic heart disease (IHD), WMI, smoker status and chronic obstructive pulmonary disease (COPD).

Survival status was assessed in November 2008 through the Danish Civil Registration System, which has complete information on the survival status of all Danish citizens [24].

The ECHOS study was approved by the authorities in participating countries and the relevant ethics committees. It was conducted in accordance with the Declaration of Helsinki III and Guidelines for Good Clinical Practice in the European Union. Informed consent to analysis was obtained, and all data were anonymized before undertaking the present analysis.

Genetic analysis (genotyping)

Genotyping was carried out using the Sequenom MassARRAY Genotyping system (Sequenom, San Diego, CA). Primers for PCR and extension probes were designed using the MassARRAY Assay Design 3.1 software (Sequenom). Multiplex PCR was performed in 5 μl reactions containing 10 ng of genomic DNA, 1.25× PCR buffer (Qiagen, Valencia, CA), 0.5 mM dNTP (Roche, Geneva, Switzerland), 100 nM of each primer (Metabion, Martinsried, Germany) and 0.5 U Taq polymerase (Qiagen), using the standard cycling conditions described by Sequenom. The PCR products were treated with arctic shrimp alkaline phosphatase (SAP) and the probe extension reaction (iPLEX) was carried out in accordance with its standard protocol (Sequenom). The iPLEX reactions were desalted using resin and spotted on a SpectroCHIP (Sequenom) using a nanodispenser. The samples were analyzed using a Bruker matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer (Sequenom) and the genotypes were determined using the MassARRAY Type 4.0 software (Sequenom). The primer sequences used were as follows: for Arg389Gly (*ADRB1*, rs1801253) 5′-ACGTTGGATGAGCCCTGC GCGCGCAGCAGA was used as the forward primer, 5′-ACGTTGGATGCCTTCAACCCCATCATCTAC as the reverse primer and 5′-CGCAAGGCCTTCCAG as the extension primer. For Gln27Glu (*ADRB2*, rs1042714) 5′-ACGTTGGATG AAGCCATGCGCCGGACCA was used as the forward primer, 5′-ACGTTGGATGAGACATGACGATGCCCATGC as the reverse primer and 5′-ACACCTCGTCCCTTT as the extension primer.

Quality control

Of the 629 samples genotyped, 11 were removed because of inconsistent patient identification. The call rate was 97% for both SNPs. Seventeen samples failed in the analysis of Arg389Gly (rs1801253), 14 samples failed in the analysis of Gln27Glu (rs1042714) and one sample failed in that of both SNPs.

Functional genotype group stratification

A priori, we set up three functional genotype combinations (Table 1). Statistical analysis did not show any difference in survival between these three groups among metoprolol-treated patients. Only seven carvedilol-treated patients had genotype combination C (*ADRB1* Gly389-carrier and *ADRB2* Glu27-homozygous; Table 1). To minimize the risk of type I error interpretation, subsequent statistical analysis and interpretation were made on a *post hoc* stratification, in which two groups of functional genotype combinations were defined (Table 1). Functional genotype group 1 consisted of patients homozygous for the Arg389 allele (*ADRB1*) and heterozygous or homozygous for the Gln27 allele (*ADRB2*) (identical with genotype group A). Functional genotype group 2 contained all other patients (genotype group B and C).

Statistical analysis

The chi-square test was used to calculate *P* values for the association of categorical covariates with the strata of functional genotype groups and β-adrenoceptor blocker treatments, and to establish whether allele frequencies

Table 2A

Baseline clinical characteristics according to *post hoc* functional genotype groups

Variable	Functional genotype group 1 (*n* = 269)	Functional genotype group 2 (*n* = 317)	*P* value
Demographic characteristic			
Age, mean (SD) (years)	69.9 (11.6)	69.8 (11.8)	0.96
Female gender, *n* (%)	71 (26)	87 (27)	0.78
Caucasian, *n* (%)	264 (98)	308 (97)	0.43
Medical history, *n* (%)			
DM	46 (17)	54 (17)	0.98
COPD	49 (18)	54 (17)	0.71
IHD	116 (43)	129 (41)	0.53
Atrial fibrillation	88 (33)	102 (32)	0.91
Previous coronary procedure, n (%)			
PCI	13 (5)	21 (7)	0.35
CABG	50 (19)	47 (15)	0.22
Medical status, *n* (%)			
β-adrenoceptor blocker	147 (55)	155 (49)	0.15
Metoprolol	91 (34)	104 (33)	0.75
Carvedilol	45 (17)	37 (12)	0.076
Bisoprolol	5 (2)	7 (2)	0.77
ACE inhibitor	208 (77)	256 (81)	0.35
ATII antagonist	28 (10)	23 (7)	0.17
Spironolactone	155 (58)	169 (53)	0.27
Diuretics	265 (99)	309 (97)	0.14
Insulin	21 (8)	21 (7)	0.57
Nolomirole	126 (47)	144 (45)	0.97
Clinical characteristic, mean (SD)			
WMI	0.85 (0.23)	0.84 (0.23)	0.79
NYHA class	2.30 (0.59)	2.25 (0.64)	0.35
Current smoker, *n* (%)	79 (39)	104 (33)	0.35

SD standard deviation; *n* number; ACE angiotensin-converting enzyme; AT angiotensin.

were in Hardy-Weinberg (HW) equilibrium. Univariate analysis of variance was used to calculate *P* values for the association of continuous covariates with the strata of functional genotype groups and *b*-adrenoceptor blocker treatments.

The end-point was defined as time to death from any cause. Survival data were graphically represented by Kaplan-Meier curves and statistically analyzed using the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (95% CIs) were calculated by Cox's proportional hazards model.

HRs and interaction analyses were adjusted in two multivariate models. In model 1, the following covariates associated with mortality at a significance level of $P < 0.15$ were included: NYHA class, gender, age, WMI, IHD, DM, COPD and use of angiotensin-converting enzyme (ACE) inhibitors, angiotensin-II (ATII) antagonists, insulin and spironolactone. In model 2, age, gender and covariates with an unequal distribution between genotype groups ($P < 0.05$) in Table 2B (carvedilol-treated patients) were included (NYHA class, ACE inhibitor).

In the ECHOS trial, the effects of nolomirole and placebo were compared. Nolomirole did not have an effect on the clinical outcome. There was no interaction between nolomirole and functional genotype group ($P = 0.39$). We therefore did not include placebo *vs*. nolomirole treatment in our analyses.

All statistical analyses were performed using SAS version 9.1.3 and the level of significance was pre-specified as $P < 0.05$ (two-sided). This level of significance was maintained for the *post hoc* stratification, since the primary variable was unchanged (A resp. 1).

Results

Allele distribution

No deviation from Hardy-Weinberg equilibrium was seen in the genotype distribution of Arg389Gly (rs1801253) ($P = 0.99$) or Gln27Glu (rs1042714) ($P = 0.31$).

Study population characteristics of the post hoc functional genotype group strata (Table 1)

Of a total of 586 patients, 269 (46%) belonged to functional genotype group 1 (high β_1-adrenergic receptor activity and down regulated β_2-adrenergic receptor) and 317 (54%) were classified into functional genotype group 2 (Table 2A). A total of 373 patients died during a median follow-up of 6.7 years (range 5.2 to 7.8 years). Three hundred and five patients were treated with β-adrenoceptor blockers at the

Table 2B

Baseline clinical characteristics for carvedilol-treated patients according to *post hoc* functional genotype groups

Variable	Functional genotype group 1 (*n* = 45)	Functional genotype group 2 (*n* = 37)	*P* value
Demographic characteristic			
Age, mean (SD) (years)	67.0 (13.1)	63.8 (13.2)	0.27
Female gender, *n* (%)	4 (9)	6 (16)	0.31
Caucasian, *n* (%)	45 (100)	37 (100)	1.00
Medical history, *n* (%)			
DM	10 (22)	9 (24)	0.82
COPD	9 (20)	6 (16)	0.70
IHD	21 (47)	13 (35)	0.25
Atrial fibrillation	14 (31)	7 (19)	0.20
Previous coronary procedure, *n* (%)			
PCI	1 (2)	2 (5)	0.44
CABG	9 (20)	3 (8)	0.12
Medical status, *n* (%)			
ACE inhibitor	35 (78)	35 (95)	0.025
ATII antagonist	6 (13)	2 (5)	0.22
Spironolactone	33 (73)	27 (73)	0.97
Diuretics	45 (100)	36 (97)	0.21
Insulin	5 (11)	3 (8)	0.65
Nolomirole	22 (49)	22 (59)	0.34
Clinical characteristic, mean (SD)			
WMI	0.79 (0.23)	0.80 (0.26)	0.92
NYHA class	2.58 (0.50)	2.27 (0.73)	0.027
Current smoker, *n* (%)	12 (27)	16 (43)	0.089

SD standard deviation; *n* number; ACE angiotensin-converting enzyme; AT angiotensin.

time of enrolment (195 with metoprolol, 82 with carvedilol, 17 with bisoprolol, 11 with other β-adrenoceptor blockers). No overall difference in the distribution of covariates was seen between functional genotype groups (Table 2A). Among carvedilol-treated patients 45 (55%) belonged to functional genotype group 1 and 37 (45%) belonged to functional genotype group 2 (Table 2B). In functional genotype group 1 there was a higher mean NYHA class (2.58 *vs.* 2.28) and fewer were treated with ACE inhibitors (78% *vs.* 95%) (Table 2B).

Compared with metoprolol-treated patients, those treated with carvedilol were characterized by higher mean NYHA-class (2.47 *vs.* 2.19), younger age (65.7 *vs.* 69.7 years), greater incidence of COPD (18% *vs.* 7%), smoking behaviour (34% *vs.* 23%) and male gender (88% *vs.* 68%) and were more likely to be receiving spironolactone treatment (73% *vs.* 54%).

Survival analysis according to post hoc *functional genotype group stratification (Table 1)*

An analysis of the interaction between functional genotype group (1 *vs.* 2) and carvedilol treatment (with *vs.* without treatment) revealed a significant interaction ($P = 0.003$; adjusted$_{\text{model 1}}$ $P = 0.033$, adjusted$_{\text{model 2}}$ $P = 0.040$). There was no interaction between functional genotype group and metoprolol treatment ($P = 0.61$; adjusted$_{\text{model 1}}$ $P = 0.92$).

In patients treated with carvedilol, survival was lower for those in functional genotype group 1 than those in group 2 (Figure 1A, $P = 0.004$). Twenty-seven of 45 patients in genotype group 1 died, and of 37 patients 16 died from genotype group 2. No difference in survival was seen between the two functional genotype groups among the metoprolol-treated patients (Figure 1B, $P = 0.82$): 47 of 91 patients died *vs.* 53 of 104 patients (genotype group 1 *vs.* 2).

No difference in overall survival was found between the two functional genotype groups (Figure 1C, $P = 0.69$): 163 of 269 patients died *vs.* 187 of 317 patients (genotype group 1 *vs.* 2).

Unadjusted and adjusted hazard ratios of post hoc *functional genotype groups*

In patients treated with carvedilol there was a significantly greater hazard of belonging to functional genotype group 1, as indicated by the unadjusted analysis (HR 2.30, 95% CI 1.28, 4.14; $P = 0.005$; Figure 2A) and when adjusted according to model 1 (HR 2.67, 95% CI 1.27–5.59; $P = 0.010$; Figure 2B) and model 2 (HR 2.05, 95% CI 1.06, 3.95; $P = 0.033$; Figure 2C). For patients treated with metoprolol there was no decreased hazard from belonging to functional genotype group 1 (unadjusted HR 0.96, 95% CI 0.66, 1.40; $P = 0.82$; adjusted HR$_{\text{model 1}}$ 1.02, 95% CI 0.68, 1.52; $P = 0.94$).

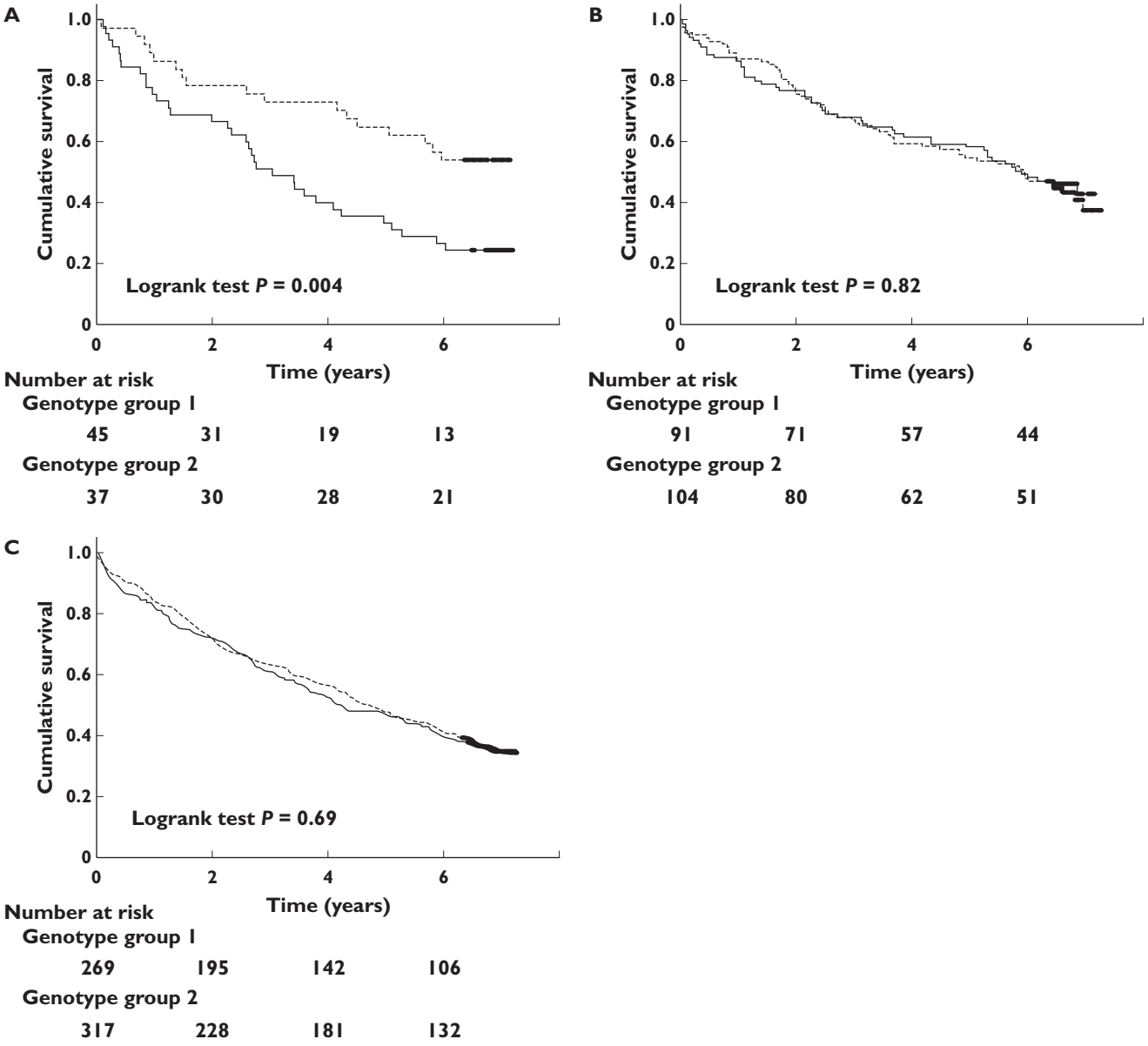

Figure 1

(A) Survival of carvedilol-treated patients according to *post hoc* functional genotype group stratification. Genotype group 1: *ADRB1* Arg389-homozygous and *ADRB2* Gln27-carrier. Genotype group 2: any other combination. (B) Survival of metoprolol-treated patients according to *post hoc* functional genotype group stratification. Genotype group 1: *ADRB1* Arg389-homozygous and *ADRB2* Gln27-carrier. Genotype group 2: any other combination. (C) Overall survival of patients according to *post hoc* functional genotype group stratification. Genotype group 1: *ADRB1* Arg389-homozygous and *ADRB2* Gln27-carrier. Genotype group 2: any other combination. Genotype group 1 (——); Genotype group 2 (----)

Other analyses

Survival according to a priori *functional genotype group stratification* (Table 1) Among metoprolol-treated patients there was no difference in survival between the three genotype groups ($P = 0.83$). For the patients treated with carvedilol there was a difference in survival between genotype group A (34 of 45 patients died) and B (12 of 30 patients died) ($P = 0.001$). Unadjusted the HR of genotype group A *vs.* B was 2.90 (95% CI 1.50, 5.63; $P = 0.0016$) and adjusted$_{\text{model 1}}$ 3.04 (95%CI 1.31, 7.04; $P = 0.0095$) and adjusted$_{\text{model 2}}$ 2.63 (95% CI 1.22, 5.66; $P = 0.014$) (data not shown). There was also a difference in survival between group C (five of seven patients died) and B ($P = 0.036$). Unadjusted HR of C *vs.* B was 2.91 (95% CI 1.02, 8.32; $P = 0.0095$), but this was insignificant when adjusting according to model 1 ($P = 0.15$) and model 2 ($P = 0.095$).

Survival and Arg389Gly and Gln27Glu polymorphisms The Arg389Gly polymorphism (Arg-homozygous *vs.* Gly-carrier) was not associated with overall mortality ($P = 0.77$),

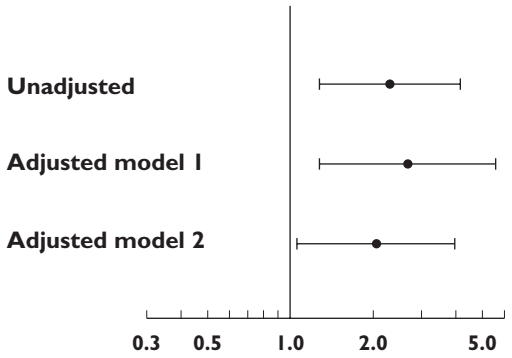

Figure 2

Hazard ratios (HR) (genotype group 1 *vs.* 2) in carvedilol-treated patients (bars represent 95% CI). Unadjusted (HR 2.30, 95% CI 1.28, 4.14; $P = 0.005$). Adjusted model 1 (HR 2.67, 95% CI 1.27, 5.59; $P = 0.010$). Adjusted model 2 (HR 2.05, 95% CI 1.06, 3.95; $P = 0.033$)

and no drug–gene-specific interaction was seen between this SNP and mortality in regard to treatment with carvedilol ($P = 0.13$) or metoprolol ($P = 0.62$). Moreover, for the Gln27Glu polymorphism (Gln-carrier *vs.* Glu-homozygous) no association was found with overall mortality ($P = 0.96$), mortality in carvedilol-treated patients ($P = 0.32$) or metoprolol-treated patients ($P = 0.33$).

Drug–gene and drug–drug interaction with nolomirole
There was no interaction between nolomirole treatment and genotype group ($P = 0.39$) or nolomirole and carvedilol treatment ($P = 0.76$). Adjusting for nolomirole had no effect on the HR (model 1 adjusted HR 2.65, 95% CI 1.26, 5.58; model 2 adjusted HR 2.04, 95% CI 1.06, 3.96).

Discussion

The main finding of this study is that carvedilol treatment of HF patients, who are characterized by a combination of high β_1-adrenergic receptor activity (Arg389-homozygous) and down regulated β_2-adrenergic receptors (Gln27-carrier), have a significant 2.30-fold (adjusted, 2.67-fold$_{\text{model 1}}$, 2.05-fold$_{\text{model 2}}$) greater mortality than that of patients with any other genotype combination. Patients from the two functional genotype groups appear to have the same response to metoprolol treatment. In our cohort, patients with the combination of high β_1-adrenergic receptor activity and down regulated β_2-adrenergic receptors (functional genotype group 1, Table 1) constituted 46% of the population. Our results suggest that there is a clinically important gene–β-adrenoceptor blocker interaction, and that carvedilol in a genetically defined subgroup of HF patients may result in less clinical benefit than in other HF patients.

The β-adrenoceptor blockers metoprolol and carvedilol have both proven effective in the treatment of heart failure [2, 3]. Although their pharmacodynamic profiles are different, a distinction between these two β-adrenoceptor blockers is not made in clinical practice. The COMET trial found a superior effect of carvedilol over metoprolol, but this is one in only few clinical studies that addresses the different efficacies of the two β-adrenoceptor blockers in question [25]. On a molecular level carvedilol is a non-selective β-adrenoceptor blocker with agonist binding properties, whereas metoprolol is a selective β_1-receptor blocker with typical antagonist binding properties [12]. Lately, research in ligand-directed signalling has shown that metoprolol and carvedilol act differently on the same receptor [26].

The present study implies the existence of a drug-specific interaction between carvedilol and a combination of two SNPs in *ADRB1* and *ADRB2* that does not seem to occur with metoprolol. It is suggested that carvedilol may have a less beneficial effect in a subgroup of HF patients with a physiologically 'active' β_1-adrenergic receptor (Arg389-homozygous) in combination with a physiologically down regulated β_2-adrenergic receptor (Gln27-carrier) [5, 9, 27].

We believe that this specific drug–gene interaction is plausible, because carvedilol indirectly might stimulate the phenotypically active β_1-receptor through further down regulation of this receptor. In fact, it has been shown that carvedilol does not restore the catecholamine-induced receptor down regulation or might even induce further down regulation [4, 12, 14, 15], and that down regulation of myocardial adrenergic β-receptors makes them supersensitive to agonist stimulation [13, 28, 29]. In contrast, metoprolol is known to up regulate the receptors [4, 12, 14, 15]. The increased level of catecholamines and the subsequent stimulation of the β-adrenergic receptors are believed to be crucial elements in the deterioration of heart failure [30], and it is important to block the β-adrenergic receptors that have the strongest response to catecholamine stimulation. Though speculative, carvedilol might have a paradoxical effect on the adrenergic β_1-receptor in the setting of an increased intracellular cAMP-production which sensitizes the receptor to agonist stimulation. It is also likely that carvedilol exerts its effects mainly through the action on the adrenergic β_2-receptor as indicated by Molenaar *et al*. [31], and therefore in the setting of down regulated adrenergic β_2-receptors lacks this way of functioning.

As these studies indicate that metoprolol and carvedilol have different effects in regard to the two distinct phenotypic profiles of the two receptors in question, *in vivo* data also suggest differences in drug–gene interaction between these two β-adrenoceptor blockers. With regards to LVEF, blood pressure and heart rate responses metoprolol has been shown mainly to interact with the *ADRB1* Arg389Gly-polymorpism [6, 8, 32], whereas carvedilol most consistently has been shown to interact with the *ADRB2* Gln27Glu-polymorphism [10, 11].

Despite these convincing data on SNP–drug interaction no studies concerning metoprolol or carvedilol have been able to show a stratified survival response when stratifying for the two SNPs in question as well as other SNPs in the two genes [16–19]. Only treatment with the β-adrenoceptor blocker bucindolol have been able to stratify patients according to a single SNP (*ADRB1* Arg389Gly) [33], and it has therefore been suggested that stratifying for specific genotype combinations might prove better [20, 21]. This is why we conducted the present study.

The present study is limited by its size and design. A larger population would allow further grouping of genotype combinations, making it possible to reveal interactions of metoprolol or carvedilol with other specific genotype combinations. Only seven patients with the *a priori* genotype combination C (Table 1) were on carvedilol treatment, so we were not able to verify our hypothesis that this genotype combination was associated with increased mortality in metoprolol-treated patients and decreased mortality in carvedilol-treated patients. On the contrary, increased mortality was indicated for the carvedilol-treated patients, but because of the aforementioned reasons (small sample size, not prespecified) we consider this a chance finding. Furthermore, we were not able to verify our hypotheses of interaction between metoprolol and the two specific genotype combinations A and C (Table 1). This may also be explained by the low statistical power of the sample, and the lack of information about pharmacokinetic conditions, e.g. metoprolol concentration. The groups of metoprolol- and carvedilol-treated patients were not matched, and because of confounding by indication (i.e. more ill patients tend to be treated with carvedilol), it is not possible to determine whether either of the β-adrenoceptor blockers is superior to the other in functional genotype group 1. This comparison of metoprolol and carvedilol needs to be made in a randomized study with matching groups.

The results would have been strengthened by supplementary end-points such as hospitalization and death due to HF and sudden cardiac death. Since the mortality rate due to HF is high even with the benefit of β-adrenoceptor blockers [34], it is nevertheless likely that the main cause of death is HF or sudden cardiac death.

Since we only have information about β-adrenoceptor blocker treatment at the time of enrolment, the possibility that changes in β-adrenoceptor blocker treatment and dose affected our results cannot be discounted. However, this is not expected to be a major problem though, since a Danish register-based study concluded that the persistence with β-adrenoceptor blocker treatment specifically in HF patients is high [35].

In conclusion, this is the first study to indicate a drug–gene-specific interaction of β-adrenoceptor blocker treatment with a functional genotype combination of the single nucleotide polymorphisms Arg389Gly (*ADRB1*) and Gln27Glu (*ADRB2*). It is one of only a few cardiovascular pharmacogenetic studies to show that functional genotype combinations of interacting SNPs in the genes *ADRB1* and *ADRB2* contribute to the understanding of individual responses to β-adrenoceptor blocker treatment in HF patients.

We acknowledge the findings of the present study to be hypothesis-generating. Nevertheless, it is apparent that before beginning β-adrenoceptor blocker treatment, HF patients might benefit from a genetic test for the genotype combinations of Arg389Gly and Gln27Glu, since this would enable the optimal choice of treatment with the three recommended β-adrenoceptor blockers, especially carvedilol. A properly designed trial in which patients with this genotype combination are randomized to carvedilol or metoprolol treatment is required to verify whether patients in functional genotype group 1 might benefit more from metoprolol than from carvedilol.

Competing Interests

All authors declare that they have no competing interests.

This study did not receive any funding or aid from any pharmaceutical company.

The study was supported by grants from the Danish Heart Foundation, the Grosserer Valdemar Foersoms Foundation, the Research Board of Rigshospitalet (Copenhagen University Hospital), the Capital Region of Denmark, and the Danish Agency for Science, Technology and Innovation.

We would like to thank Michael Christiansen (Statens Serum Institut, Copenhagen) for DNA purification.

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