Abstract

The Role of the Beta-1,2-adrenergic and GRK5 Receptor Single-Nucleotide-Polymorphisms (SNPs) on the Treatment of Heart Failure with Beta-blockers

Hobart Lee Rogers Jr., Doctor of Philosophy 2010

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Despite recent advances in medical treatment, heart failure still remains a primary cause of mortality in the U.S. Pharmacologic β-blockade using carvedilol, metoprolol succinate, or bisoprolol in heart failure is associated with reduced mortality and morbidity and considered a class I recommendation in the consensus guidelines for the treatment of systolic heart failure. Nevertheless, β-blockers are still underused in this patient population. Additionally, significant interpatient variability in response to these agents exists. The differential response to β-blockade may be due to genetic heterogeneity of the β-adrenergic receptor (β-AR), in the form of single-nucleotide-polymorphisms (SNPs). SNPs with functional differences have been identified on the β1-AR at positions 49 (Ser/Gly) and 389 (Arg/Gly) and β2-AR at positions 16 (Gly/Arg) and 27(Gln/Glu). Furthermore, a functional SNP on the G-protein coupled receptor kinase (GRK) has also been identified. These SNPs have all been linked to significant differences in receptor expression and function in experimental models. The objective of this study was to characterize the clinical implications of the β1,2-AR and GRK SNPs on the treatment of heart failure with β-blockers. Eighty-six NYHA class II-III heart failure patients receiving maximum titrated doses of either carvedilol or metoprolol succinate were enrolled in this study.

Heart rate (HR) was measured at rest and immediately following a 6-minute walk test, and a blood sample was obtained for genotyping. Genotypes were determined by Taqman genotyping assays. To account for plasma β-blocker concentrations, two novel enantioselective HPLC assays with fluorescence detection were developed. Our primary finding was that metoprolol-treated patients who were homozygous for Arg at position 389 on the β1-AR had both an enhanced negative chronotropic and antihypertensive response to β-blockade when corrected for plasma *S*-metoprolol concentrations. When evaluating the β2-AR SNPs, both the carvedilol-treated Gly16 homozygotes and the Glu27 carriers had a significantly enhanced antihypertensive response compared to their subsequent counterparts. These findings suggest that SNPs in both the β1,2-ARs are associated with clinical response to either carvedilol or metoprolol succinate. Moreover, our findings emphasize the importance of accounting for pharmacokinetic differences when evaluating pharmacodynamic relationships.

THE ROLE OF THE BETA-1,2-ADRENERGIC AND GRK5 RECEPTOR SINGLE-NUCLEOTIDE-POLYMORPHISMS ON THE TREATMENT OF HEART FAILURE WITH BETA-BLOCKERS

by

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Foreward

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Introduction: Heart Failure and the β-Adrenergic Receptor Single-Nucleotide-Polymorphisms

1.1 Introduction - Heart Failure

1.1.1 Pathophysiology

According to the American Heart Association, heart failure is defined as a pathophysiological state in which the heart is unable to pump blood at a rate that is sufficient to meet the metabolic needs of the body. Heart failure arises from a number of cardiovascular disorders, with the most common etiologies being hypertension, ischemic heart disease and dilated cardiomyopathy. Regardless of the cause, heart failure patients tend to follow a similar progression once they develop the disease. Clinically, signs and symptoms of heart failure include shortness of breath, coughing, peripheral and pulmonary edema and exercise intolerance.

Despite the initial precipitating event, heart failure progressively becomes a systemic disease that eventually leads to death or cardiac failure. The progression of heart failure is driven by neurohormones and autocrine/paracrine factors. Amongst these neurohormones are the cardiotoxic catecholamines, norepinephrine and epinephrine, which play a central role in the deleterious compensatory process in heart failure. Heart failure is characterized by an upregulation of catechols as a response of organs sensing decreased perfusion. This heightened sympathetic nervous system activity acts to increase cardiac contractility and output initially, but consequently places more stress on a failing heart. In addition, this compensatory mechanism also causes ventricular hypertrophy and remodeling and if left untreated eventually leads to death.[1-3] Animal studies further support this role as transgenic mice over expressing beta-

1-adrenergic receptors (β1-ARs) develop myocardial hypertrophy and ventricular dilation. They also have increased amounts of apoptotic myocytes and eventually develop heart failure.[4]

1.1.2 Heart Failure Statistics

As of 2009, heart failure remains a major cause of mortality and hospitalizations. According to the American Heart Association, in 2009 the estimated total cost of heart failure will be over 37.2 billion dollars.[5] Currently, almost 5 million Americans are diagnosed with heart failure, with overall 5-year survival of approximately 40%.[6] This mortality rate increases with New York Heart Association (NYHA) stage of heart failure. There are four stages of heart failure based upon the patient's functional limitations. Patients with NYHA stage IV heart failure have the lowest functional status based on exercise capacity and have a one-year mortality rate of approximately 50%.[7]

1.1.3 Treatment of Heart Failure with β-blockers

There are numerous drug therapies used in the treatment of heart failure. (Table 1.1) Some of the benefits of drug therapy include reduced mortality/morbidity and hospitalizations as well as symptomatic relief. Of these agents, pharmacologic β-antagonists (β-blockers) are the mainstay of therapy for treating heart failure. Certain β-blockers have been shown to decrease mortality and hospitalizations and provide symptomatic improvement. Bisoprolol, metoprolol and carvedilol have not only been shown to decrease mortality and hospitalizations, but also improve quality-of-life and hemodynamic measures. [8]

Table 1.1 Cardiovascular drugs used in the treatment of heart failure

Drug Class	Examples
β-blocker	Carvedilol, Metoprolol (succinate)
Angiotensin converting enzyme (ACE) inhibitor	Lisinopril, Enalapril, Ramipril
Angiotensin receptor blocker (ARB)	Lostartan, Candesartan, Valsartan
Diuretics	Furosemide, Metolazone, Bumetanide
Cardiac Glycosides	Digoxin
Aldosterone Antagonists	Spironolactone, Aldactone

Numerous large-scale clinical trials have indicated an approximate 30-40% reduction in mortality for both carvedilol and metoprolol succinate.[9, 10] Both agents are currently approved by the FDA for treatment of heart failure.

Despite being in the same pharmacological class, there are some important differences in the pharmadynamic and pharmacokinetic properties of metoprolol succinate and carvedilol (Table 1.2). Metoprolol is administered as a racemic mixture of *S*-metoprolol and *R*-metoprolol. Metoprolol is a second generation β-blocker that has an approximate 75-fold greater affinity for the β1-AR compared to the β2-AR.[8] However, at higher doses (> 200 mg/day) metoprolol is known to also bind to the β2-AR.[11,12]

Metoprolol is primarily metabolized via the highly polymorphic cytochrome P450D6 (CYP2D6) pathway, which can significantly vary the rate of metabolism.[13] Zineh *et al.* showed that there was a 30-fold variation in plasma *S*-metoprolol concentrations within patients that were identified as CYP2D6 extensive metabolizers.[14] Hence, wide variations in the pharmacokinetic response to metoprolol are common.

Similar to metoprolol, carvedilol is also administered as a racemic mixture. The *S*-enantiomer is responsible for the β-blocking activity, whereas the *R*-enantiomer is responsible for the α-1-blocking activity of the drug. Unlike metoprolol, carvedilol has similar affinity for both the β1 and β2-ARs.[8, 15] Carvedilol is also primarily metabolized via the liver via the CYP2D6 pathway. As previously mentioned, polymorphisms of the CYP2D6 metabolizing enzyme can alter serum levels of carvedilol. Previous studies have found that the oral

Table 1.2 Pharmacodynamic and pharmacokinetic properties of Metoprolol and Carvedilol

	Metoprolol succinate	Carvedilol
β-AR affinity	β1 > β2	β1 = β2
R-enantiomer	No α-1 activity	α1-antagonist
Max dose	200mg once daily	25mg twice daily
Metabolism	Hepatic via CYP4502D6	Hepatic via CYP4502D6
t1/2 (hours)	3-7; 24 sustained release	7-10
Vd_{ss} (L/kg)	5.6	1.6

clearance of carvedilol is highly correlated with volume of distribution, which suggests that the interindividual difference in bioavailability is largely responsible for the pharmacokinetic variability of carvedilol.[16]

Fortunately, pharmacotherapy using pharmacologic β-antagonists is an effective means of blocking the deleterious effects of the cardiotoxic catecholamines that are upregulated in heart failure. These agents not only help to prevent detrimental cardiovascular remodeling and hypertrophy, but also act to lower heart rate. The magnitude of heart rate reduction due to β-blockade is closely tied with mortality in heart failure patients.[17] Hence, β-blockers are one of the primary treatments for patients with heart failure.

Despite their purported benefits, β-blockers are still underutilized in the heart failure patient population mainly due to fear of decompensation.[8,18] Moreover, β-blocker therapy is known to produce widely variable responses in patients.[8] A component of this variability may be attributed to genetic heterogeneity at the level of the beta-adrenergic receptor (β-AR). Numerous single-nucleotide polymorphisms (SNPs) have been identified at both β1 and β2-ARs. Differences in expression and functionality of β1,2-AR SNPs have been well documented. However, their role in the variability of β-blocker treatment in heart failure is still unclear.

1.1.4 β-ARs and Their Role in Heart Failure

β-ARs are 7-transmembrane G-protein coupled receptors (GPCRs) with an extracellular amino terminus and an intracellular carboxy terminus. There are three different signaling pathways associated with GPCRs, stimulatory G-protein

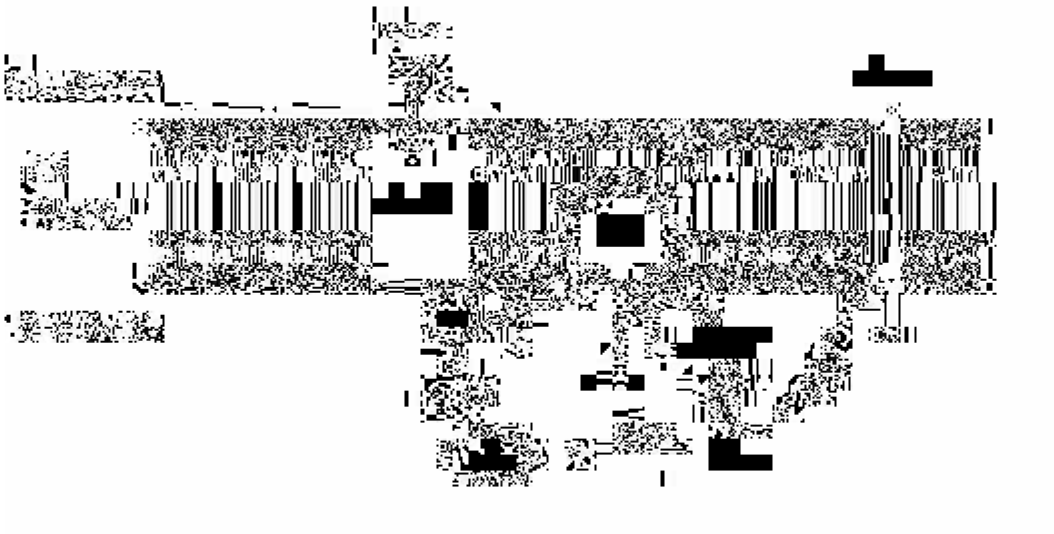
(G_s), inhibitory G-protein (G_i) and $G_{q/11}$ which has variable roles. Of these, β-adrenergic receptors (β-ARs) are mainly coupled to G_s, which mediate downstream signaling through adenylyl cyclase-generated cAMP.

In addition, there are three known subtypes (β1,β2, and β3) of the β-adrenergic receptors (β-ARs) found in cardiac tissue. β1 and β2 play a role in both chronotropy (heart rate) and ionotropy (contractility). However, the role of the β_3 in the heart still has not been elucidated.[19, 20] β1,2-ARs are primarily coupled to the stimulatory G-protein (G_s), while the β2-AR may also activate the inhibitory G_i pathway.[21]

As mentioned, the β1-ARs are located mainly on cardiac myocytes and comprise about 80% of all β-receptors in the healthy heart.[8] However, in the case of heart failure, downregulation of β1-ARs due to chronic adrenergic signaling drops the ratio of β1 to β2-ARs to approximately 60:40.[20] In heart tissue, their key function is to regulate blood pressure through both positive inotropic and chronotropic effects. Normal agonist-induced stimulation by norepinephrine or epinephrine leads to activation of adenylyl cyclase (through its coupling to Gs-protein), which increases intracellular cAMP levels. This activates protein kinase A to phosphorylate troponin I, the L-type calcium channels, and phospholamban. The final result of this pathway is a greater influx of calcium into the cardiac myocyte and consequent enhanced contractility.[22] (Figure 1.1)

The β2-AR is also located on cardiac myocytes and highly expressed throughout the cardiovascular system and airway smooth muscle with lower expression in the brain and adipose tissue. Similar to β1-ARs, they act to

Figure 1.1 Schematic diagram of the β-AR and downstream signaling



Pathway depicting activation of the β-AR by agonist. 1. Agonist binds activating receptor. 2. Stimulatory G-protein (Gs) activates adenylyl cyclase (AC). 3. Adenylyl cyclase phosphorylates cyclic adenosine monophosphate (cAMP). 4. cAMP then phosphorylates protein kinase A (PKA). 5. PKA phosphorylates L-type calcium channels allowing for increased intracellular calcium for greater ionotropic and chronotropic effects.   6. In the case of chronic agonist signaling, PKA also phosphorylates G-protein receptor kinase (GRK) which activates beta-arrestin which uncouples Gs from the β-AR.

regulate blood pressure and contractility as well as arterial vasodilation. Like the β1-AR the β2-AR is also coupled to G_s-protein, however it is also coupled to the G_i-protein. The β2-AR exists in multiple polymorphic forms. The role of these polymorphic forms and their G_i-protein coupling have not been fully elucidated in heart failure.[23]

1.1.5 The Role of Genetic Heterogeneity in the β-AR

β1-AR SNPs

The β1-AR is encoded by an intronless gene located at chromosome 10q24-26.[24] To date, 12 single-nucleotide polymorphisms (SNPs) for the β1-AR have been discovered, occurring primarily in either the extracellular or intracellular portion. Thus, the transmembrane region of the receptor appears to be highly conserved. The two most common SNPs occur at codon positions 49 (extracellularly) and 389 (intracellularly) on the β1-AR. (Table 1.3)
The remaining SNPs do not occur at high enough frequencies (less than 0.1%) to be of major clinical significance. At codon 49, the most commonly occurring amino acid residue is Serine substituted by the variant Glycine (Ser49Gly). At codon 389, the most commonly occurring amino acid residue is Arginine replaced by a Glycine (Arg389Gly). Following the definition of a polymorphism, both these variants occur in frequencies greater than 1%. The variant allele at position 49 Gly occurs with a frequency of about 0.15 in Caucasians and Asians and approximately 0.29 in African Americans, while at position 389 Gly occurs in about 0.27 in Caucasians and Asians and approximately 0.42 in African Americans.[25] The extracellular Ser49Gly polymorphism of the protein is

Table 1.3 Common SNPs of the β1,2-ARs and their frequencies

Receptor	Location	Position	Receptor Variant	Racial Frequency of Variant Allele (White/Asian/Black)	Variant Allele Effect
β1-AR	extracellular	49	Ser→Gly	0.15 / 0.15 / 0.29	Increased agonist-promoted downregulation
β1-AR	intracellular	389	Arg→Gly	0.27 / 0.27 / 0.42	Hyporesponsive receptor
β2-AR	extracellular	16	Gly→Arg	0.46 / 0.59 / 0.49	Decreased agonist-induced desensitization
β2-AR	extracellular	27	Gln→Glu	0.35 / 0.07 / 0.20	Resistance to desensitization

responsible for receptor expression and regulation. Conversely, the intracellular Arg389Gly is responsible for receptor functioning and G-protein coupling.[26]

The functional relevance of each of these receptor polymorphisms has been studied using *in vitro* techniques. At position 49 on the β1-AR the Gly variant undergoes an increased amount of agonist promoted down-regulation compared to the Ser variant.[22, 25] Thus it suggests that the Gly49 variant could offer some protective effect from the cardio-toxic catecholes in heart failure.

With respect to the β1-AR polymorphism at codon 389, Rochais discovered that both the Arg and Gly variants have similar basal constitutive activity in terms of cAMP generation.[27] However, *in vitro* studies using Chinese Hamster Fibroblast cells have demonstrated that the Gly389 variant has 3-fold lower levels of isoprenaline stimulated adenylyl cyclase activity when compared to the Arg389 genotype.[28] Further investigation found that these differences were due to greater coupling of the Arg389 receptor to G_s-protein.[29] Hence, the Gly389 variant is deemed as "hyporesponsive" in terms of agonist induced activation.

Rochais *et al*. also demonstrated that carvedilol, metoprolol and bisoprolol all act as inverse agonists at both of the 389 variants in living rat myocytes. However, in terms of secondary messengers, only carvedilol decreased basal levels of cAMP and significantly decreased cAMP concentrations in the Arg variants compared to the Gly variants. The Arg389 variant seems to be uniquely sensitive to carvedilol.[27] Nonetheless, the clinical implications of these findings are unknown.

β2-AR SNPs

The β2-AR is encoded by an intronless gene located at chromosome 5q31-32. Similar to the β1-AR, the β2-AR not only couples to the stimulatory protein

G_s, but also couples to the inhibitory protein G_i. In case of prolonged agonist activation, the β2-AR does not undergo downregulation like the β1-AR, but rather desensitization.[30] Genetic heterogeneity in the form of SNPs also occurs on the codon for the β2-AR. Of the polymorphisms identified for the β2-AR, two (position 16 and 27) have been shown to alter receptor expression and functioning.[25] At codon 16, the commonly occurring Gly residue is substituted with Arg and at codon 27, the commonly occurring Gln is substituted with Glu. It is estimated that the Arg16 allele occurs with a frequency of about 0.46 in Caucasians and 0.49 in African Americans, and is often referred to as the wild type. The Glu27 occurs with a frequency about 0.35 in Caucasians and about 0.20 in African Americans. (See Table 1.3)

The functional relevance of each of these β2-AR polymorphisms has been studied using *in vitro* techniques. Both of the mentioned SNPs on the β2-AR occur in the extracellular portion thus affecting ligand binding and receptor desensitization. At position 16 the Arg variant depressed agonist-promoted receptor desensitization. At position 27 the Glu variant also exhibits depressed agonist-promoted desensitization.[23, 31]

1.1.6 The Role of the β1,2-AR SNPs in Heart Failure

Numerous clinical studies have evaluated the role of the β1-AR SNPs in heart failure. In terms of hemodynamics, studies suggest that patients homozygous for the Arg389 allele had an increased resting heart rate as well as increased exercise capacity compared to the Gly389 carriers.[32-34] At codon 49 no significant associations between hemodynamics have been identified. Taken together the β1-AR polymorphisms alone are not directly related to development of heart failure. However, they may modify the disease and its response to drug treatment.

Current research suggests that both the Arg16 and Glu27 variants are associated with increased exercise capacity in heart failure patients.[23] In a study to examine associative properties of the β2-AR SNPs and risk of heart failure, Liggett *et al*. found no association with prognosis of heart failure at either position 16 or 27.[35] In concurrence with previous results, Covolo *et al*. found no role of any of the mentioned β1,2-AR SNPs as risk factors for heart failure.[36] Overall, the β1,2-ARs appear to have no predictive role of the development of heart failure.

1.1.7 The Role of the β1,2-AR SNPs in Heart Failure Patients Treated With β-Blockers

Few published studies have evaluated the relationship between β1,2-AR polymorphisms and response to β-blockers in heart failure. In practice, β-blockers are widely used in the treatment of both heart failure and hypertension.[8, 37] In heart failure, β1-AR antagonists have been associated with increases in myocardial β1-AR density and improved hemodynamic responses.[36] It could be expected that variations in β1-AR expression due to the Ser49Gly polymorphism or response to agonist via the Arg389Gly polymorphism might have clinical implications. In one of the few studies evaluating β1-AR polymorphisms in response to initial titration of β-blockers in heart failure patients, Terra *et al*. found that a greater percentage of Gly389 carriers required an increase in heart failure medications when compared to the Arg389 homozygotes. Thus, suggesting it may be necessary to treat heart failure patients with other medications based upon their β1-AR genotype. It is also important to note that this study was conducted during the initial titration phase of metoprolol and not in patients on chronic doses.

Similar work was conducted by de Groote *et al*. who analyzed the association between β1,2-AR polymorphisms and the effects of the β-blockers bisoprolol and carvedilol in patients with heart failure.[38, 39] This group reported a lack of an association between the 4 previously mentioned β1,2-AR SNPs and resting heart rate or change in LVEF following β-blockade. Limitations of this study are that it only evaluated carvedilol and resting heart rate and failed to correct for plasma β-blocker concentrations.

In contrast, Kaye *et al*. reported a positive association between β2-AR genotype and carriers of the Glu27 allele (both hetero and homozygotes) and improvement in LVEF following treatment with carvedilol. It is important to note that this study found no associations to either genotype at position 16 and response to carvedilol.[40] This study opposes the findings of de Groote *et al*., and offers the first evidence that β2-AR genotype may be associated with the clinical response of heart failure patients receiving carvedilol.

In one of the most compelling studies to date, Liggett *et al*. genotyped patients who had participated in a previous clinical trial (BEST), testing the efficacy of the β-blocker bucindolol on mortality in heart failure patients. The initial finding of the trial showed that bucindolol resulted in no favorable effects in heart failure patients when compared to placebo. However, when the patient's data was reanalyzed and patients stratified by β1-AR genotype, numerous significant findings resulted. Based on the β1-AR genotype of these patients, they found that the Arg389 homozygotes had an age, sex and race-adjusted 38% mortality reduction (*P* = 0.03), while the Gly389 carriers had no clinical response to bucindolol similar to placebo.[41] This study is the first to demonstrate a difference in clinical response based solely upon genotype at position 389 of the β1-AR.

1.1.8 G-Protein-Coupled Receptor Kinases

There are numerous pathways that mediate adrenergic signaling through the βARs that play a significant role in heart failure. Of these, G protein-coupled receptor kinases (GRKs) are known to desensitize ligand bound βARs and are part of an important feedback mechanism in heart failure (see Figure 1.1). GRKs act by turning-off the destructive feedback of excessive catechol stimulation in early heart failure.[42] Thus, GRKs can function as an endogenous form of β-blockade by switching off both active β1,2-ARs.[43, 44]

Within the GRK superfamily there are 2 isoforms that are especially important in cardiac functioning. Both GRK2 and GRK5 are the predominate forms found in the myocardium.[45, 46] More specifically, GRK2 and GRK5 are important in heart failure as they downregulate βAR signaling though phosphorylation of cardiac βAR, which activates β-arrestin, thus leading to receptor uncoupling from G proteins and subsequent downstream activation.[47] Unlike GRK2 however, GRK5 does not undergo upregulation or downregulation as acutely.[48] Therefore, GRK5 is thought to be more important than GRK2 for chronic βAR regulation as in the case of heart failure.[48] It has been postulated that GRK5-mediated βAR desensitization may be beneficial during ventricular decompensation before overt heart failure occurs.[42]

1.1.9 GRK5 Polymorphism

Similar to the β-ARs, genetic variability occurs in the form of SNPs on the GRK5 codon and plays a significant role in GRK function. A functional polymorphism in the GRK5 isoform was recently discovered at codon 41 in which a wild type glutamine (Glu) residue was substituted by leucine (Leu). In chinese hamster ovary (CHO) cells, GRK5-Leu41 variants were shown to have an approximately 25% lower rate of cAMP accumulation compared to the wild type Gln41 cells. This finding is indicative that the GRK5-Leu41 polymorphism decreases β1-AR signaling by increasing agonist-promoted desensitization, similar in nature to pharmacological β-blockade.[44, 49] Thus, *in vitro* work has identified the GRK5 polymorphism at codon 41 to be of functional significance.

1.1.10 GRK5 Polymorphism and Heart Failure

Despite large differences in allele frequencies of the GRK5 codon 41 SNP between African Americans and Caucasians, no predictive association of the SNP with the development of either heart failure or ischemic cardiac disease has been identified within either race.[42] While not predisposing individuals for heart failure, the GRK5-Leu41 polymorphism was however shown to confer a survival benefit similar to pharmacological β-blockade in African Americans with heart failure.[44] This same benefit was not observed in European Americans, most likely due to their lower allele frequency (0.02) compared to (0.35) in African Americans respectively.[42] Currently, no other prosepective studies have been conducted evaluating the pharmacogenomic interaction of the GRK5 SNP at position 41 and response to β-blockade.

1.2 Hypotheses and Specific Aims

Based on this background, it was hypothesized that genetic heterogeneity within the β1,2-ARs and GRK5 are partly responsible for variable responses to drug treatment with β-blockers. More specifically, it was hypothesized that heart failure patients being treated with a β-blocker (carvedilol or metoprolol) who were homozygous for the Arg allele at position 389 on the β1-AR would have an enhanced response (i.e. decreased heart rate following sub-maximal exercise) when compared to carriers of the Gly allele.

These hypothesis were tested with the following specific aims presented in this dissertation:

Aim 1. Determine the relationship between β1,2-AR and GRK5 genotype and clinical response in heart failure patients treated with β-blockers.

The primary hypothesis of this study is that patients being treated with a β-blocker (carvedilol or metoprolol) who are homozygous of the Arg allele at position 389 on the β1-AR will have an enhanced response (i.e. decreased heart rate following sub-maximal exercise) when compared to the Gly389 carriers. In addition the other previously mentioned β1,2-AR and GRK5 SNPs were also evaluated for all endpoints of the study. Hence, this study characterized the relationship between genetic heterogeneity at the level of the β-AR and clinical response to β-blockade with either carvedilol or metoprolol extended release.

Aim 2. Determine whether β1,2-AR genotype is associated with the clinical degree of β-blockade between metoprolol and carvedilol.

It was hypothesized that genetic variability at the β1-AR would not play a role in the clinical degree of β-blockade between metoprolol and carvedilol. Thus, it was predicted that there would be no difference in the clinical degree of β-blockade between metoprolol and carvedilol based upon β1-AR genotype. The ability to lower heart rate during exercise (negative chronotropism) was used as the primary means of assessing the clinical degree of β-blockade. Additionally, a number of other endpoints were used to characterize the clinical response in heart failure patients receiving either agent.

Aim 3. Determine the relationship between β1,2-AR and GRK5 genotype and β-blocker dose level.

It was hypothesized that patients who are homozygous for the Arg389 allele would require lower doses of β-blocker (carvedilol or metoprolol succinate) to maintain a similar resting heart rate as their Gly389 carrying counterparts. Hence, this study investigated the role of β-AR genotype on the dose of β-blocker received in heart failure patients maximally titrated to either carvedilol or metoprolol succinate.

Aim 4. Evaluate the role of pharmacokinetic variability in response to β-blockade.

The degree of pharmacokinetic variability was characterized relative to pharmacodynamic outcomes observed based on the genetic heterogeneity at the β-AR. It was hypothesized that there would be no significant difference in β-blocker pharmacokinetics based upon β1-AR genotype. To account for pharmacokinetic variation between patients, plasma carvedilol or metoprolol concentrations were determined using a novel stereospecific HPLC assay.

Chapter 2

The Clinical Implications of the β1,2-adrenergic and GRK5 Receptor SNPs on the Treatment of Heart Failure with β-blockers

2.1 Evaluation of the relationship between β1-AR genotype and clinical response in heart failure patients treated with β-blockers.

2.1.1 Introduction

Pharmacologic β-blockade using carvedilol, metoprolol succinate, or bisoprolol in heart failure is associated with reduced mortality and morbidity and considered a class I recommendation in the consensus guidelines for the treatment of systolic heart failure.[50] Meta-analysis has shown that the magnitude of HR lowering of β-blockers in heart failure is significantly associated with survival benefits.[17] Despite their purported benefits, β-blockers are still underutilized in patients with heart failure most likely due to a perceived risk of decompensation.[8,18] It is also known that individual response to β-blocker therapy is highly variable.[8] A component of this variability may be attributed to genetic heterogeneity of the β1-AR. Numerous single-nucleotide polymorphisms (SNPs) have been identified on the β1-AR.[51] Of these, two common polymorphisms Ser49Gly and Arg389Gly have been most widely studied. Differences in expression and functionality of these β1-AR SNPs have been well documented.[28, 29, 52-54]

Several studies have shown significant associations between β-AR polymorphisms and blood pressure response to β-blockers in healthy and hypertensive subjects.[55-58] Additionally, studies have also shown initial tolerability and left-ventricular remodeling responses based upon β1-AR genotype in heart failure patients.[37, 52, 59] However, few studies have yet to evaluate the role of these polymorphisms on negative chronotropic response to β-blockade in heart failure. Thus far, the role of pharmacogenetic variability on HR response to β-blocker treatment in heart failure has yet to be fully elucidated.

Negative chronotropic response on HR during exercise is considered the gold standard for assessing the clinical degree of β1-AR blockade.[60] In addition to HR, functional capacity is a critical determinant of prognosis in heart failure patients, which is partially related to β1-AR function. It is known that β1-AR polymorphisms are a significant determinant of exercise capacity in heart failure patients.[32] Previous studies have shown significant difference in terms of distance covered on the 6-minute walk test (6MWT) in relation to β-AR genotype.[33]

The objective of this study was to test the hypothesis that genetic heterogeneity at position 389 within the β1-AR is related, in part, to variable responses observed in β-blockade as assessed by change in HR from rest during exercise. Specifically, it was predicted that heart failure patients being treated with either carvedilol or metoprolol succinate who are homozygous for the Arg389 would have an enhanced response to the negative chronotropic effects of β-blockers when compared to the carriers of the Gly389 allele.

2.1.2 Materials and Methods

Patient Population

85 (36 carvedilol treated, 49 metoprolol treated) NYHA class II-III heart failure patients were recruited from the heart failure clinics at the University of Maryland Medical Systems as well as the Veterans Administration.

Inclusion criteria

1. Receiving a stable dose (unchanged in last 4 weeks) of either carvedilol or metoprolol succinate.
2. Ambulatory and able to take 6 minute walk test.

Exclusion criteria

1. Left-ventricular ejection fraction > 40%
2. Receiving any anti-arrhythmic drug therapy other than carvedilol or metoprolol extended-release
3. History of an ischemic incident (requiring emergency care) or myocardial infarction in the previous month
4. Requiring inotropic support other than digoxin
5. Changes in other heart failure medications such as (ACEI's, ARB's, diuretics, digoxin, sprinolactone) during the previous 2 weeks.
6. Having a resting HR greater than 90 bpm.

Sample Size

Sample size was determined using the following parameter estimates: α = 0.05, power = 0.80, change in resting HR (ΔHR, primary endpoint) of 6 bpm between groups, with a standard deviation of ± 10 bpm and a ratio of β1-AR Arg389 homozygotes to Gly389 carriers of 1:1. A sample size of 26 patients in each genotype group (homozygote or variant carrier) has 80% power to detect a difference of 8 bpm. change in HR between groups. Hence, this study was powered to detect a difference of 8 bpm change in HR from rest following the 6MWT (ΔHR) between groups based upon genotype at position 389 on the β1-AR.

Carriers were considered to have at least one copy of the variant allele in the case of all genotypes. Due to the low frequency of the variant alleles, it was planned *a priori* to combine both variant hetero- and homozygotes.

This protocol and consent forms were approved *(04/19/2007, #H-28288, Appendix I)* by the human subjects research committee and Institutional Review Board (IRB) at both the University of Maryland and the Veterans Administration. This research was conducted in compliance with all HIPAA regulations regarding privacy and confidentiality.

Genotyping

Blood samples for genotyping were obtained at the time of informed consent. Genomic DNA was extracted from whole blood using a commercial kit (Roche DNA Isolation Kit for Mammalian Blood, Indianapolis, IN, USA). β1-AR genotypes at positions Ser49Gly (rs1801252) and Arg389Gly (rs1801253) were determined by Taqman genotyping assays on the ABI 7900HT-Fast using a 384 well microplate (Applied Biosystems, Foster City, CA, USA)**.** Thus, each 384-well tray contained patient samples, of which were run in duplicate, along with positive controls for both homozygous and the heterozygous genotypes, as well as negative controls. 384-well plates were dried in a speed-vac concentrator, sealed with aluminum sealing tape and stored at -20° C until the assays were set up. At the time of the assay, an assay master mix was added to each 5 µl sample and then plates were sealed with optical adhesive film, and cycled in an MJ Tetrad using the following cycles: 10 minutes at 95° C, followed by 50 cycles of (15 seconds at 92° C followed by 60° C for 1 minute). After 50 cycles, the plates were held at 4° C on the thermal cycler until plates were scanned by the 7900HT. Based on marker fluorescence, genotypes were assigned via the 7900HT. The primer sequences for position 49 was 5'-CTCGTTGCTGCCTCCCGCCAGCGAA[A/G]GCCCCGAGCCGCTGTCTCAGCAGTG-3' and position 389 was 5'-CCCCGACTTCCGCAAGGCCTTCCAG[C/G]GACTGCTCTGCTGCGCGCGCAGGGC-3'. Genotypes were evaluated in accordance with Hardy-Weinberg equilibrium and previously reported allelic frequencies.

Outcomes

The primary endpoint of this prospective study was ΔHR from rest in relation to β1-AR genotype at position 389. For this evaluation, the 6MWT was employed as a measure of submaximal exercise. The 6MWT is a well known, safe and reliable measure of exercise capacity in patients with heart failure.[61] The 6MWT has also been shown to be a predictor of maximal oxygen consumption, functional capacity and prognosis in certain heart failure patients.[62-65] Secondary outcomes included resting HR and distance walked during the 6MWT.

The 6MWT was performed at the heart failure clinic along an enclosed private corridor 30m in length. Turnaround points were designated by orange cones. Patients were instructed to begin walking and a timer was started. Patients were permitted to slow down, stop and rest as necessary. No coaching or motivational talk was permitted. This test was conducted in the manner outlined by the American Thoracic Society Guidelines.[66,67] Resting HR was measured before the administration of the 6MWT over 30 seconds using a (*Nonin Onyx*™, Nonin Medical, Plymouth, MN) commercial heart-rate monitor. Patients were seated for 5 minutes before resting HR was measured. Patients then completed the 6MWT. The first HR recorded immediately following the 6MWT was used as the endpoint to calculate the change in HR (resting – exercise induced HR). If at any point the patient was unable to complete the 6MWT, a HR was recorded at the moment the patient deemed they were no longer able to continue. This heart rate was then used as the endpoint to calculate change and it was documented that the patient was unable to complete the test. The distance completed in the 6MWT was also evaluated as a secondary endpoint.

Statistical Analysis

Patients were segregated by genotype at position 389 and were evaluated with regards to ΔHR, resting HR, 6-minute walk distance, dose of β-blocker received, and systolic and diastolic blood pressures. Baseline demographics were compared based upon both genotype at position 389 and self-assigned race. Comparisons were made by unpaired *t*-test. Patient groups, demographics, racial makeup and Hardy-Weinberg equilibrium were also compared by the use of the unpaired *t*-test, Fisher exact test, or χ^2 test as appropriate. In addition the primary endpoint and resting HR were both corrected for their respective plasma β-blocker concentrations. The relationships between both ΔHR and resting HR with β-blocker plasma concentrations were analyzed by linear regression. Statistical analyses were carried out using SAS (version 9.2; SAS institute INC, Cary, North Carolina). A *P*-value less than 0.05 was considered statistically significant.

2.1.3 Results

β1-AR genotype

A total of 86 patients were enrolled in this study and the β1-AR genotype at position 389 was available for 85 of these patients. Baseline demographics based upon genotype at position 389 are shown in Table 2.1. There were 42 Arg389 homozygotes and 43 Gly389 carriers. Baseline characteristics did not differ by genotype at position 389 with the exception of race and weight. As expected, more African Americans were carriers of the Gly389 allele, consistent with known racial differences in position 389 allelic frequencies.[68] The overall racial breakdown was 40 Caucasians and 45 African Americans. Allelic frequencies were 0.69 for Arg389. All genotype frequencies were in Hardy-Weinberg equilibrium.

Based upon genotype at position 389 no initial difference between groups combined was observed except for systolic blood pressure (Table 2.2). As hypothesized an interesting trend ($P = 0.07$) is noted for the Arg389 homozygotes having a lower resting HR. No significant differences in any of the study endpoints were observed based upon genotype at position 49.

In the metoprolol treated group (n = 49), Arg389 homozygotes had a significantly lower systolic BP compared to the Gly389 carriers (106 ± 16 mmHg and 121 ± 16 mmHg respectively (Tables 2.3 & 2.4)**.** However, this difference was not observed in the carvedilol treated group (n = 36). Furthermore, metoprolol treated Arg389 homozygotes had a significantly lower systolic blood pressure than carvedilol treated Arg389 homozygotes (106 ± 16 mmHg vs. 118 ±

Table 2.1 Baseline characteristics of patients according to genotype at position 389

Variable	Arg 389 Homozygous (n = 42)	Gly 389 Carrier (n = 43)	*P*-value
Age (yrs)	**60 ± 13**	**57 ± 12**	**0.28**
Male Gender (%)	**90**	**81**	**0.35**
Caucasian (%)	**62**	**30**	**0.005**
Weight (lbs)	**189 ± 43**	**221 ± 52**	**0.003**
Ischemic etiology (%)	**43**	**49**	**0.67**
NYHA functional class			
II (%) / III(%)	**57/43**	**70/30**	**0.27**
Medications			
Loop Diuretic (%)	**71**	**70**	**0.99**
ACE Inhibitor or ARB (%)	**88**	**91**	**0.74**
Digoxin (%)	**50**	**44**	**0.67**
Aldosterone Antagonist (%)	**45**	**40**	**0.66**
Hydralazine (%)	**7**	**23**	**0.07**

Table 2.2 Outcomes by β1-AR genotype

Outcome	Arg 389 Homo (n = 42)	Gly 389 Carrier (n = 43)	*P* -value	Ser 49 Homo (n = 64)	Gly 49 Carrier (n = 21)	*P* -value
Systolic B.P. (mmHg)	**111 ± 18**	**122 ± 17**	**0.009**	**117 ± 19**	**116 ± 17**	**0.89**
Diastolic B.P. (mmHg)	**67 ± 10**	**70 ± 12**	**0.13**	**68 ± 11**	**71 ± 13**	**0.21**
Resting HR (bpm)	**64 ± 11**	**68 ± 11**	**0.07**	**65 ± 11**	**70 ± 10**	**0.08**
ΔHR (bpm)	**27 ± 12**	**27 ± 14**	**0.94**	**26 ± 12**	**31 ± 16**	**0.12**
6-min Walk (ft)	**1187 ± 307**	**1110 ± 228**	**0.19**	**1134 ± 273**	**1189 ± 268**	**0.43**

Table 2.3 Metoprolol group outcomes

Endpoint	Arg 389 Homo (n = 24)	Gly 389 Carrier (n = 25)	*P*-value	Ser 49 Homo (n = 36)	Gly 49 Carrier (n = 13)	*P*-value
Systolic B.P. (mmHg)	**106 ± 16**	**121 ± 16**	**0.002**	**117 ± 18**	**107 ± 13**	**0.08**
Diastolic B.P. (mmHg)	**65 ± 10**	**71 ± 11**	**0.06**	**68 ± 11**	**66 ± 10**	**0.52**
Resting HR (bpm)	**62 ± 10**	**67 ± 9**	**0.10**	**63 ± 10**	**68 ± 8**	**0.10**
ΔHR (bpm)	**28 ± 12**	**28 ± 17**	**0.90**	**27 ± 12**	**32 ± 20**	**0.42**
6-min Walk (ft)	**1152 ± 320**	**1060 ± 206**	**0.70**	**1086 ± 273**	**1156 ± 263**	**0.43**

Table 2.4 Carvedilol group outcomes

Endpoint	Arg 389 Homo (n = 18)	Gly 389 Carrier (n = 18)	*P*-value	Ser 49 Homo (n = 28)	Gly 49 Carrier (n = 8)	*P*-value
Systolic B.P. (mmHg)	**118 ± 19**	**122 ± 20**	**0.53**	**117 ± 20**	**132 ± 11**	**0.06**
Diastolic B.P. (mmHg)	**69 ± 11**	**70 ± 14**	**0.77**	**67 ± 10**	**80 ± 14**	**0.006**
Resting HR (bpm)	**66 ± 12**	**70 ± 12**	**0.33**	**67 ± 12**	**72 ± 12**	**0.35**
ΔHR (bpm)	**27 ± 12**	**26 ± 10**	**0.94**	**25 ± 11**	**30 ± 10**	**0.23**
6-min Walk (ft)	**1233 ± 291**	**1180 ± 244**	**0.56**	**1196 ± 265**	**1242 ± 285**	**0.70**

19 mmHg; *P* = 0.03, respectively). Similar outcomes were observed for both genotypes groups at position 49.

No significant differences in any of the endpoints based upon genotype at position 389 were observed in the carvedilol group. At position 49, the Ser49 homozygotes had a significantly lower diastolic blood pressure compared to the Gly49 carriers (Table 2.4). Additionally, the differences in systolic blood pressure approached significance (*P* = 0.06) for this position. Given the rarity of the Gly49 allele, unfortunately only 8 patients were in this group.

Although there was a significant positive relationship between 6-minute walk distance and ΔHR (*P* = 0.006), there was no significant difference based upon genotype in either resting HR or ΔHR when corrected for 6-minute walk distance.

Plasma concentrations of both *S*-metoprolol and *S*-carvedilol were measured to account for differences in pharmacokinetic variability. No differences in drug concentration based upon genotype at position 389 were observed for either drug group (Tables 2.5 & 2.6). However, significant differences were observed in both the primary endpoint of ΔHR and the secondary endpoint of resting HR when these values were corrected for *S*-metoprolol concentrations. This difference was only observed in the metoprolol treated group. To further investigate this, the relationships between *S*-metoprolol concentrations and outcomes (ΔHR and resting HR) were plotted as shown in Figure 2.1**.** A weak association was observed based upon genotype at position 389, and regression analysis showed that the relationship between ΔHR and *S*-metoprolol concentration for

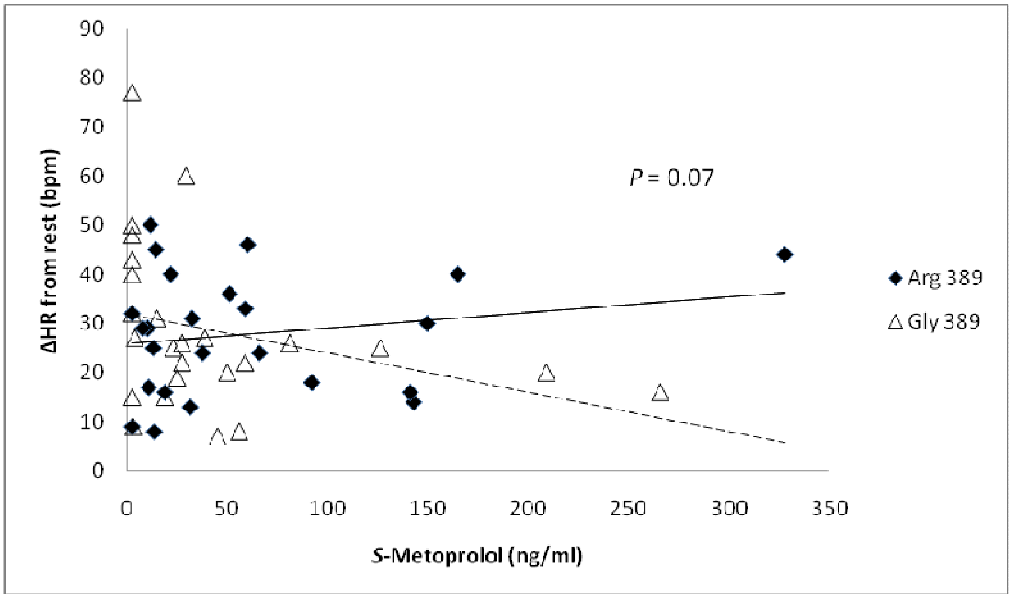
Table 2.5 Endpoints corrected for pharmacokinetic differences in the metoprolol treated group

Variable	Arg 389 Homo (n = 24)	Gly 389 Carrier (n = 25)	*P* - value	Ser 49 Homo (n = 36)	Gly 49 Carrier (n = 13)	*P* - value
***S*-metoprolol (ng/ml)**	**61.9 ± 76.2**	**44.9 ± 65.7**	**0.41**	**48.8 ± 59.7**	**65.3 ± 97.3**	**0.58**
***S*-metoprolol (mg/kg)**	**1.72 ± 0.90**	**1.75 ± 0.77**	**0.88**	**1.76 ± 0.89**	**1.67 ± 0.65**	**0.74**
Resting HR/*S*-met (bpm/ng/ml)	**4.5 ± 7.2**	**10.6 ± 12.4**	**0.04**	**6.0 ± 8.9**	**12.1 ± 13.4**	**0.07**
ΔHR /*S*-met (bpm/ng/ml)	**1.77 ± 2.68**	**5.71 ± 8.47**	**0.04**	**2.91 ± 4.95**	**6.19 ± 9.65**	**0.26**

Table 2.6 Endpoints corrected for pharmacokinetic differences in the carvedilol treated group

Variable	Arg 389 Homo (n = 18)	Gly 389 Carrier (n = 18)	*P*-value	Ser 49 Homo (n = 28)	Gly 49 Carrier (n = 8)	*P*-value
***S*-carvedilol (ng/ml)**	**16.6 ± 18.6**	**14.9 ± 15.6**	**0.77**	**13.6 ± 9.6**	**23.3 ± 31.6**	**0.42**
***S*-carvedilol (mg/kg)**	**0.50 ± 0.18**	**0.49 ± 0.12**	**0.73**	**0.52 ± 0.13**	**0.40 ± 0.18**	**0.06**
Resting HR/*S*-car (bpm/ng/ml)	**6.9 ± 4.4**	**11.7 ± 11.9**	**0.12**	**8.5 ± 6.8**	**13.5 ± 14.7**	**0.35**
ΔHR/*S*-car (bpm/ng/ml)	**3.2 ± 3.0**	**5.3 ± 7.4**	**0.27**	**3.3 ± 3.9**	**7.3 ± 9.5**	**0.28**

A



B

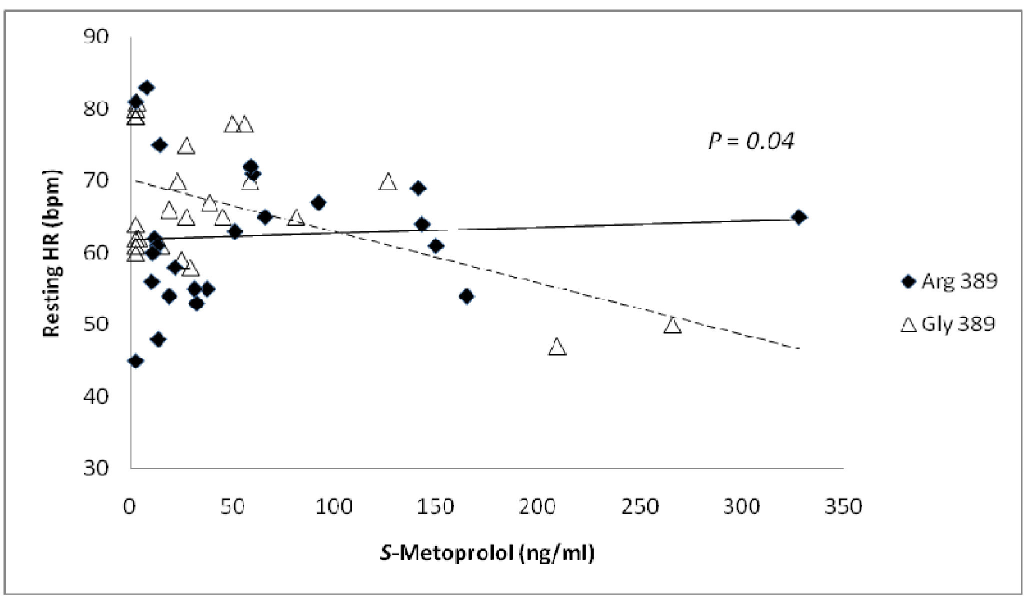
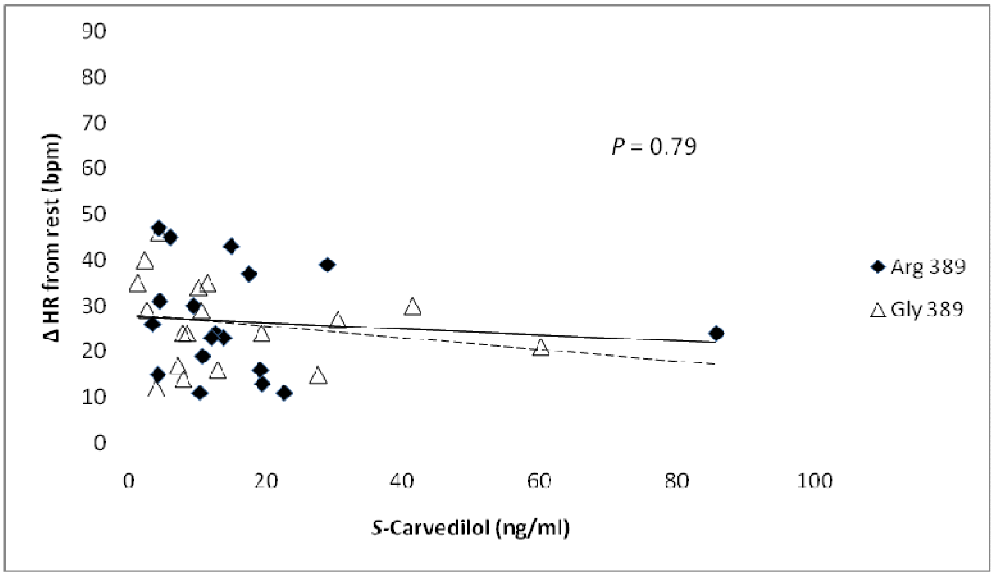


Figure 2.1 Relationship between change in HR from rest, ΔHR (A) or resting HR (B) and plasma *S*-metoprolol concentrations for (n = 24) Arg389 homozygotes (diamonds) and (n = 25) Gly389 carriers (triangles). Solid line represents regression line for Arg389, and broken line represents regression line for Gly389. The decrease in resting HR was significantly different between the two genotypes ($P = 0.04$).

metoprolol for the Arg389 homozygotes and Gly389 carriers was not significantly different ($\gamma = 0.03$ vs. $\gamma = -0.08$, respectively; $P = 0.07$). Regression analysis showed that there was a significant difference in the relationship between resting HR and plasma *S*-metoprolol concentration based upon genotype at position 389 (Arg389 $\gamma = 0.009$, Gly389 $\gamma = -0.07$; $P = 0.04$). In the carvedilol-treated group, no significant differences in the slopes of either of these relationships were observed based upon genotype at position 389 (Figure 2.2).

Given the racial differences in SNP frequencies, baseline demographics were also compared by race (Table 2.7). There were a few significant differences between the two groups based upon race. The Caucasian group was significantly older by an average age of 7 years. This same group also had a significantly lower usage of hydralazine (5% vs. 24%, respectively) compared to the African American group. Notably, no significant difference in the baseline weight between groups was observed.

A



B

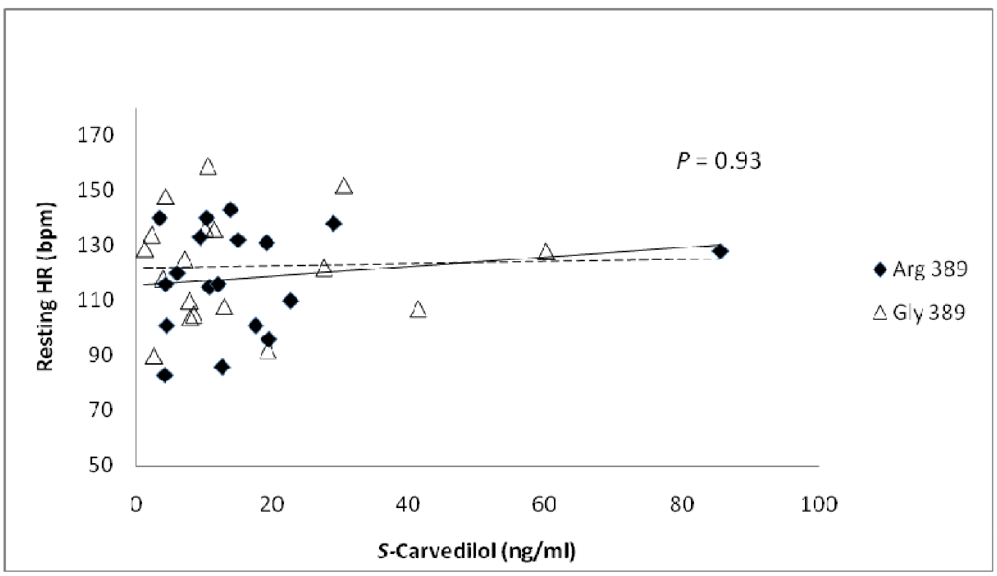


Figure 2.2 Relationship between change in HR from rest, ΔHR (A) or resting HR (B) and plasma *S*-carvedilol concentrations for (n = 18) Arg389 homozygotes (diamonds) and (n = 18) Gly389 carriers (triangles). Solid line represents regression line for Arg389, and broken line represents regression line for Gly389. The change in HR from rest, ΔHR (P = 0.79) and resting HR (P = 0.93) was not significantly different between the two genotypes.

Table 2.7 Baseline demographics by race

Variable	Caucasian (n = 40)	African American (n = 45)	*P* – Value
Age (y)	**62 ± 12**	**55 ± 12**	**0.02**
Male Gender (%)	**93**	**80**	**0.13**
Weight (lbs)	**197 ± 40**	**213 ± 56**	**0.29**
Ischemic Etiology	**65**	**44**	**0.08**
Drug (carvedilol/metoprolol)	**40/60**	**45/55**	**0.83**
NYHA functional class			
II (%) / III(%)	**58/42**	**69/31**	**0.38**
Medications			
Loop Diuretic (%)	**60**	**80**	**0.06**
ACE Inhibitor or ARB (%)	**90**	**89**	**0.99**
Digoxin (%)	**53**	**42**	**0.39**
Aldosterone Antagonist (%)	**43**	**42**	**0.99**
Hydralazine (%)	**5**	**24**	**0.02**

2.1.4 Discussion

The results of the present study suggest that β1-AR genotype at position 389 contributes, in part, to the degree of β-blockade observed in patients with heart failure receiving metoprolol. In patients who were Arg389 homozygotes at the β1-AR, an enhanced response (as measured by a decreased ΔHR/*S*-metoprolol) was observed compared to the Gly389 carriers. These same patients also had a lower resting HR/*S*-metoprolol in response to chronic metoprolol therapy, compared to the Gly389 carriers. It is therefore likely that enhanced negative chronotropic response to metoprolol can be expected in patients who are Arg389 homozygotes.

This is the first study to demonstrate a negative chronotropic response to β-blockade following exercise and resting HR in heart failure based upon β1-AR genotype. Previous studies have reported a relationship between β1-AR genotype at position 389 and response to metoprolol in healthy individuals and hypertensive patients. Liu *et al.* reported a significant difference in change in exercise-induced HR in healthy individuals treated with metoprolol. Here the Arg389 homozygotes had a significantly greater decrease in exercise HR after treatment with metoprolol compared to the Gly389 homozygotes (14.0% vs. 11.7%; $P = 0.02$) [56] In the current study, a significant association between ΔHR and Arg389Gly genotype after correcting for plasma *S*-metoprolol concentrations was observed. A trend was also observed for lower resting HR in the Arg389 homozygotes, but only after correcting for *S*-metoprolol concentrations was a significant difference revealed for both resting and ΔHRs. A similar approach was used by Beitelshees *et al*. to evaluate metoprolol treatment in hypertensive patients. Here, a significant difference in resting HR based upon genotype at β1-AR position 49 was observed with the Ser49 homozygotes having a 6% greater reduction in resting HR compared to the Gly49 carriers ($P = 0.05$); however, after correcting this data for plasma *S*-metoprolol concentration, this difference was not significant.[69] In the present study, a significant difference in response based upon genotype at position 389 was observed only after plasma *S*-metoprolol concentrations were taken into account.

The differential findings in the negative chronotropic response between carvedilol and metoprolol treated patients are potentially due to the different pharmacological profiles of these two agents. Metoprolol is β1 selective, while carvedilol has a higher affinity for the β2-Ars.[70, 71] Furthermore, recent evidence suggests that β-blockers have differential effects related to the Arg389Gly polymorphism. Studies by Rochais *et al*. in rat myocytes demonstrated that carvedilol, metoprolol and bisoprolol act as inverse agonists at both of the Arg and Gly389 variants. However, the β-blockers act differentially upon secondary messengers: only carvedilol decreased basal levels of cAMP and significantly decreased cAMP concentrations in the Arg variants compared to the Gly variants.[27] Thus, β-blocker specific pharmacogenomic associations in the current study should not be discounted.

A significant association between genotype at position 389 and blood pressure response to metoprolol was also observed. More specifically, the metoprolol treated Arg389 homozygotes had a significantly lower systolic blood pressure compared to their Gly389 counterparts. These results are consistent with previous studies in healthy and hypertensive patients.[55, 56, 58] However, as metoprolol is not typically noted for its antihypertensive effects in heart failure, the clinical implications of this pharmacogenomic association are especially noteworthy.[72] Moreover, this difference was not observed in those receiving carvedilol, which is unexpected since the *R*-enantiomer of carvedilol has a high affinity for the α_1 adrenergic receptor. This differential antihypertensive response following β-blocker treatment based upon genotype at 389 may be due to a mechanism separate from the negative chronotropic response, such as the rennin-angiotensin system. Bruck *et. al*, published a report of bisoprolol markedly suppressing the dobutamine-induced plasma rennin-activity increase in Arg389 homozygotes but only marginally in Gly389 homozygotes.[73] Hence, the antihypertensive response may be related to pharmacological blockade on the β-AR in the kidney rather than the β-ARs on the heart.

In the present study there were no significant associations between genotype and 6-minute walk distance. Exercise performance on the 6MWT is closely tied to survival in heart failure patients.[62,74] Despite this, no significant associations between the 6-minute walk distance and β1-AR genotype at position 389 were observed. A significant association between the total distance walked during the 6MWT and ΔHR was observed. As expected, patients who walked a further distance during the 6-minutes had a significantly larger ΔHR. Hence, ΔHR was corrected for 6-minute walk distance, but no significant associations were observed.

Overall, the groups were well matched based upon genotype at position 389. However the significant difference in weight was unpredicted. The only other significant difference between the groups was racial makeup, as expected given the known racial differences in allelic frequencies (see Table 2.2).[68] When comparing the groups by race and no significant difference in weight was observed. Thus, the difference in weight may be related to another factor. To circumvent this, the doses of both β-blockers were corrected by weight (mg/kg) and no significant differences based upon genotype were observed. Additionally, there were no significant differences in the dose of either β-blocker received based upon genotype at position 389.

Given the significant degree of linkage disequilibrium between position 49 and 389 on the β1-AR[25], the relationship of the SNP at position 49 on both primary and secondary endpoints was also analyzed. Although this study was not specifically powered to evaluate this relationship, it is of interest to note that genotype at position 49 was not associated with either the primary endpoint or secondary endpoints even when corrected for plasma β-blocker concentrations. A significant difference was observed with carvedilol-treated Ser49 carriers having a significantly lower diastolic blood pressure than their Gly49 carrying counterparts. This study is the first to make this association between genotype at position 49 and diastolic blood pressure. Previous studies have found an association between systolic blood pressure and position 49, but no differences regarding diastolic blood pressure were observed.[56, 57] Hence, the current results should be interpreted with caution given the relatively small amount of patients (n = 8) in the Gly49 carrying group.

Given the known differences in racial allelic frequencies, both groups were compared at baseline for racial differences. The Caucasian patients were significantly older (P = 0.02), by 7 years of age. The only other difference based upon race was the significantly higher usage of hydralazine in the African American patients compared to the Caucasians (24% vs. 8%; P = 0.02). This differential finding is expected following the clinical guidelines that recommend hydralazine for heart failure patients that identify their race as African American.[50]

Racial differences in the antihypertensive response to β-blockers have been previously documented. [75, 76] Currently, no racial differences in the response of heart failure patients to either carvedilol or metoprolol succinate have been identified. It has been postulated that the racial differences in response to β-blockade may be due in part to the allelic differences at position 389. Notably, the frequency of the hyperfunctional Arg389 allele is lower in African Americans than Caucasians (~37% vs. ~52%). Johnson *et al*. observed that genotype at position 389 largely accounted for the racial variance in the antihypertensive response to metoprolol.[57] The current study was not specifically powered to evaluate the complex relationship between race and response to β-blockade in heart failure. However, apart from racial differences a genetic difference was identified that was able to account for significant differences in clinical response to β-blockade that may partially explain some of the previously noted racial differences in response.

This study further demonstrates the importance of accounting for plasma β-blocker concentrations. Beitelshees *et al.* were the first to demonstrate the importance of correcting for β-blocker concentrations when investigating the negative chronotropic response to metoprolol.[69] In the current study, while there was a trend (P =0.07) for lower resting HR in the Arg389 homozygotes compared to the Gly389 carriers in the overall analysis, significant differences in resting HR and ΔHR were only observed after correcting for plasma *S*-metoprolol concentrations. The absence of these findings in the carvedilol group is consistent with other studies.[39] As predicted there were no significant differences in the plasma concentrations of either *S*-metoprolol or *S*-carvedilol based upon genotype at position 389. (Tables 2.5 & 2.6) *S*-metoprolol concentrations were higher on average than those reported in a similar study by Terra *et al.* They observed average steady-state *S*-metoprolol concentrations of 31ng/ml for the Arg389 homozygotes and 19 ng/ml for the Gly389 carriers.[37] The steady-state *S*-metoprolol concentrations in the current study were higher (62 ng/ml and 45 ng/ml; Arg389 and Gly389 carriers respectively). However, the average dose of metoprolol in both the Arg389 and Gly389 groups (139 mg and 164 mg) was also higher than those reported by Terra (136 mg, and 104 mg). Notably, the average metoprolol dose was 152 mg similar to the average 153 mg received in the MERIT trial.[77] Tenero *et al*. reported that heart failure patients receiving 25mg of carvedilol twice daily had average steady-state *S*-carvedilol C_{max} plasma concentrations of (30.8 ± 16.1 ng/ml).[78] *S*-carvedilol steady-state concentrations were lower on average, but the sampling times were random and not necessarily around the t_{max}. Although, the current study did not control for the time of plasma β-blocker collection relative to dose, the steady-state plasma concentrations of each β-blocker were still able to account for pharmacokinetic variation at the time of testing. It is noteworthy, that the vast majority of patients took their medicine approximately 6-8 hours before the blood draw. Hence, both ΔHR and resting HR were corrected for plasma β-blocker concentrations.

Although this study is the first to show these pharmacogenomic associations in metoprolol but not carvedilol, there are a few limitations to consider. One limitation was the inability to test all pharmacogenomic associations with adequate power. Nonetheless, statistically significant associations between genotype at position 389 and both the clinical degree of β-blockade and resting hemodynamics were observed. Unfortunately, the study lacked power to test the primary outcome for pharmacogenomic associations with position 49 on the β1-AR, as the study was powered based upon position 389. Thus, the lack of significant associations at positions at position 49 cannot be taken as evidence that such an association does not exist.

Another potential limitation is the inability to account for differences in time of drug dosing relative to the time of the study and plasma sampling. Patients were assumed to have steady-state levels of either β-blocker at the time of sampling. Regardless, large variations in both plasma *S*-metoprolol (53 ± 71 ng/ml) and *S*-carvedilol (15 ± 17 ng/ml) concentrations were detected.

This study was the first to assess this negative chronotropic response phenotype in heart failure patients treated with either carvedilol or metoprolol. The primary finding was that homozygousity for Arg at position 389 on the β1-AR enhanced the clinical degree of β-blockade to metoprolol. Additionally, a significant difference in the antihypertensive response to metoprolol between genotype groups was observed. Thus, the Arg389Gly polymorphism of the β1-AR may be able to account for some of the variability in clinical response seen in heart failure patients treated with β-blockers. Future studies are needed to more thoroughly investigate the differential pharmacogenomic effect of these β-blockers in heart failure.

2.2 Evaluation of the Relationship Between β2-AR Genotype and Clinical Response in Heart Failure Patients Treated with Carvedilol or Metoprolol

2.2.1 Introduction

Although the β2-AR is not the primary β-AR expressed in cardiac myocytes, it still plays a critical role in cardiac functioning and can mediate both inotropic and chronotropic effects.[19] When faced with chronic agonist signaling, the β2-AR undergoes desensitization rather than downregulation contrary to the β1-AR.[79] Likewise, genetic heterogeneity in the form of SNPs also occurs on the codon for the β2-AR. Of the polymorphisms identified for the β2-AR, two (position 16 and 27) have been shown to alter receptor expression and functioning.[25] Hence, genetic heterogeneity at the level of the β2-AR could also play a role in the variability of clinical response to β-blockade.

In the case of chronic adrenergic signaling typical of heart failure, the β1-Ars are down regulated so that β2-Ars can represent up to 40% of the total β-AR population.[19, 34] Therefore, the β2-Ars may play a more functionally relevant role in heart failure patients than in the case of healthy subjects. Few studies have yet to evaluate the role of these β2-AR SNPs in heart failure. Both Liggett *et al*. and De Groote *et al*. failed to find any association between the aforementioned β2-AR SNPs and survival in heart failure patients.[35, 38] In another published report, De Groote *et al*. also failed to find any association with heart rate and either of the β2-AR SNPs in heart failure patients treated with carvedilol.[39]

Although the functional relevance of each of these β2-AR polymorphisms has been studied using *in vitro* and *in vivo* techniques, relatively little is known about their pharmacogenomic associations with β-blockade in heart failure. Thus, the goal of this study was to characterize the role of these β2-AR SNPs in heart failure patients treated with either carvedilol or metoprolol.

2.2.2 Materials and Methods

Gentoyping

Genomic DNA was isolated from whole blood using a commercially available kit (Roche DNA Isolation Kit for Mammalian Blood®, Indianapolis, IN, USA). β2-AR genotype at positions Gly16Arg (rs1042713) and Gln27Glu (rs1042714) were determined by Taqman Genotyping Assays on the ABI 7900HT-Fast as described in Chapter 2.1.2. The primer sequences for position 16 was 5’-

CAGCGCCTTCTTGCTGGCACCCAAT[A/G]GAAGCCATGCGCCGGACCA

CGACGT-3' and position 27 was 5'-TGCGCCGGACCACGACGTCACGCAG[C/G]AAAGGGACGAGGTGTGGGTGGTGGG-3'. Genotypes were evaluated in accordance with Hardy-Weinberg equilibrium and previously reported allelic frequencies.

2.2.3 Results

β2-AR genotype

A total of 86 patients were enrolled in this study. The β2-AR genotype at position 16 was available for 85 whereas genotype at position 27 was determined for all 86 patients. Baseline demographics based upon genotype at position 27 are shown in Table 2.8. There were 44 Gln27 homozygotes and 42 Glu27 carriers. Variant allele frequencies were 0.44 for Arg at position 16 and 0.30 for Glu27. All genotype frequencies were in Hardy-Weinberg equilibrium.

Based upon genotype at either position 16 or 27 no initial difference between genotype groups for any endpoint except diastolic blood pressure was found (Table 2.9). The Glu27 carriers had a significantly lower diastolic blood pressure of 6 mmHg. Furthermore the Arg16 carriers also had a significantly lower diastolic blood pressure (P = 0.06) that approached significance.

In the metoprolol treated group (n = 49), no significant difference based upon genotype at either β2-AR SNP was observed (Table 2.9). Conversely, when evaluating the carvedilol treated group (n = 26) several significant differences in resting hemodynamics were observed (Table 2.10). Significant differences in both systolic blood pressure and diastolic blood pressure were observed based

Table 2.8 Outcomes by β2-AR genotype

Endpoint	Gly 16 Homo (n = 57)	Arg 16 Carrier (n = 28)	*P* – value	Gln 27 Homo (n = 44)	Glu 27 Carrier (n = 42)	*P* – value
Systolic B.P. (mmHg)	**119 ± 19**	**112 ± 16**	**0.08**	**120 ± 20**	**112 ± 17**	**0.07**
Diastolic B.P. (mmHg)	**70 ± 12**	**65 ± 10**	**0.06**	**71 ± 13**	**65 ± 9**	**0.01**
Resting HR (bpm)	**66 ± 11**	**67 ± 11**	**0.52**	**67 ± 10**	**66 ± 11**	**0.72**
ΔHR (bpm)	**28 ± 14**	**26 ± 10**	**0.46**	**29 ± 13**	**26 ± 13**	**0.23**
6-min Walk (ft)	**1144 ± 279**	**1156 ± 264**	**0.84**	**1155 ± 278**	**1147 ± 266**	**0.89**

Table 2.9 Metoprolol group outcomes

Endpoint	Gly 16 Homo (n = 31)	Arg 16 Carrier (n = 18)	*P* – value	Gln 27 Homo (n = 25)	Glu 27 Carrier (n = 25)	*P* – value
Systolic B.P. (mmHg)	**115 ± 18**	**113 ± 16**	**0.64**	**113 ± 20**	**113 ± 16**	**0.95**
Diastolic B.P. (mmHg)	**68 ± 11**	**68 ± 11**	**0.96**	**68 ± 13**	**67 ± 10**	**0.58**
Resting HR (bpm)	**64 ± 10**	**66 ± 9**	**0.56**	**66 ± 10**	**64 ± 9**	**0.42**
ΔHR (bpm)	**28 ± 17**	**28 ± 11**	**0.83**	**29 ± 15**	**27 ± 15**	**0.72**
6-min Walk (ft)	**1060 ± 256**	**1181 ± 282**	**0.13**	**1086 ± 273**	**1156 ± 263**	**0.37**

Table 2.10 Carvedilol group outcomes

Endpoint	Gly 16 Homo (n = 26)	Arg 16 Carrier (n = 10)	*P* – value	Gln 27 Homo (n = 19)	Glu 27 Carrier (n = 17)	*P* – value
Systolic B.P. (mmHg)	**124 ± 19**	**110 ± 17**	**0.05**	**129 ± 16**	**111 ± 18**	**0.004**
Diastolic B.P. (mmHg)	**73 ± 13**	**61 ± 5**	**0.005**	**75 ± 13**	**63 ± 7**	**0.002**
Resting HR (bpm)	**67 ± 12**	**70 ± 14**	**0.59**	**67 ± 11**	**69 ± 14**	**0.76**
ΔHR (bpm)	**28 ± 11**	**23 ± 8**	**0.23**	**29 ± 11**	**23 ± 9**	**0.08**
6-min Walk (ft)	**1243 ± 277**	**1111 ± 220**	**0.19**	**1260 ± 283**	**1147 ± 241**	**0.21**

upon genotype at position 16 and 27. In each case, the Arg16 and Glu27 carriers had significantly lower resting blood pressures.

To account for pharmacokinetic variability, plasma concentrations of both *S*-metoprolol and *S*-carvedilol were measured. No significant differences were observed in the *S*-metoprolol concentrations, even when corrected for weight, based upon β2-AR genotype (Table 2.11). Furthermore, when both the primary endpoint (ΔHR) and secondary endpoint (resting HR) were corrected for *S*-metoprolol concentrations, no significant differences were observed. Similar to the metoprolol treated group, no significant differences in *S*-carvedilol concentrations between either β2-AR genotype groups were found. (Table 2.12) Likewise, there were no significant differences in correcting for the primary and secondary endpoints.

Table 2.11 Endpoints corrected for pharmacokinetic differences in the metoprolol treated group

Variable	**Gly 16 Homo (n = 31)**	**Arg 16 Carrier (n = 18)**	***P* – value**	**Gln 27 Homo (n = 36)**	**Glu 27 Carrier (n = 13)**	***P* – value**
S-metoprolol (ng/ml)	**53.1 ± 64.3**	**53.3 ± 82.8**	**0.99**	**49.0 ± 55.6**	**55.4 ± 83.8**	**0.75**
S-metoprolol (mg/kg)	**1.80 ± 0.83**	**1.63 ± 0.84**	**0.49**	**1.76 ± 0.73**	**1.69 ± 0.92**	**0.77**
Resting HR/S-met (bpm/ng/ml)	**7.24 ± 10.1**	**8.27 ± 11.4**	**0.75**	**8.6 ± 11.4**	**6.9 ± 9.6**	**0.56**
ΔHR/S-met (bpm/ng/ml)	**4.00 ± 7.2**	**3.50 ± 5.6**	**0.81**	**3.9 ± 5.9**	**3.6 ± 7.2**	**0.90**

Table 2.12 Endpoints corrected for pharmacokinetic differences in the carvedilol treated group

Variable	**Gly 16 Homo (n = 26)**	**Arg 16 Carrier (n = 10)**	***P* – value**	**Gln 27 Homo (n = 19)**	**Glu 27 Carrier (n = 17)**	***P* – value**
S-carvedilol (ng/ml)	**17.3 ± 19.3**	**12.0 ± 8.1**	**0.41**	**18.1 ± 21.0**	**13.2 ± 10.9**	**0.39**
S-carvedilol (mg/kg)	**0.48 ± 0.16**	**0.53 ± 0.10**	**0.99**	**0.51 ± 0.17**	**0.48 ± 0.12**	**0.49**
Resting HR/S-car (bpm/ng/ml)	**8.9 ± 9.2**	**10.4 ± 9.4**	**0.69**	**9.8 ± 11.4**	**8.8 ± 6.0**	**0.73**
ΔHR/S-car (bpm/ng/ml)	**4.2 ± 5.8**	**4.3 ± 5.6**	**0.97**	**5.2 ± 7.3**	**3.1 ± 2.9**	**0.25**

2.2.4 Discussion

The aim of this portion of the study was to evaluate the clinical degree of β-blockade based upon β2-AR genotype. Again, the gold standard of negative chronotropic response on HR during exercise was used for assessing the degree of β2-AR blockade.[80] This was analyzed by assessing the degree of HR reduction following submaximal exercise (ΔHR).

Overall, no significant differences in either the primary or secondary endpoints were observed based upon genotype at either β2-AR studied SNP. When analyzing the data by drug treatment group, once again no significant differences were observed in either the carvedilol or metoprolol-treated groups based upon genotype at either β2-AR position. Furthermore, no significant difference based upon β2-AR genotype was observed in either the carvedilol or metoprolol treated patients even when these values were corrected for their respective plasma concentrations.

Although not the initial focus of this study, it is important to address the significant differences observed in resting hemodynamics based upon both genotype at β2-AR positions 16 and 27. A significant difference was observed in diastolic blood pressure based upon genotype at position 27, with the Glu27 carriers having 6 mmHg average lower diastolic blood pressures compared to the Gln27 homozygotes. Upon further analysis by drug treatment group, this same significant association was only observed in the carvedilol-treated patients. As seen in Figure 2.10, both the carvedilol-treated Glu27 carriers and Arg16 carriers had a significantly lower systolic and diastolic blood pressure. This was foreseeable given the previously mentioned linkage disequilibrium between the 2 SNPs. These same associations were not observed in the metoprolol-treated patients.

The differential findings in the resting hemodynamics between carvedilol and metoprolol treated patients are potentially due to the different pharmacological profiles of these two agents. As previously mentioned, metoprolol is β1 selective, while carvedilol has a higher affinity for the β2-ARs.[70, 71] Hence, these results are in accordance with the established low affinity of metoprolol for the β2-AR. Furthermore, the β1-ARs are known to down-regulate in heart failure. Therefore, β2-AR may represent up to 40% of the total β-AR population.[34, 81] Thus, these β2-ARs SNPs may play a greater role in heart failure than in healthy subjects or hypertensive patients.

These results are consistent with prior reports in healthy patients. McCaffrey *et al.* previously showed that healthy subjects who were Gly16 homozygotes had a higher resting diastolic blood pressure.[82] Additionally, Kaye *et al.* found a positive association between Glu27 homozygous heart failure patients and improvement in LVEF following treatment with carvedilol.[40] Perhaps the current finding of significantly lower systolic and diastolic blood pressures in the carvedilol treated Glu27 homozygotes may play a modulatory role in this positive change in LVEF.

This study is the first to evaluate a pharmacogenomic association of a negative chronotropic phenotype and β2-AR genotype in heart failure patients receiving either carvedilol or metoprolol. The primary finding was that β2-AR genotype at either position 16 or 27 was not significantly associated with response to β-blockade as defined by ΔHR from rest following sub-maximal exercise. Although, this study was not initially designed to evaluate the influence of these SNPs on resting hemodynamics, the present findings concerning the difference in resting hemodynamics based upon β2-AR genotype at positions 16 and 27 in the carvedilol treated group are of interest. Notably, there is a well-documented linkage disequilibrium between these two SNPs.[83, 84] Most patients who are homozygous for Glu27 are also homozygous for Gly16. However, patients who are homozygous for Gly16 can be either homozygous or carriers of the Gln27 allele. Hence, the results of one group could be driving the other. Accordingly, a larger sample size to include more patients in each group and to allow for haplotype analysis given the strong degree of linkage disequilibrium between these two SNPs may be required to validate these findings in future studies.

Once again, the main limitation of this study was the relatively small sample size. Although statistically significant associations between β2-AR genotype and resting hemodynamics were observed, this study was not specifically powered to evaluate these β2-AR associations. When analyzing the drug treatment groups alone, this portion of the study is at greater risk for type II error given the relatively small sample sizes. These significant findings suggest that a larger more adequately powered study to further clarify the associations of these β2-AR SNPs on resting hemodynamics is necessary.

In conclusion, the current findings indicate that β2-AR SNPs at positions 16 and 27 are not associated with the clinical degree of β-blockade in heart failure patients treated with either carvedilol or metoprolol. Rather, β2-AR SNPs at positions 16 and 27 are significantly associated with and resting hemodynamics in carvedilol treated heart failure patients. Thus, β2-AR genotype could potentially predict both systolic and diastolic blood pressure response in carvedilol treated heart failure patients. These findings are the first to indicate that β2-AR genotype may be clinically relevant when evaluating carvedilol treated heart failure patients.

2.3 Evaluation of the Relationship Between GRK5 Genotype and the Clinical Response in Heart Failure Patients Treated with Carvedilol or Metoprolol

2.3.1 Introduction

The GRK polymorphism

There are numerous pathways that mediate adrenergic signaling and thus play a crucial role in heart failure. Of these, G-protein-coupled receptor kinases (GRKs) are known to desensitize ligand bound βARs and are part of an essential feedback mechanism in heart failure. GRKs help to desensitize the destructive feedback of excessive catechol stimulation in early heart failure.[42] GRKs function as an endogenous form of β-blockade by inactivating active β-ARs.

Within the GRK superfamily, there are 2 isoforms of particular significance in cardiac functioning. Both GRK2 and GRK5 are the predominate forms found in the myocardium.[45, 46] More specifically, GRK2 and GRK5 play a crucial role in heart failure functioning and downregulate βAR signaling though phosphorylation of cardiac βAR. This, in turn, activates β-arrestin and leads to receptor uncoupling from G proteins and subsequent downstream activation.[47] However, unlike GRK2, GRK5 does not undergo upregulation or downregulation as acutely.[48] Therefore, GRK5 is thought to be more important for chronic βAR regulation.[48, 85] It has been postulated that GRK5-mediated βAR desensitization may be beneficial during ventricular decompensation before overt heart failure occurs.[42]

GRK5 Polymorphism

A functional polymorphism in the GRK5 isoform was recently discovered at amino acid residue 41 in which a wild type glutamine (Gln) is substituted by leucine (Leu). In CHO cells, GRK5-Leu41 variants were shown to have an approximately 25% lower rate of cAMP accumulation compared to the wild type Gln41 cells. This finding is indicative that the GRK5-Leu41 polymorphism decreases β1-AR signaling by increasing agonist-promoted desensitization similar in nature to pharmacological β-blockade.[42]

GRK5 Polymorphism and Heart Failure

The GRK5-Leu41 polymorphism was subsequently shown by Liggett *et al*. to confer a survival benefit similar to pharmacological β-blockade in African Americans with heart failure. This same benefit was not observed in European Americans, most likely due to their lower allelic frequency [(0.02 compared to 0.35) in African Americans].[42]

Although the GRK5-Leu41 polymorphism has been shown to confer a survival benefit in African Americans with heart failure, little else is known about its role and pharmacogenomic interaction in heart failure patients receiving β-blockade. Hence, this polymorphism was selected for the current study to gain better insight into its role in the pharmacogenomics of the β-blockers in heart failure. The research set to evaluate the relationship between GRK5 genotype at position 41 and clinical response to β-blockade (defined by ΔHR following the 6MWT). The study also investigated the relationship between GRK5 genotype at position 41 and both resting HR and 6-minute walk distance achieved by heart failure patients. The goal of this study was to further quantify the role of the GRK5-41 polymorphism in heart failure patients receiving maximal tolerated levels of β-blockers.

2.3.2 Materials and Methods

Genotyping

Genomic DNA was isolated from whole blood using a commercially available kit (Roche DNA Isolation Kit for Mammalian Blood®, Indianapolis, IN, USA). GRK5 genotype at position Gln41Leu (rs17098707) was determined by a Taqman Genotyping Assay on the ABI 7900HT-Fast as described in Chapter 2.2. The GRK position 41 sequence was 5’-ATCCTGAAGTTCCCTCACATTAGCC[A/T]GTGTGAAGACCTCCGAAGGACCATA -3’. Genotypes were evaluated in accordance with Hardy-Weinberg equilibrium and previously reported allelic frequencies.

2.3.3 Results

No significant differences were observed in any of the endpoints based upon GRK5 genotype at position 41 (Table 2.13). Given the extremely low allelic variant (Leu) frequency (< 2%) in Caucasians, the data were also evaluated solely in the African American patients (Table 2.14). No significant differences were observed based upon genotype in this group either. Of note, all (n = 26) of the Leu41 carriers were African American.

Results of the metoprolol treated arm (n = 48), were consistent with our *a priori* predictions. Where the Gln41 homozygotes had a significantly lower resting HR compared to carriers of the Leu allele (Table 2.15). No other significant differences were observed between either genotype group.

In the carvedilol treated arm (n = 36), the only significant difference observed based upon GRK5-41 genotype was a significantly longer 6-minute walk distance demonstrated by the Leu 41 carriers (Table 2.16). No significant differences in resting hemodynamics or HR were observed. It is important to note that sample size was limited to (n = 9) patients.

Plasma concentrations of both *S*-metoprolol and *S*-carvedilol were measured to account for differences in pharmacokinetic variability. No differences in drug concentration based upon genotype at position 41 were observed for either drug group suggesting similar drug exposure between groups (Tables 2.17 & 2.18). No significant differences were observed in both the primary endpoint of ΔHR and the secondary endpoint of resting HR when these values were corrected for their respective *S*-metoprolol or *S*-carvedilol concentrations.

Table 2.13 Outcomes by GRK5 genotype

Endpoint	**Gln 41 Homo (n = 58)**	**Leu 41 Carrier (n = 26)**	***P*-value**
Systolic B.P. (mmHg)	**115 ± 19**	**121 ± 17**	**0.12**
Diastolic B.P. (mmHg)	**68 ± 11**	**70 ± 13**	**0.33**
Resting HR (bpm)	**65 ± 11**	**69 ± 9**	**0.14**
ΔHR (bpm)	**27 ± 12**	**29 ± 114**	**0.56**
6-min Walk (ft)	**1152 ± 266**	**1165 ± 282**	**0.84**

Table 2.14 GRK5 Outcomes in African American group

Endpoint	Gln 41 Homo (n = 18)	Leu 41 Carrier (n = 26)	*P*-value
Systolic B.P. (mmHg)	**121 ± 17**	**121 ± 17**	**0.95**
Diastolic B.P. (mmHg)	**74 ± 13**	**70 ± 13**	**0.33**
Resting HR (bpm)	**71 ± 12**	**69 ± 9**	**0.46**
ΔHR (bpm)	**29 ± 15**	**29 ± 114**	**0.90**
6-min Walk (ft)	**1169 ± 228**	**1165 ± 282**	**0.96**

Table 2.15 Metoprolol group outcomes

Endpoint	**Gln 41 Homo (n = 31)**	**Leu 41 Carrier (n = 17)**	***P* –value**
Systolic B.P. (mmHg)	**111 ± 19**	**119 ± 15**	**0.16**
Diastolic B.P. (mmHg)	**66 ± 11**	**70 ± 12**	**0.25**
Resting HR (bpm)	**62 ± 9**	**69 ± 9**	**0.01**
ΔHR (bpm)	**28 ± 14**	**29 ± 17**	**0.79**
6-min Walk (ft)	**1149 ± 274**	**1063 ± 255**	**0.29**

Table 2.16 Carvedilol group outcomes

Endpoints	**Gln 41 Homo (n = 27)**	**Leu 41 Carrier (n = 9)**	***P* – value**
Systolic B.P. (mmHg)	**118 ± 19**	**126 ± 21**	**0.31**
Diastolic B.P. (mmHg)	**69 ± 12**	**71 ± 15**	**0.78**
Resting HR (bpm)	**68 ± 13**	**69 ± 9**	**0.82**
ΔHR (bpm)	**26 ± 11**	**28 ± 9**	**0.57**
6-min Walk (ft)	**1156 ± 261**	**1359 ± 232**	**0.05**

Table 2.17 Endpoints corrected for pharmacokinetics in the metoprolol treated group

Variable	**Gln 41 Homo (n = 31)**	**Leu 41 Carrier (n = 17)**	***P* –value**
S-metoprolol (ng/ml)	**50.6 ± 76.4**	**56.8 ± 64.4**	**0.79**
S-metoprolol (mg/kg)	**1.65 ± 0.84**	**1.90 ± 0.79**	**0.32**
Resting HR/S-met (bpm/ng/ml)	**6.3 ± 8.4**	**11.1 ± 13.5**	**0.20**
ΔHR/S-met (bpm/ng/ml)	**3.0 ± 4.9**	**5.6 ± 8.9**	**0.27**

Table 2.18 Endpoints corrected for pharmacokinetic differences in the carvedilol treated group

Variable	**Gln 41 Homo (n = 27)**	**Leu 41 Carrier (n = 9)**	***P* –value**
S-carvedilol (ng/ml)	**17.3 ± 19.2**	**11.1 ± 5.5**	**0.34**
S-carvedilol (mg/kg)	**0.48 ± 0.15**	**0.55 ± 0.13**	**0.18**
Resting HR/S-car (bpm/ng/ml)	**9.3 ± 9.3**	**9.6 ± 9.0**	**0.93**
ΔHR/S-car (bpm/ng/ml)	**4.3 ± 5.9**	**4.1 ± 5.1**	**0.92**

2.3.4 Discussion

Overall, the GRK5-41 Gln to Leu polymorphism was not significantly associated with any of the study endpoints. Given the relatively low frequency (< 0.02) of the Leu allele in Caucasians the overall results were evaluated in just the African American patients. Despite adjusting for race, no significant differences in any of the endpoints were observed based upon GRK5-41 genotype. Of note, all of the Leu41 carriers were African American.

In the metoprolol-treated group the Leu41 carriers had a significantly higher resting heart rate by 7 bpm on average. However, the result lost significance after correcting for plasma *S*-metoprolol differences between the Gln41 homozygotes and the Leu41 carriers (table 2.17). By accounting for pharmacokinetic variation this study was able to identify a potential spurious finding, which further emphasizes the importance of accounting for pharmacokinetic variation. The implications of this finding should however be investigated further by an adequately powered study as the Gln41 homozygotes still had on average a lower corrected resting heart rate compared to the Leu41 carriers.

The carvedilol arm of the study also revealed a significant difference based upon GRK5-41 genotype on the endpoint of distance walked during the 6MWT. Although this finding was in opposition to what would have been predicted, it may be confounded by type I error due to the relatively small (n=9) size of the Leu41 group. No additional significant differences based upon GRK5-41 genotype were observed.

Although the GRK5-41 Leu polymorphism may confer a survival benefit in heart failure patients,[44] it does not appear to be associated with any of the hemodynamic outcomes evaluated in the current study. These results are similar to those of Kurnik *et al.* who also found no significant differences in resting heart or exercise HR when accounting for GRK5-41 genotype in healthy subjects given atenolol.[86] Notably, no differences were observed when correcting for plasma atenolol levels similar to the current study.

The current study did reveal a significant association with a lower resting HR in the Gln41 metoprolol-treated patients, however this difference was not significant when plasma *S*-metoprolol levels were accounted for. Furthermore, given the low reported (~ 2%) allele frequencies[44] in Caucasians this study may have been inadequately powered to evaluate these associations. Regardless, the African American portion of this study was analyzed separately and once again no significant differences in any of the study endpoints based upon GRK5-41 genotype were observed. The results should be interpreted with caution given that the study was not specifically powered to evaluate these associations.

Although the current novel findings do offer some insight on the role of the GRK5-41 genotype in heart failure patients treated with β-blockers, these findings should be interpreted cautiously given the relatively small sample size of the study. This limitation was expected as the study was not initially powered to evaluate the GRK5-41 genotype and the associated clinical pharmacogenomic relationships. Given the relatively uncommon (< 2%) occurrence of the variant

Leu allele in Caucasians, perhaps a study specifically powered to evaluate this relationship solely in African Americans in necessary.

In summary, these results would indicate that the GRK5 Glu41Leu polymorphism is not significantly associated with the clinical degree of β-blockade in heart failure patients. The survival benefits associated with this polymorphism may be mediated through some other mechanism.

Chapter 3

Evaluation of the Relationship Between β1,2-AR and GRK5 Genotype and β-blocker Dose

3.1 Evaluation of the relationship between β1,2-AR and GRK5 genotype and β-blocker dose

3.1.1 Introduction

The mortality benefits of β-blockers in heart failure are well established. However, many heart failure patients are unable to tolerate these agents due to side effects including bradycardia, symptomatic hypotension and decompensation of heart failure. As a result, β-blockers are often underutilized in a population that could clearly benefit from them.[37, 87, 88] Given the high degree of interindividual response to β-blockade it is unknown whether intolerance to these drugs is directly related to their β1,2-AR genotype. Recommendations put forth from the MERIT-HF study group recommend that β-blockers be titrated to their maximal tolerable dose in heart failure patients.[89] Resting HR is commonly used as a measurement of this titration. Hence, any differential response to β-blockade may be reflected in the actual maximal titrated dose that the patient receives. It is therefore important to investigate the relationship between β-blocker dose and β1,2-AR genotype. Thus, it was hypothesized that patients who are homozygous for the Arg389 allele on the β1-AR will require lower doses of β-blocker (carvedilol or metoprolol) in order to maintain a similar resting heart rate as their Arg389 counterparts.

3.1.2 Methods

Outcomes

Daily β-blocker dose was compared to β1,2-AR genotype at the 4 mentioned SNPs.

Genotyping

Genotyping was conducted as previously described *(see chapter 2.1.2).*

Statistical Analysis

The unpaired *t*-test was used to compare groups in terms of β1,2-AR genotype and β-blocker dose. This test was conducted separately for each of the β1,2-AR SNPs previously mentioned. Mean daily dose of the homozygotes was compared to the mean daily dose of the “variant carriers” at each position.

3.1.3 Results

When assessing each drug treatment group (carvedilol or metoprolol), no significant difference in the dose of β-blocker received based upon genotype at position 49 or 389 was observed (Table 3.1). In the metoprolol group, the Arg 389 homozygotes received 139 ± 69 mg on average, whereas the Gly389 carriers received 164 ± 60 mg ($P = 0.18$). In the carvedilol group, the Arg389 homozygotes received 44 ± 16 mg while the Gly389 carriers received 50 ± 15 mg ($P = 0.25$).

No significant difference in the daily dose of either β-blocker was observed when stratified by genotype at position 16 or 27 on the β2-AR (Table 3.2). At SNP positions studied, average carvedilol doses approached the recommended maximum of 50 mg daily given as 25 mg twice a day.

Table 3.1. β1-AR genotypes and β-blocker dose

	Arg 389 Homo	Gly 389 Carrier	*P* - value	Ser 49 Homo	Gly 49 Carrier	*P* - value
Metoprolol	**(n = 24)**	**(n = 25)**		**(n = 36)**	**(n = 13)**	
Dose (mg/day)	**139 ± 69**	**164 ± 60**	**0.18**	**151 ± 71**	**154 ± 48**	**0.88**
Carvedilol	**(n = 18)**	**(n = 18)**		**(n = 28)**	**(n = 8)**	
Dose (mg/day)	**44 ± 16**	**50 ± 15**	**0.25**	**48 ± 11**	**45 ± 27**	**0.84**

Table 3.2 β2-AR genotypes and β-blocker dose

	Gly 16 Homo	**Arg 16 Carrier**	***P* - value**	**Gln 27 Homo**	**Glu 27 Carrier**	***P* - value**
Metoprolol	**(n = 31)**	**(n = 18)**		**(n = 25)**	**(n = 25)**	
Dose (mg/day)	**156 ± 68**	**144 ± 62**	**0.57**	**161 ± 60**	**141 ± 69**	**0.28**
Carvedilol	**(n = 26)**	**(n = 10)**		**(n = 19)**	**(n = 17)**	
Dose (mg/day)	**46 ± 18**	**50 ± 0**	*****	**49 ± 19**	**46 ± 10**	**0.61**

Table 3.3 GRK-41 genotype and β-blocker dose

GRK5 41	Gln 41 Homo	Leu Carrier	*P* - value
Metoprolol Arm	**(n = 31)**	**(n = 17)**	
Dose (mg/day)	**144 ± 66**	**165 ± 63**	**0.29**
Carvedilol Arm	**(n = 18)**	**(n = 18)**	
Dose (mg/day)	**47 ± 17**	**47 ± 8**	**0.95**

3.1.4 Discussion

Based upon β1,2-AR genotype, no significant differences in the dose of either β-blocker was observed. Previous studies have indicated that the Arg389 homozygotes may require lower doses of β-blocker.[37] Although the average daily dose of metoprolol in the current study was lower in both the Arg389 and Ser49 homozygotes, this difference was not significant. However, it is important to note that these same patients had lower mean resting heart rates, despite receiving a higher mean dose of metoprolol. No significant difference in the dose of either carvedilol or metoprolol was observed for either SNP on the β2-AR. Both the average doses of carvedilol and metoprolol were similar to previous clinical trials with these agents, suggesting that the study patients were optimally titrated to maximal tolerable doses.[77, 90]

An important caveat of this study is that the maximal dose of β-blocker received was higher than previous studies[40, 56, 37, 91, 92] (47 ± 15mg, 152 ± 64 mg for both carvedilol and metoprolol treated patients respectively). This is a reliable indicator that the patients in this study were maximally titrated to β-blocker.

These data suggest that neither β1,2-AR nor GRK5 genotype at the 5 mentioned SNPs can predict the dose of either carvedilol or metoprolol that a heart failure patient should receive. This further justifies the findings in Chapter 2 suggesting that despite receiving a similar dose of β-blocker significant clinical differences in both the negative chronotropic and antihypertensive effects based upon β1,2-AR genotype do indeed exist.

Chapter 4

A Rapid and Sensitive Method for the Enantioselective Determination of Metoprolol in Human Plasma Using Chiral High-Performance Liquid Chromatography

4.1 Introduction

Metoprolol {1-isopropylamino-3-[4-(2-metoxyethyl)phenoxy]-2-propranol}is a β1-adrenergic receptor antagonist that has both negative ionotropic and chronotropic properties on the heart. Metoprolol is widely used in the treatment of heart failure due to its negative chronotropic properties. Similar to other β-blockers, the affinity for the β1-receptor is significantly higher for the (*S*) enantiomer.[71] Metoprolol is mainly metabolized by the cytochrome P4502D6 (CYP2D6) enzyme system in the liver with metabolites resulting from O-demethylation, oxidative deamination and α-hydroxylation. Of these, O-demethylation product undergoes further oxidation to the corresponding carboxylic acid, which is responsible for elimination of 65% of the dose in humans[93]. Metabolism of metoprolol is both polymorphic and stereospecific, and pharmacogenomic studies have shown that extensive metabolizers (EMs) have 35% higher clearance of *R*-metoprolol compared to *S*-metoprolol, whereas the opposite occurs in poor metabolizers (PMs).[94, 95]

Various HPLC methods for the direct enantiomeric separation of metoprolol in plasma using fluorescence detection have been previously reported.[96-103] Limitations of prior assays included expensive solid-phase extraction, [96, 98, 103] lengthy sample run times, [96, 99, 100] lack of an internal standard [98, 101] and poor sample resolution (LLOQ > 10 ng/ml).[96, 99, 102] The current method was developed to optimize sample preparation time without the need for expensive solid-phase extractions using a commercially available internal standard (*R*-propranolol).

This report describes a novel chiral specific HPLC method for determining concentrations of both enantiomers (*S*/*R*) of metoprolol in plasma utilizing a simplified liquid-phase extraction technique with sensitive fluorescence detection.

4.2 Materials and Methods

Reagents and Chemicals

Metoprolol and the internal standard *R*-propranolol (Figure 4.1) were purchased from Sigma (St. Louis, MO, USA). Diethylamine and ethanol were also purchased from Sigma. HPLC grade methanol, hexane, and methylene chloride were purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized, distilled water was obtained from a Millipore purification system (Bedford, MA, USA). Drug free human plasma was obtained from the Blood Bank at University of Maryland Medical Center.

Equipment and Instrumentation

The HPLC system consisted of a Waters model 2695 separations module and a model 474 fluorescence detector set at excitation and emission wavelengths of 230nm and 310nm, respectively (Waters Assoc., Milford, MA, USA). The chromatographic data was collected and analyzed using Waters Empower software (Waters Assoc., Milford, MA, USA**.** Separation was achieved at ambient temperature with a Chiralpak-AD (3,5-dimethylphenylcarbamate, 10μm particle size, 250 mm x 4.6 mm ID) chiral HPLC column preceded by a Chiralpak-AD guard column (10 μm particle size 50 mm x 4.6 mm ID)(Daicel

Chemical Industries Ltd., Tokyo Japan). The mobile phase used for analysis consisted of hexane, ethanol, diethylamine and deionized water (90:10:0.1:0.05).

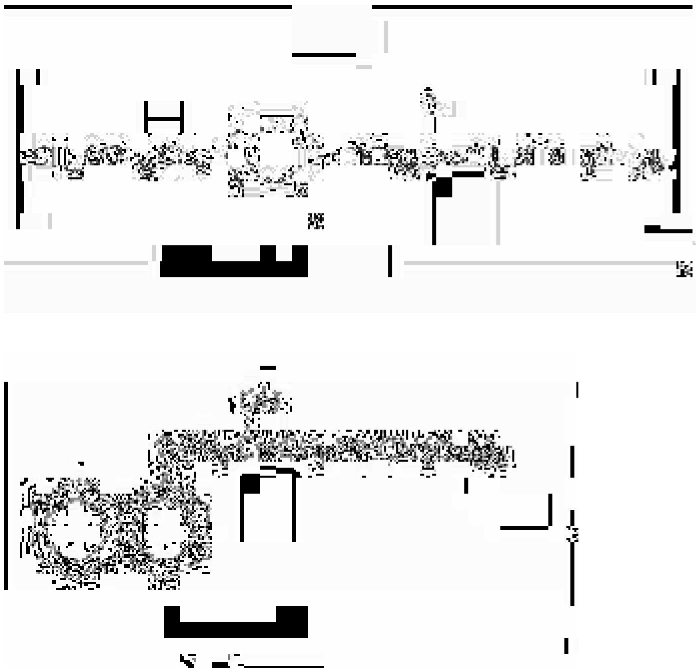


Figure 4.2 Molecular structures of metoprolol and propranolol

*denotes chiral center

The mobile phase was filtered through a 0.45 μm filter and was delivered at a rate of 1.2 ml/min yielding a pump pressure of approximately 430 psi. The total analysis time was 15 minutes.

Preparation of stock solutions and spiked standards

Stock solutions of metoprolol (1 mg/ml and 10,000 ng/ml) and *R*-propranolol (1 mg/ml, 10,000 ng/ml and 1,000 ng/ml) were prepared in methanol and stored at 4°C. These solutions were used to spike plasma in the preparation of standards. Standard and quality control samples were made by addition of the determined quantity of stock solution to drug-free plasma stored at -20°C in aliquots.

Preparation of plasma samples

Plasma (1 ml) was combined with 50 µl of internal standard stock solution (*R*-propranolol 1,000 ng/ml) in a 5 ml conical polypropylene tube. The sample was vortexed briefly, followed by the addition of 4 ml of methylene chloride. The tubes were capped and vortexed for approximately 5 seconds, followed by centrifugation for 5 minutes at 2500 g. The upper inorganic layer was vacuum-removed and the remaining mixture evaporated to dryness under a stream of nitrogen. The sample was then reconstituted with 250 µl of mobile phase, vortexed gently, transferred to an autosampler vial and 200 µl was injected into the HPLC system.

Calibration and Linearity

Calibration curves were constructed using six standard concentrations in plasma and run in duplicate. Curves were generated daily for three days using peak-height ratios of *S*-metoprolol or *R*-metoprolol to the internal standard against the corresponding concentration of each metoprolol enantiomer. Linear calibration curves were generated by weighted ($1/x^2$) linear regression analysis and obtained over the concentration range of 5.0 to 150 ng/ml. All standards and QC samples were stored at -20°C until analysis.

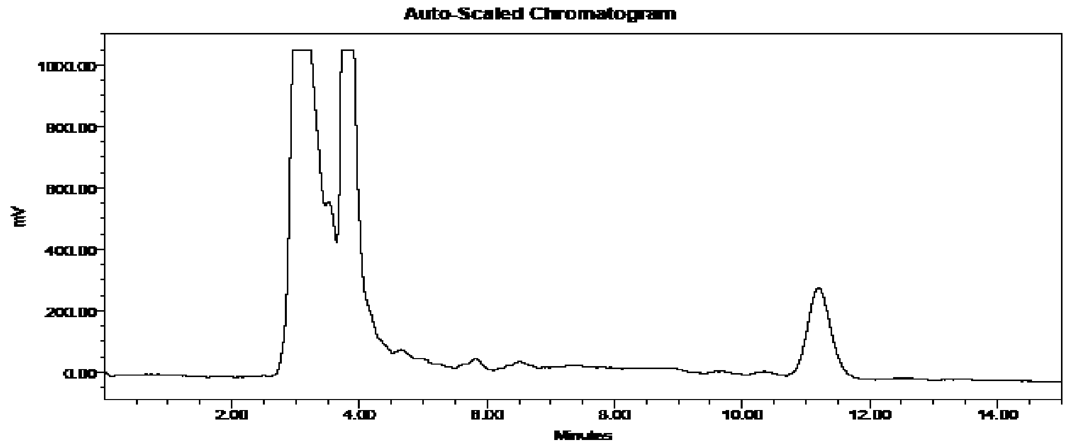
Precision and Accuracy

The precision and accuracy of the assay was determined by analysis of plasma quality control (QC) samples. Plasma QC concentrations for each enantiomer of metoprolol were 12.5, 37.5, and 125 ng/ml respectively. Six replicate QC samples at each concentration were analyzed over three days after which inter- and intra-day means, standard deviations and coefficients of variation (C.V.'s) were calculated by standard methods.

4.3 Results

Representative chromatograms of a blank sample, spiked standard and a patient sample are shown in Figure 4.2. Retention times for *R* and *S*-metoprolol were 8.9 and 10.2 minutes, respectively. The retention time for *R*-propranolol was 5.5 minutes. Calibration curves were linear over the concentration range of 5.0-150 ng/ml with correlation coefficients (r^2) > 0.995 (Figure 4.3); mean standard curve parameters for each analyte are reported in Table 4.1. The intra- and inter-day precision and %CV (Tables 4.2 & 4.3) were less than 8.3 and 10.3 for *S* and *R*-metoprolol respectively. The recovery of *S* and *R*-metoprolol in plasma was 72.1 ± 3.9 and 66.1 ± 2.1% respectively. The lower limit of detection

A



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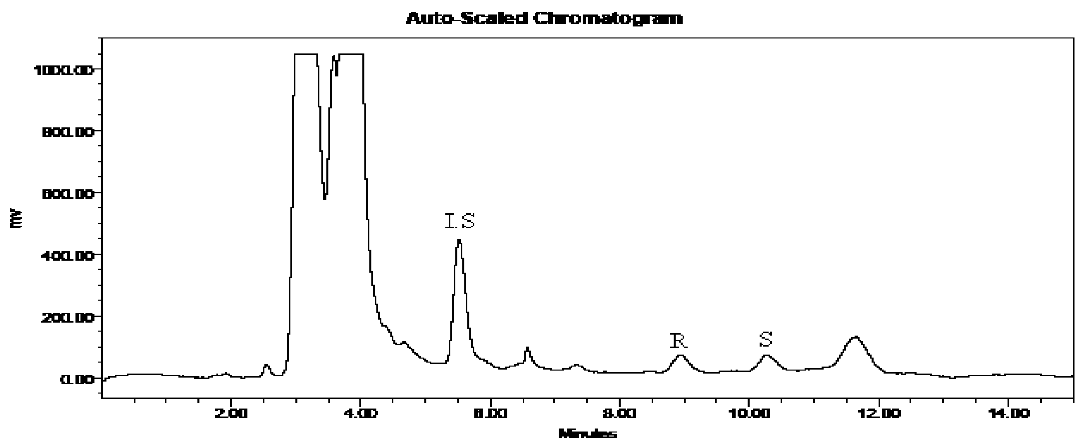

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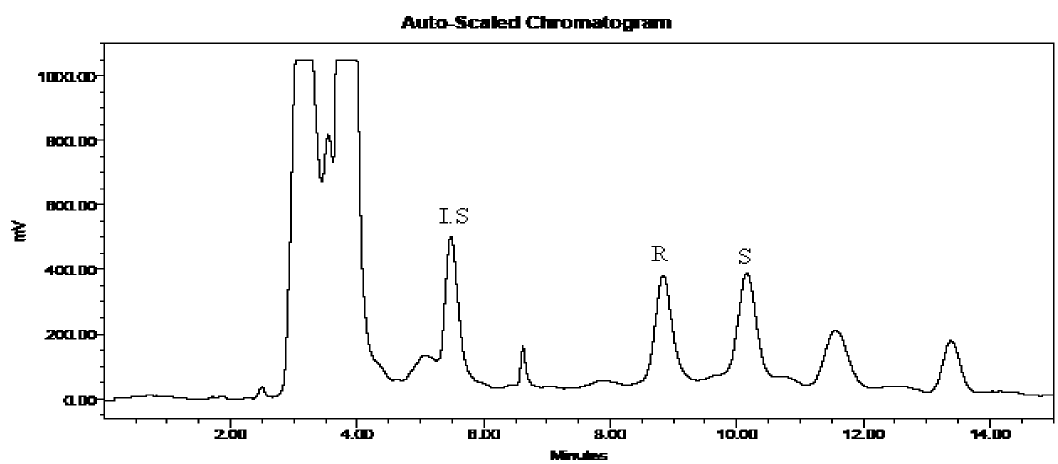
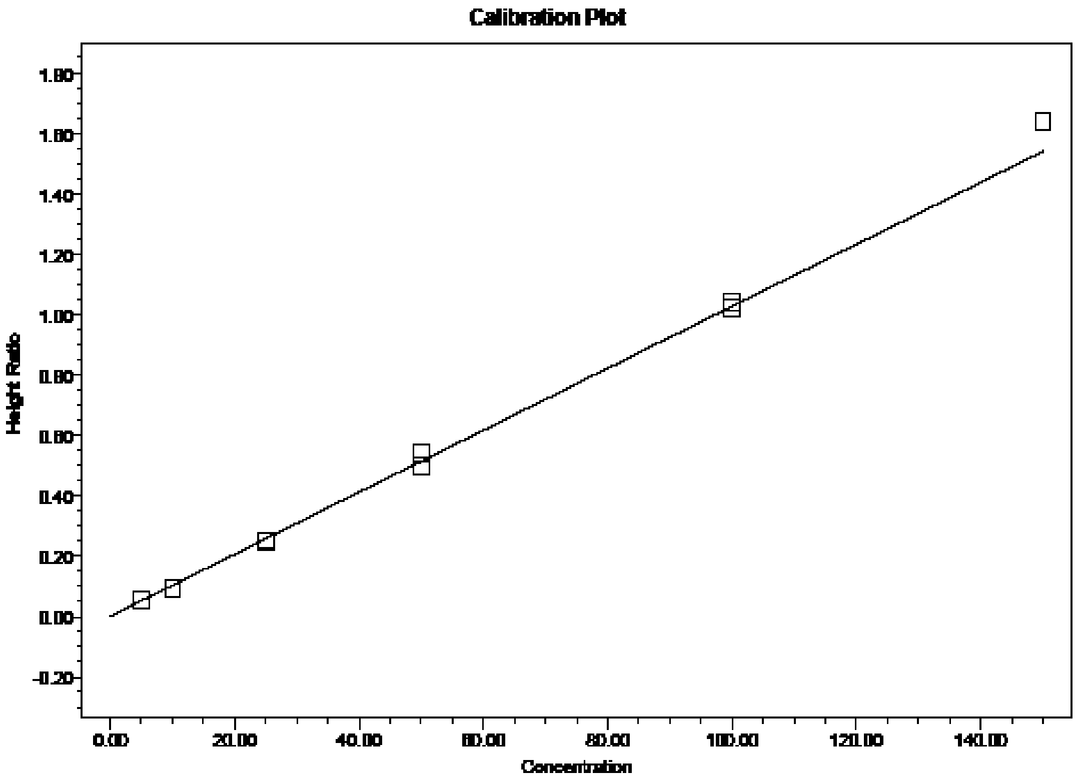


Figure 4.3 Representative chromatograms of Metoprolol (A) blank plasma, (B) plasma low control (12.5 ng/ml, each enantiomer), (C) patient sample (75 ng/ml *S*-metoprolol, 63 ng/ml *R*-metoprolol) (R = *R*-metoprolol, S = *S*-metoprolol, I.S. = internal standard)

A



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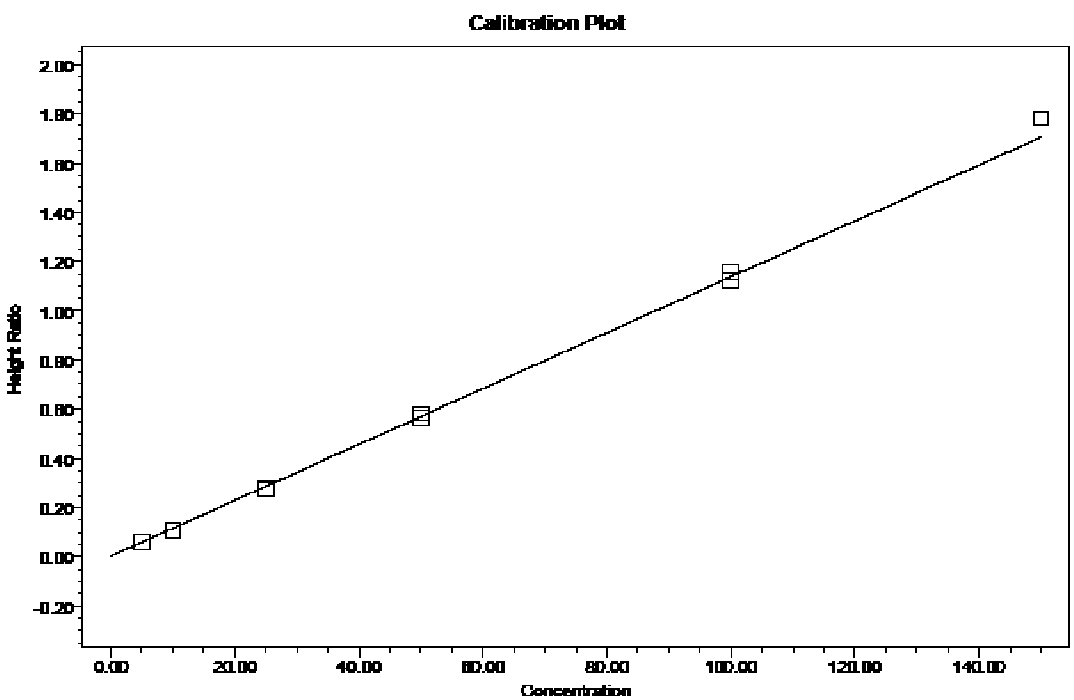


Figure 4.4 Representative standard curves for (A) *S*-metoprolol and (B) *R*-metoprolol concentration expressed as (ng/ml)

Table 4.1 Mean parameters of the calibration curves for *S*, *R*-Metoprolol

	$y = mx + b$		
Plasma (n=3)	m	b	r^2
S-Metoprolol	-0.0034± 0.004	0.010 ± 0.002	0.996
R-Metoprolol	-0.0007± 0.002	0.011 ± 0.0002	0.997

Table 4.2 Intra- and inter-day precision and accuracy for *S*-metoprolol in plasma

	Concentration (ng/ml)		CV(%)	% Deviation
	Added	Found (mean +/- S.D.)		
Intra-assay reproducibility[a]				
Quality Controls	12.5	12.3 ± 0.5	4.0	-2.0
	37.5	37.6 ± 1.2	3.3	0.3
	125	131.2 ± 5.9	4.5	4.9
Inter-assay reproducibility[b]				
Quality Controls	12.5	12.3 ± 0.2	5.1	-1.8
	37.5	39.5 ± 0.3	2.4	5.2
	125	135.3 ± 6.0	6.9	8.3
Standards	5.0	5.3 ± 0.3	6.2	5.5
	10.0	9.5 ± 0.6	6.2	-5.5
	25.0	24.4 ± 2.0	8.3	-2.3
	50.0	50.9 ± 3.1	6.1	1.9
	100.0	95.3 ± 6.3	6.6	-4.7
	150.0	160.7 ± 4.3	2.6	7.1

[a] Six quality control samples per concentration
[b] Six quality control samples or two standards per day per concentration for 3 days

Table 4.3 Intra- and Inter-day precision and accuracy for *R*-metoprolol in plasma

	Concentration (ng/ml)		CV(%)	%Deviation
	Added	Found (mean +/- S.D.)		
Intra-assay reproducibility[a]				
Quality Controls	12.5	12.3 ± 0.3	2.6	-1.7
	37.5	39.7 ± 1.5	3.7	5.8
	125	140.7 ± 8.0	5.7	12.5
Inter-assay reproducibility[b]				
Quality Controls	12.5	12.0 ± 0.6	6.2	-3.9
	37.5	38.3 ± 0.3	3.2	2.2
	125	136.1 ± 5.8	7.9	8.9
Standards	5.0	5.2 ± 0.5	10.3	4.4
	10.0	10.0 ± 0.8	8.0	-0.2
	25.0	24.3 ± 1.6	6.4	-2.8
	50.0	51.2 ± 2.5	4.9	2.4
	100.0	95.4 ± 6.7	7.0	-4.6
	150.0	156.9 ± 5.0	3.2	4.6

[a] Six quality control samples per concentration.
[b] Six quality control samples or two standards per day per concentration for 3 days

for both *S* and *R*-metoprolol were determined with a limit of sensitivity defined as a signal to noise ratio of 4:1. The analysis of 6 different blank plasma samples collected showed no interference of endogenous components with the metoprolol enantiomers or internal standard (Figure 4.2).

4.4 Discussion

As mentioned, a variety of assays have been developed for the direct enantiomeric quantification of metoprolol in plasma.[96, 98-103] However, limitations of these methods compared to the method presented here include extensive multi-step processing or expensive sample preparation using solid phase extraction,[96, 98, 99, 103] lack of an internal standard,[98, 101] lengthy sample run times (exceeding 20 minutes)[96, 98-100] and poor sample quantification (LLOQ > 10 ng/ml).[96, 98, 99, 102] In addition, Mistry et. al, utilized a Teicoplanin chiral stationary phase, which is relatively less stable than the current chiral stationary phase.[101]

The primary advantage of the present method over previously published assays is simplified plasma processing using a single step LLE procedure coupled with shorter analyte retention times this allows for a more time efficient and high-throughput sample analysis, while still maintaining good sample resolution. Furthermore, the current method avoids the unnecessary addition of sodium hydroxide[98-100, 102] to the extraction while maintaining excellent and consistent analyte recovery of 72.1% and 66.1% for *S* and *R* metoprolol respectively.

This assay employed a direct chiral stationary phase as a means of enantiomeric separation. This has several advantages over indirect chiral separation, and avoids the lengthy derivitization steps necessary for indirect separation. In addition, use of a chiral stationary phase yielded high sensitivity using the natural fluorescence of metoprolol with an overall shorter sample preparation time using a simplified LLE method. Sample preparation took approximately 20 minutes and did not include a lengthy extraction process (10 seconds compared to 30 minutes)[98, 100, 102] or various washing steps common to solid phase extraction.[96, 103]

The present assay was used to determine steady-state metoprolol levels in an ongoing pharmacogenomic clinical trial to evaluate the relationship between β1 adrenergic receptor variants and response in patients with heart failure. All *S*,*R*-metoprolol concentrations obtained in clinical samples were within the quantification limit for this assay. This patient population was receiving a number of other pharmaceutical agents, yet no interfering peaks on any of the chromatograms were observed.

A simplified, rapid, and sensitive assay has been developed and validated for determination of *R*- and *S*-metoprolol concentrations in human plasma. This method provides an efficient sample preparation step while still maintaining sensitivity and precision over a broad range of plasma concentrations that may be encountered in patients receiving various doses of metoprolol succinate. The assay has been utilized in a clinical study investigating the enantioselective pharmacokinetics of oral metoprolol succinate in heart failure patients, and is well suited for supporting clinical pharmacokinetic trials in heart failure patients receiving metoprolol.

Chapter 5

A Rapid and Sensitive Method for the Enantioselective Determination of Carvedilol in Human Plasma Using Chiral High-Performance Liquid Chromatography

5.1 Introduction

Carvedilol 1-(carbazol-4-yloxy-3[[2-(O-methoxyphenoxy)ethyl]amino]-2-propanol is a β-adrenergic and α_1 receptor antagonist. The *S*(-)-enantiomer has β-adrenoceptor blocking activity, while the racemate also has α1-receptor blocking activity due to the activity of the *R*(+)-enantiomer.[104 105] Each enantiomer has different pharmacokinetic activity in human patients.[80, 106, 107]

Carvedilol is extensively metabolized. It is mainly metabolized via the highly polymorphic Cytochrome P450 2D6 (CYP2D6) enzyme system in the liver. The main metabolic pathways are 4’, 5’and 8’ hydroxylation and O-desmethylation. In addition to CYP2D6, CYP2C9 and CYP1A2 also play minor roles in the metabolism of carvedilol. The *S*-isomer is metabolized by the same enzymes as the *R*-isomer, yet at a much higher rate.[108] Hence, it is important to measure plasma concentrations of each carvedilol enantiomer to account for the wide variation in pharmacokinetics.

Currently, only one direct method for the enantioselective separation of carvedilol in human plasma exists.[109] Other methods have employed a chiral derivitization method.[78, 110, 111] Limitations of these methods include expensive and lengthy sample preparation.[78, 110]

This report describes a chiral specific HPLC method for determining concentrations of both enantiomers (*S/R*) of carvedilol in plasma. This method utilizes 500 µl of plasma with a liquid-phase extraction using *S*-carazolol as the internal standard.

5.2 Materials and Methods

Reagents and Chemicals

Carvedilol and the racemic mixture of the internal standard *S*-carazolol (Figure 5.1) were purchased from Sigma (St. Louis, MO, USA). Diethylamine and ethanol were also purchased from Sigma. HPLC grade methanol, hexane, and diethyl ether were purchased from Fisher Scientific (Pittsburgh, PA, USA). Deionized, distilled water was obtained from a Millipore purification system (Bedford, MA, USA). Drug free human plasma was obtained from the Blood Bank at the University of Maryland Medical System.

Equipment and Instrumentation

The HPLC system consisted of a Waters model 2695 separations module, and a shimadzu wavelength fluorescence detector (RF-10AXL, Shimadzu, Columbia, MD, USA) set at an excitation wavelength of 284 nm and an emission wavelength of 343 nm (Waters Assoc., Milford, MA, USA). The chromatographic data was collected and analyzed using Waters Empower software (Waters Assoc., Milford, MA, USA). Separation was achieved at 45ºC temperature with a Daicel (West Chester PA, USA) Chiralpak-AD (3,5-dimethylphenylcarbamate, 10µm particle size, 250 cm x 4.6 mm ID) chiral HPLC column preceded by a Chiralpak-AD guard column (10 µm particle size 50 cm x 4.6 mm ID). The mobile phase used for analysis consisted of hexane, isopropanol, and diethylamine (78:22:0.1). The mobile phase was filtered through a 0.45 µm filter. The mobile phase was delivered at a rate of 1.2 ml/min with a pump pressure of approximately 690 psi. The total analysis time was 22 minutes.

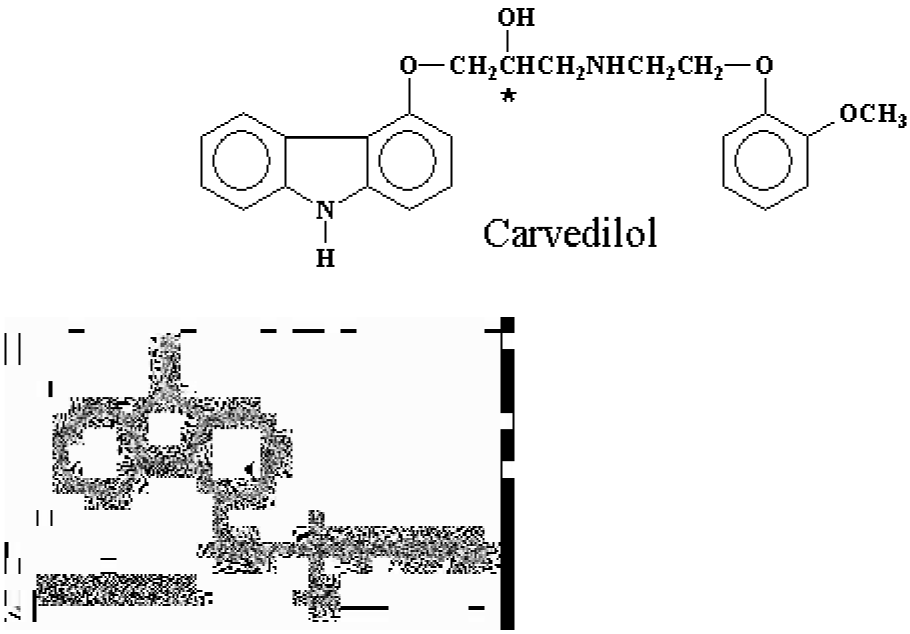


Figure 5.1 Molecular structures of Carvedilol and Carazolol
*denotes chiral center

Preparation of stock solutions and spiked standards

S-carazolol was separated from racemic carazolol (10 µg/ml in methanol) using an enantioselective column (Chiralpak AD column; 4.6 cm X 250 mm, Daicel Chemical Industries, Ltd. Tokyo, Japan). The mobile phase for the enantioselective separation of carazolol consisted of hexane:ethanol:diethylamine (90:10:0.1 v/v) at a flow rate of 1.2ml/min. The excitation and emission wavelengths were set at 284 and 343 nm respectively. The eluted *S*-carazolol was collected at 15 mins, and evaporated under a nitrogen gas (99.5%) stream. *S*-carazolol was then reconstituted with methanol to prepare the stock solution.

A stock solution of 10,000 ng/ml of carvedilol was made in methanol and stored at 4°C. This solution was stable for at least 6 months when stored at 4°C and used to spike plasma in the preparation of standards. Standard and quality control samples were made by addition of the determined quantity of stock solution to drug free plasma stored at -20°C in aliquots.

Preparation of plasma samples

Plasma (500 µl) was combined with 50 µl of internal standard stock solution (S-carazolol 1000 ng/ml) in a 5 ml conical polypropylene tube. The sample was vortexed briefly, and 4 ml of diethyl ether were added. The tubes were then capped and vortexed for approximately 5 seconds, the tubes were then mechanically shaken for 10 minutes. The tubes were then centrifuged for 5 minutes at 2500g. The upper organic layer was transferred into another 5 ml conical polypropylene tube and evaporated to dryness under a stream of nitrogen gas. The sample was then reconstituted with 150 µl of mobile phase, vortexed gently, transferred to a WISP vial and 50 µl was injected into the HPLC system.

Calibration and Linearity

Calibration curves were constructed using six standard concentrations in plasma and run in duplicate. Curves were generated daily for three days using peak-height ratios of *S*-carvedilol or *R*-carvedilol to the internal standard against the corresponding concentration of each carvedilol enantiomer. Linear calibration curves were generated by weighted ($1/x^2$) linear regression analysis and obtained over the respective concentration range. The lower limit of quantification (LOQ) for both *S* and *R*-carvedilol was selected as the concentration at which the assay precision was within 20% and the signal to noise ratio exceeded 3:1. The standard concentrations ranged from 1 to 50 ng/ml. All standards and QC samples were stored at -20°C until analysis.

Precision and Accuracy

The precision and accuracy of the assay was determined by analysis of plasma quality control (QC) samples. Plasma QC concentrations for each enantiomer of carvedilol were 3, 15 and 37.5 ng/ml respectively. Six replicate QC samples at each concentration were analyzed over three days after which inter- and intra-day means, standard deviations and coefficients of variation (C.V.) were calculated by standard methods.

5.3 Results

Representative chromatograms of a blank sample, spiked standard and patient sample are shown in Figure 5.2. Retention times for *S* and *R*-carvedilol were 14.0 and 19.5 minutes respectively. The retention time for *S*-carazolol was 5.9 minutes. Calibration curves were generated using weighted ($1/x^2$) least squares regression. Curves were linear over the concentration range 1.0-50 ng/ml with correlation coefficients (r^2) > 0.996 and 0.995 for *S* and *R*-carvedilol respectively (Figure 5.3); mean standard curve parameters for each analyte are reported in Table 5.1. The intra- and inter-day precision and %CV was less than 7.3 and 7.1 for *S* and *R*-carvedilol respectively (Tables 5.2 & 5.3).

The recovery of *S* and *R*-carvedilol in plasma was 74.4 ± 18.5 and 77.7 ± 18.1% respectively. The lower limit of detection for both *S* and *R*-carvedilol were determined with a limit of sensitivity defined as a signal to noise ratio of 4:1. The analysis of 6 different blank plasma samples collected showed no interference of endogenous components with the carvedilol enantiomers or internal standard (Figure 5.3).

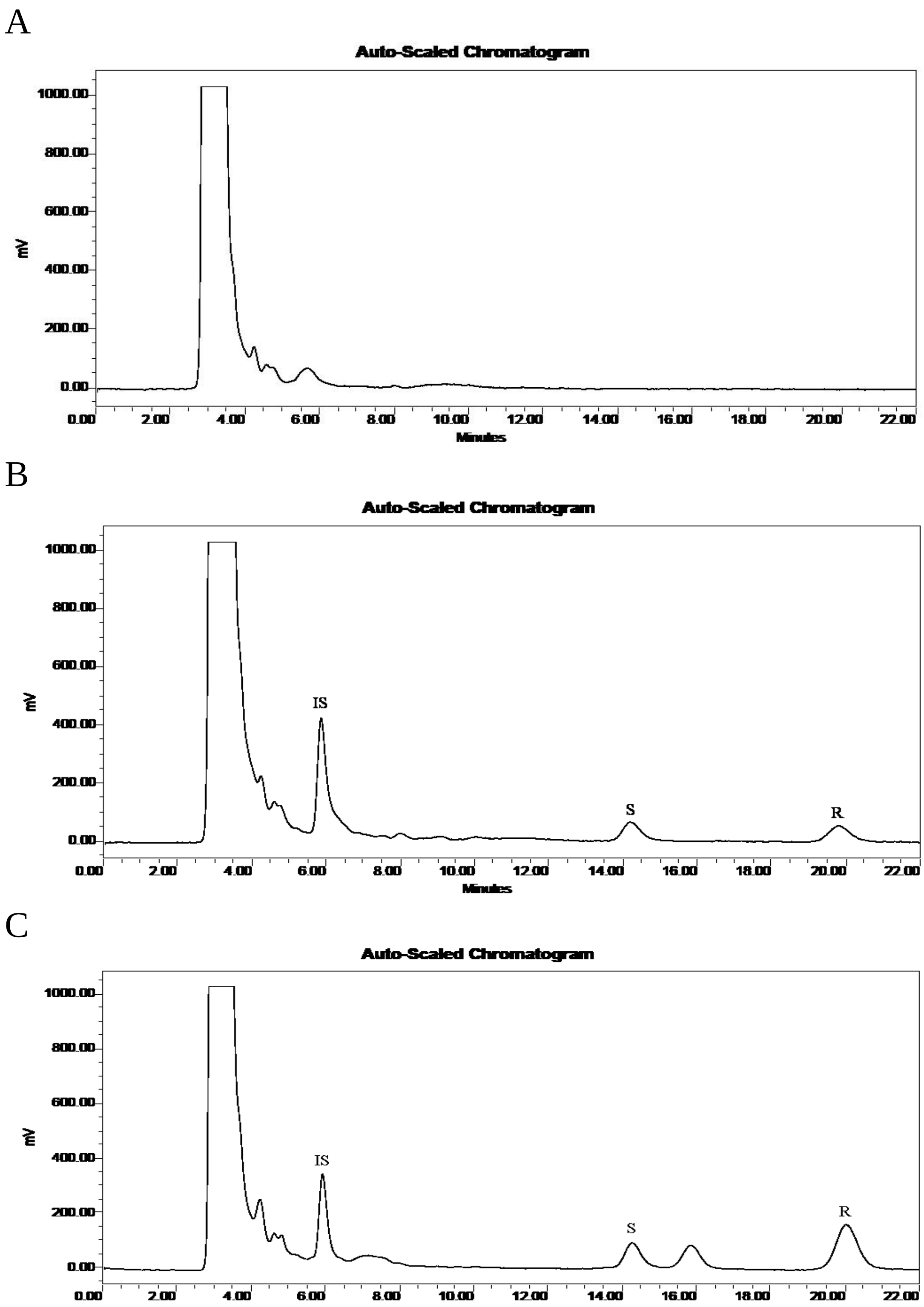
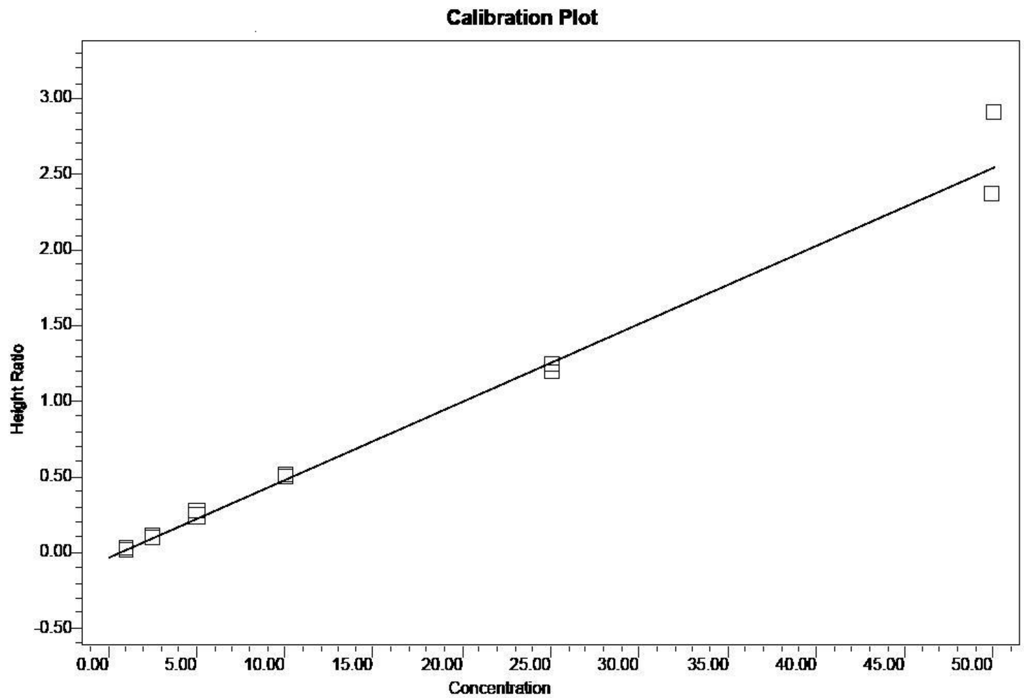


Figure 5.2 Representative chromatograms of Carvedilol (A) blank plasma, (B) plasma low control (3 ng/ml, each enantiomer), (C) patient sample (6.1 ng/ml *S*-carvedilol, 11.9 ng/ml *R*-carvedilol) (R = *R*-carvedilol, S = *S*-carvedilol, I.S. = *S*-carazolol)

A



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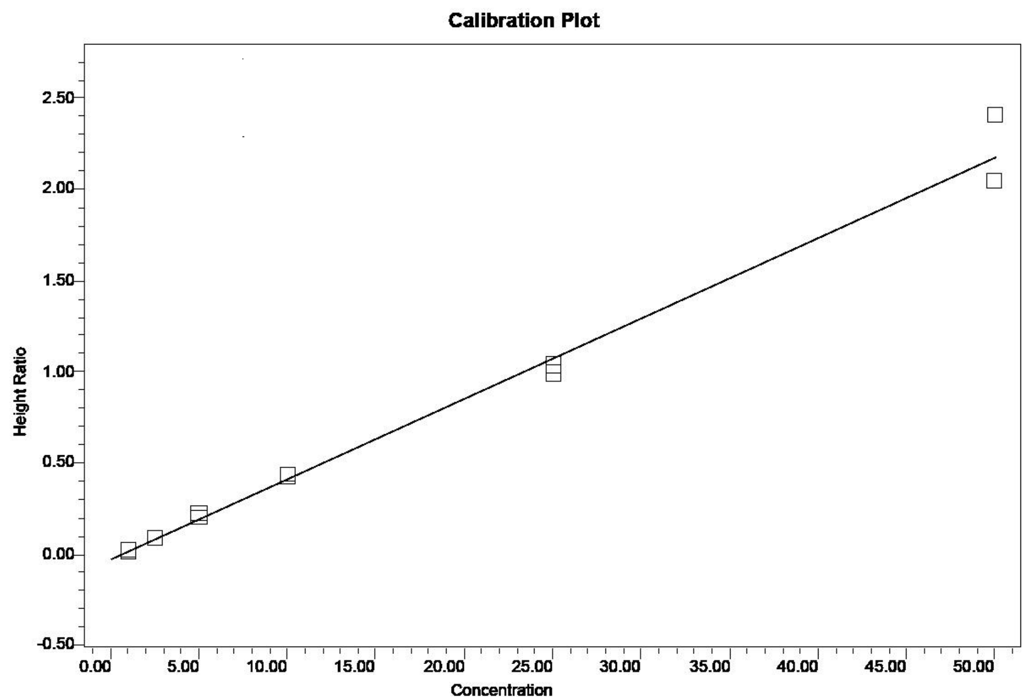

Figure 5.3 Representative standard curves for (A) S-carvedilol and (B) R-carvedilol concentration expressed as (ng/ml)

Table 5.1 Mean parameters of the calibration curves for S, R Carvedilol

	y = mx + b		
Plasma (n=3)	m	b	r^2
S-Carvedilol	-0.0291	0.059	0.996
R-Carvedilol	-0.0253	0.051	0.995

Table 5.2 Intra- and inter-day precision and accuracy for *S*-Carvedilol in plasma

	Concentration (ng/ml)		CV(%)	% Deviation
	Added	Found (mean +/- S.D.)		
Intra-assay reproducibility[a]				
Quality Controls	3.0	3.2 ± 0.1	2.0	5.3
	15.0	16.6 ± 1.0	6.0	10.4
	37.5	41.5 ± 1.7	4.2	10.6
Inter-assay reproducibility[b]				
Quality Controls	3.0	3.2 ± 0.2	7.3	6.7
	15.0	16.0 ± 0.3	5.5	6.4
	37.5	40.8 ± 0.3	5.0	8.9
Standards	1.0	1.0 ± 0.1	2.1	-1.0
	2.5	2.5 ± 0.1	3.9	0.0
	5.0	5.2 ± 0.2	3.9	4.0
	10.0	9.8 ± 0.4	4.4	-1.9
	25.0	24.0 ± 1.0	4.1	-4.0
	50.0	51.4 ± 3.8	7.3	6.7

[a] Six quality control samples per concentration
[b] Six quality control samples or two standards per day per concentration for 3 days

Table 5.3 Intra- and Inter-day precision and accuracy for *R*-carvedilol in plasma

	Concentration (ng/ml)		CV(%)	%Deviation
	Added	Found (mean +/- S.D.)		
Intra-assay reproducibility[a]				
Quality Controls	3.0	3.1 ± 0.	2.7	4.5
	15.0	16.6 ± 1.1	6.5	10.8
	37.5	42.9 ± 2.1	4.8	14.5
Inter-assay reproducibility[b]				
Quality Controls	3.0	3.2 ± 0.1	7.1	7.5
	15.0	15.7 ± 0.2	5.7	4.7
	37.5	40.7 ± 0.1	5.3	8.5
Standards	1.0	1.0 ± 0.1	4.5	-3.0
	2.5	2.6 ± 0.1	4.4	3.2
	5.0	5.2 ± 0.2	3.3	4.0
	10.0	9.9 ± 0.4	4.2	-1.4
	25.0	23.5 ± 1.2	5.2	-6.0
	50.0	50.6 ± 3.2	6.4	1.2

[a] Six quality control samples per concentration.

[b] Six quality control samples or two standards per day per concentration for 3 days

5.4 Discussion

Currently, only one other assay[109] is available that uses a chiral stationary phase for the enantioselective separation of carvedilol. The current method has the advantage being able to identify plasma carvedilol levels as high as 50 ng/ml, which may be necessary when dealing with patients taking maximal doses of carvedilol. In addition, the current method also avoids the addition of a buffer during the sample preparation phase, yet still maintains ideal sensitivity (LLOQ = 1 ng/ml) over a larger range 1 – 50 ng/ml for carvedilol. This assay was ideally suited to determine both *S* and *R*-carvedilol levels in heart failure patients receiving maximal doses of the drug.

A simplified, rapid, and sensitive assay has been developed and validated for determination of *R*- and *S*-carvedilol concentrations in human plasma. This method provides an efficient sample preparation step while still maintaining sensitivity and precision over a broad range of plasma concentrations that may be encountered in patients receiving various doses of carvedilol. The assay has been utilized in a clinical study investigating the enantioselective pharmacokinetics of oral carvedilol in heart failure patients, and is well suited for supporting clinical pharmacokinetic trials in heart failure patients receiving carvedilol.

Chapter 6

Conclusions and Future Directions

Within the next decade, the approach to treating heart failure will likely involve dose-specific and drug-specific individualization based on pharmacogenomic, pharmacokinetic and pharmacodynamic information. There are numerous potential benefits to this including improved efficacy and safety. The goal of pharmacogenomics is to select the right drug at the right dose of the medication for the patient. This not only aids to maximize efficacy, it also avoids unnecessary side effects while simultaneously decreasing health care costs.

The current study sought to clarify the role of pharmacogenomics on the treatment of heart failure with β-blockers. Agent-specific pharmacogenomics, pharmacodynamics and pharmacokinetics were examined as potential means of variation in patient response. A number of important findings were brought to light from the results of the current study. If further confirmed, the implications of this study's results could have a potent impact on the treatment of heart failure patients. Based upon the specific aims, there are a few potential clinical implications from the results of the study.

In the first two specific aims, the goal was to clarify the relationship between β-blockade in heart failure patients and the β1,2-AR and GRK5 aforementioned SNPs. When accounting for all treatment groups, no significant relationship between these SNPs and the clinical degree of β-blockade was observed. However when evaluating the β1-AR Arg389Gly polymorphism based on agent-specific β-blocker treatment, a significantly enhanced negative chronotropic response was observed in the metoprolol-treated Arg389 homozygotes after correcting for plasma *S*-metoprolol concentrations compared to the Gly389 carriers. The Arg389 homozygotes had a significantly lower resting heart rate after this correction in comparison to their Gly389 counterparts. Furthermore, a noteworthy difference in systolic blood pressure conferred by metoprolol at position 389 on the β1-AR was also reported. Specifically, metoprolol-treated Arg389 homozygotes had a significantly lower systolic blood pressure compared to the Gly389 carriers. Although this association had been previously observed in healthy and hypertensive patients, this study was the first to document this finding in heart failure patients. Additionally, a strong association between the β2-AR SNPs (16 & 27) and hemodynamic response to carvedilol was observed. Both the Arg16 and Glu27 carriers had significantly lower systolic and diastolic blood pressures compared to their counterparts. These findings provide further evidence that there are genotype-specific and drug-specific response differences in heart failure patients treated with carvedilol or metoprolol.

The third specific aim, investigated the relationship between β1,2-AR and GRK5 genotype and the maximal titrated dose of β-blocker dose received. No significant difference in the dose of either carvedilol or metoprolol was based on any of the SNPs evaluated in this study. Thus, this suggests that neither β1,2-AR nor GRK5 genotype plays a role in the maximal titrated dosing of either carvedilol or metoprolol. More importantly, it points out that despite receiving equivalent doses, differential responses were observed in the previous two specific aims.

The fourth specific aim evaluated the role of pharmacokinetic variability in response to β-blockade. To assess this, two enantioselective HPLC assays with fluorescent detection were developed. These were capable of determining plasma concentrations of either carvedilol or metoprolol that may be commonly encountered in heart failure patients receiving either of these medications. Each method offered significant advantages in both sample preparation and detection limits compared to previously published methods.

These novel detection methods were crucial for the overall goal of accounting for the wide variability in response to β-blockade. A real-world approach was used to obtain plasma *S*-carvedilol or *S*-metoprolol concentrations at the time of the endpoint measurements. This was an essential feature of this study as it allowed us to control for the wide pharmacokinetic variability while simultaneously evaluating the pharmacodynamic relationship of the clinical degree of β-blockade and β1,2-AR and GRK5 genotype. The necessity of this approach was evidenced by the findings in both the primary endpoint of ΔHR and secondary endpoint of resting HR. Both of these findings were significantly different based upon Arg389Gly genotype only when corrected for plasma *S*-metoprolol levels. Thus, this study further reinforces the fact that pharmacokinetic variability should be accounted for when evaluating pharmacodynamic differences.

The major finding of the current study was that heart failure patients who were Arg389 homozygotes at the β1-AR had a significantly enhanced response to metoprolol as measured by a negative chronotropic response on heart rate during exercise (ΔHR) when corrected for plasma *S*-metoprolol concentrations. This was not observed in the Gly389 carriers. Furthermore, the Arg389 homozygotes also had a significantly lower resting heart rate when corrected for plasma *S*-metoprolol concentrations. These findings suggest that the degree of β-blockade in metoprolol treated heart failure patients is different based upon genotype at position 389. Interestingly, these same findings did not hold true for patients in the carvedilol treated arm. This may be due to a difference in the degree of β-blockade by metoprolol and carvedilol in heart failure patients when accounting for genotype at position 389.

Further studies are needed to characterize the pharmacogenomic associations between both negative chronotropic and hemodynamic responses to specific β-blockers in heart failure. These results suggest that there may be a selective metoprolol-specific response based upon genotype at position 389 on the β1-AR. A similar association may exist between carvedilol and the β2-AR SNPs at positions 16 and 27. Therefore, this too merits further investigation.

The current findings would propose that a large prospective clinical trial comparing each of the aforementioned β-blockers while accounting for each of the aforementioned β1,2-AR SNPs would be valuable in tailoring future heart failure treatments. More specifically, a prospective, multi-center study would be necessary to confirm the results of this study based upon the β1,2-AR and GRK5 SNPs. The study should consist of β-blocker naïve heart failure patients. At baseline all vital signs would be recorded along with LVEF, 6-minute walk distance, and heart rate response to a cycle ergometry test. In addition a one-million SNP chip would be used for genotyping the β1,2-AR and GRK5 receptor SNPs as well as the CYP2D6 genotype of the patient. Patients would then be randomized to receive either carvedilol or metoprolol succinate and would be titrated to maximal tolerated doses in a standard fashion. One-year from completion of the dose titration, patient's resting HR, blood pressure as well as LVEF, 6MWT and performance on the cycle ergometry test would be recorded. The primary endpoint would be the patient's heart rate response to the cycle ergometry test based upon the aforementioned SNPs. Secondary endpoints would include the change in resting HR (ΔHR), blood pressure, 6MWT performance and LVEF from baseline.

Additionally, one-year after maximal titration, patients would submit to a one-day pharmacokinetic study to determine pharamacokinetic parameters of the drug they received. These parameters would include the AUC, tmax, Cmax and Cmin and average steady-state plasma concentrations of both the *S/R*-enantiomers of each agent. These parameters would then be compared to the patient's CYP2D6 genotype and analyzed accordingly for correlations with all study endpoints.

Hence, this prospective study would not only act to confirm the results of the current study, but it would also be sufficiently powered to analyze most sources of pharmacogenomic and pharmacokinetic variability. From this, a tailored patient-specific β-blocker regimen could be formulated for all future heart failure patients using only a saliva or blood sample to genotype them. Furthermore, using the genome wide association analysis, this study could potentially identify other sources of pharmacogenomic variability not currently identified.

In conclusion, this series of studies investigating the role of the β1,2-AR and GRK5 SNPs on the treatment of heart failure with β-blockers have shown a number of novel findings with potential clinical implications. The current study is one of the first to suggest pharmacogenomic response to β-blockers in heart failure may be drug-specific. If confirmed by future prospective studies, these findings have the potential to significantly alter the approach to heart failure treatment and guidelines. It may soon be possible to use β1,2-AR and GRK5 genotype to predict response, in numerous clinical outcomes in patients treated with either carvedilol or metoprolol succinate.

Appendix – I
Protocols and Consent Forms

Study Protocol

A. Specific Aims:

Currently, over 5 million Americans are diagnosed with heart failure, with overall 5-year survival of approximately 40%. This mortality rate increases with New York Heart Association (NYHA) stage of heart failure. Patients with NYHA stage IV heart failure have the lowest functional status, based on exercise capacity, and have a one-year mortality rate of approximately 50%.[1]

The primary treatment for heart failure is beta-blockers, which antagonize the beta-adrenergic receptor (β-AR). Metoprolol and carvedilol have not only been shown to decrease mortality and hospitalizations, but also improve quality-of-life and hemodynamic measures in heart failure patients. Despite their purported benefits, beta-blockers are still underutilized in this patient population mainly due to fear of decompensation of heart failure.[2] Moreover, β-blocker therapy is known to produce widely variable responses in patients.[2] A component of this variability may be attributed to genetic heterogeneity at the level of the β-AR. Numerous single nucleotide polymorphisms (SNPs) have been identified at both β1 and β2-ARs. Differences in expression and functionality of β1,2-AR SNPs have been well documented, however their role in the variability of β-blocker treatment in heart failure is still unclear. This study will investigate the role of genetic variants that may contribute to this variability of response.

The goal of this series of projects is to evaluate the relationship between β1 and β2-AR single nucleotide polymorphisms (SNPs) and clinical response to beta-blockade with either metoprolol or carvedilol in NYHA class II-III heart failure patients. We will accomplish this through the following four specific aims:

Aim 1. Determine the relationship between β1,2-AR and GRK5-41 genotype and clinical response in heart failure patients treated with beta-blockers.

It has been suggested that the mortality benefit of beta-blockers in heart failure is due in part to their ability to decrease heart rate in the presence of elevated catecholes.[2] Functional capacity is a critical determinant of prognosis in heart failure patients, which is partially related to β1,2-AR function. It is known that β1,2-AR polymorphisms are a significant determinant of exercise capacity in heart failure patients.[3,4] Thus, this study will evaluate the role these polymorphisms play in exercise capacity in patients being treated with a beta-blocker. The primary hypothesis of this study is that patients being treated with a beta-blocker (carvedilol or metoprolol) who are carriers of the Gly allele at position 389 on the β1-AR will not have an attenuated response (i.e. higher heart rate following sub-maximal exercise) when compared to the Arg homozygotes. Sub-maximal exercise will be measured by the 6-minute walk test and functional distance completed in the 6-minute walk test will also be measured.

Aim 2. Determine the relationship between β1,2-AR genotype and β-blocker dose level.

The mortality benefits of beta-blockers in heart failure are well established. However, many heart failure patients are unable to tolerate these agents due to side effects including bradycardia, symptomatic hypotension and decompensation of heart failure. As a result, beta-blockers are often underutilized in a population that could clearly benefit from them.[5,6] Given the high degree of interindividual response to beta-blockade it is unknown whether intolerance to these drugs is directly related to their β1,2-AR genotype. It is therefore important to identify the relationship between beta blocker dose and β1,2-AR genotype.

This specific aim will test the hypothesis that patients homozygous for Arg at position 389 on the β1-AR will be able to tolerate higher levels of beta-blockers compared to the Gly carriers. Accordingly, β1,2-AR receptor genotype will be determined at the 4 specified SNPs, and will be compared to each patient's current stable beta-blocker dose.

Aim 3. Evaluate the role of pharmacokinetic variability in response to β-blockade.

Significant interpatient variability in pharmacokinetic parameters has been reported with both metoprolol and carvedilol.[7,8,9,10] This portion of the study will identify and account for the sources of underlying pharmacokinetic variability. To account for pharmacokinetic variation between patients, plasma carvedilol or metoprolol concentrations will be determined using a novel stereospecific HPLC assay. Plasma samples will be taken from the patients immediately following the 6-minute walk test. Thus, plasma levels of both carvedilol and metoprolol will be used to test for correlation with heart rate and exercise performance.

Aim 4. Determine whether genotype at position 389 on the β1-AR is associated with the clinical degree of beta-blockade between metoprolol and carvedilol.

A recent study suggests that there may be a difference between carvedilol and metoprolol in terms of level of beta-blockade in relation to the specific β1-AR genotype at position 389.[11] This aim will investigate whether a difference in beta-blockade exists in relation to each genotype (Arg homozygotes/Gly carriers) at position 389 for both carvedilol and metoprolol. The clinical degree of β-blockade will be assessed by change in heart rate from rest following the 6-minute walk test. Negative chronotropic response on heart rate during exercise is considered the gold standard for assessing degree of β1-AR blockade.[12,13] It is still unknown whether a clinical difference remains in terms of the treatment of heart failure between carvedilol and metoprolol. This specific aim will further clarify the relationship between β1-AR genotype at position 389 and specific drug response.

B. Background and Significance:

B1. Heart Failure and Treatment

There are numerous drug therapies used in the treatment of heart failure. Benefits of drug therapy include reduced mortality/morbidity and hospitalizations as well as symptomatic relief. Of these agents, β-blockers are the mainstay of therapy for treating heart failure, and certain β-blockers have been shown to decrease mortality and hospitalizations and provide symptomatic improvement. Furthermore bisoprolol, metoprolol and carvedilol have not only been shown to decrease mortality and hospitalizations, but also improve quality-of-life and hemodynamic measures.[2] Numerous large-scale clinical trials (MERIT, COPERNICUS, MOCHA) have indicated an approximate 30-40% reduction in mortality for both carvedilol and metoprolol. Both agents are currently approved by the FDA for treatment of heart failure.

Polypharmacy and drug interactions also contribute to the complexity of treating heart failure. Pharmacogenomics provides a mechanism to approach these problems. A major area of interest within the field of cardiovascular pharmacogenomics is evaluation of β-AR expression and function. Single-nucleotide-polymorphisms (SNPs) of the β1, 2 and 3 adrenergic receptors have been elucidated and evaluated in a number of pathophysiological conditions such as hypertension, heart failure, and asthma.

B2. β-adrenergic Receptors

Beta-adrenergic receptors (β-AR's) are Gs and Gi protein coupled receptors that play critical roles in cardiovascular functioning. While similar in configuration, the beta receptors differ in terms of secondary messaging and downstream activation. Like all Gs protein coupled receptors, the β-AR is a seven transmembrane spanning receptor with an extracellular amino terminus and an intracellular carboxy terminus. Although polymorphisms have been identified in all three beta-adrenergic receptors, this research will focus on the polymorphisms of the β1 and β2-AR's.

The β1-AR's are located mainly on cardiac myocytes and comprise about 80% of all β-receptors in the healthy heart.[2] In heart tissue, their key function is to regulate blood pressure through both positive inotropic and chronotropic effects. Normal agonist-induced stimulation by norepinephrine or epinephrine leads to activation of adenylyl cyclase (through its coupling to Gs-protein), which increases intracellular cAMP levels. This activates protein kinase A to phosphorylate troponin I, the L-type calcium channels, and phospholamban. The final result of this pathway is a greater influx of calcium into the cardiac myocyte and consequent enhanced contractility.[14]

The β2-AR is also located on the cardiac myocytes and highly expressed throughout the cardiovascular system and airway smooth muscle with lower expression in the brain and adipose tissue**.** Similar to β1-AR s, they act to regulate blood pressure and contractility as well as arterial vasodilation. Like the β1-AR the β2-AR is also coupled to Gs-protein, however it is also coupled to the Gi-protein. The β2-AR exists in multiple polymorphic forms. The role of these polymorphic forms and their Gi-protein coupling have not been fully elucidated in heart failure.[4]

B3. β1-AR Single Nucleotide Polymorphisms

The β1-AR is encoded by an intronless gene located at chromosome 10q24-26.[15] To date, approximately 12 single-nucleotide polymorphisms (SNPs) for the β1-AR have been discovered, occurring primarily in either the extracellular or intracellular portion. Thus, the transmembrane region of the receptor appears to be highly conserved. The two most common SNPs occur at codon positions 49 (extracellularly) and 389 (intracellularly) on the β1-AR .**(Table 1)** The remaining SNPs do not occur at high enough frequencies (less than 0.1%). to be of a major clinical significance. At codon 49, the most commonly occurring SNP is Serine substituted by the variant Glycine (Ser49Gly), with the wild type being the homozygous Ser49Ser. At residue 389, the most commonly occurring SNP is Arginine replaced by a Glycine (Arg389Gly), with the wild type being the homozygous Arg389Arg. Following the definition of a polymorphism, both these variants occur in frequencies greater than 1%. The variant allele Ser49Gly occurs with a frequency of about 0.15 in whites and Asians and approximately 0.29 in blacks, while the Arg389Gly occurs in about 0.27 in whites and Asians and approximately 0.42 in blacks.[16] The extracellular Ser49Gly polymorphism of the protein is necessary for receptor expression and regulation. Conversely, the intracellular Arg389Gly is important for receptor functioning and G-protein coupling.[3]

The functional relevance of each of these receptor polymorphisms has been studied using *in vitro* techniques. At position 49 on the β1-AR it appears that the Gly variant undergoes an increased amount of agonist promoted down-regulation compared to the Ser variant.[14,16] Thus it appears that the Gly49 variant could offer some protective effect from the cardio-toxic catecholes in heart failure.

With respect to the β1-AR polymorphism at position 389, it was recently discovered that both the Arg and Gly variants have similar basal constitutive activity in terms of cAMP generation.[11] However, *in vitro* studies using Chinese Hamster Fibrolasts cells have demonstrated that the Gly389 variant has 3-fold lower levels of isoprenaline stimulated adenylyl cyclase activity when compared to the Arg389 type.[17] Further investigation found that these differences were due to greater coupling of the Arg389 receptor to Gs-protein.[18] Hence, it appears that the Gly389 variant is hyporesponsive in terms of agonist induced activation.

Rochais et al. recently reported that carvedilol, metoprolol and bisoprolol all act as inverse agonists at both of the 389 variants in living rat myocytes. In terms of secondary messengers, only carvedilol decreased basal levels of cAMP and significantly decreased cAMP concentrations in the Arg variants compared to the Gly variants. The Arg389 variant seems to be uniquely sensitive to carvedilol.[11] However, the clinical implications of these findings are unknown.

Numerous clinical studies have evaluated the role of the B1-AR SNP's in heart failure. In terms of hemodynamics, it appears that patients homozygous for the Arg389 allele had an increased resting heart rate as well as increased exercise capacity compared to the Gly389 carriers.[3,19,20] At position 49 no significant associations between hemodynamics have been identified. Taken together it appears that the β1-AR polymorphisms alone are not directly related to development of heart failure. However, they may modify the disease and its response to drug treatment.

B4. β2-AR Single Nucleotide Polymorphisms

The β2-AR is encoded by an intronless gene located at chromosome 5q31-32. Similar to the β1-AR , the β2-AR not only couples to the stimulatory protein Gs, but also couples to the inhibitory protein Gi. Of the polymorphisms identified for the β2-AR , two (position 16 and 27) have been shown to alter receptor expression and functioning.[16] At position 16, the commonly occurring Gly is substituted with Arg and at position 27, the commonly occurring Gln is substituted with Glu. It is estimated that the Arg16 allele occurs with a frequency of about 0.46 in whites and 0.49 in blacks, and is often referred to as the wild type. The Glu27 occurs with a frequency about 0.35 in whites and about 0.20 in blacks.**(Table 1)**

The functional relevance of each of these receptor polymorphisms has been studied using in vitro techniques. Both of the mentioned SNP's on the β2-AR occur in the extracellular portion thus affecting ligand binding and receptor desensitization. At position 16 the Arg variant depressed agonist-promoted receptor downregulation. At position 27 the Glu variant also exhibits depressed agonist-promoted downregulation.[4]

Current research suggests that both the Arg16 and Glu27 variants are associated with increased exercise capacity in heart failure patients.[4] In a study to examine associative properties of the B2-AR SNP's and risk of heart failure, Liggett et al. found no association with prognosis of heart failure at either position 16 or 27.[21] In concurrence with previous results, Covolo et al. found no role of any of the mentioned B1,B2-AR SNP's as risk factors for heart failure.[20] Overall, the B2-AR s appear to have no predictive role of the development of heart failure.

Table 1. Common SNPs of the β1,2-ARs and their frequencies [16]

Receptor	Location	Position	Receptor Variant	Racial Frequency of Variant Allele (White/Asian/Black)	Variant Allele Effect
β1-AR	extracellular	49	Ser→Gly	0.15 / 0.15 / 0.29	Increased agonist-promoted downregulation
β1-AR	intracellular	389	Arg→Gly	0.27 / 0.27 / 0.42	Hyporesponsive receptor
β2-AR	extracellular	16	Gly→Arg	0.46 / 0.59 / 0.49	Decreased agonist-induced desensitization
β2-AR	extracellular	27	Gln→Glu	0.35 / 0.07 / 0.20	Resistance to desensitization

B5. Pharmacogenomic studies: β1,2-AR SNPs and β-Blockers

Few published studies have evaluated the relationship between β1,2-AR polymorphisms and response to beta-blockers in heart failure. In practice, β-blockers are widely used in the treatment of both heart failure and hypertension.[2] β1-AR antagonists have been associated with increases in myocardial β1-AR density and improved hemodynamic responses.[22] It could be expected that variations in β1-AR expression due to the Ser49Gly polymorphism or response to agonist via the Arg389Gly polymorphism could be clinically relevant. In one of the few studies evaluating β1-AR polymorphisms in response to initial titration of beta-blockers in CHF patients, Terra et al. found that a greater percentage of Gly389 carriers required an increase in heart failure medications when compared to the Arg389 homozygotes. Another remarkable finding of this study was the diplotype analysis between the β1-AR polymorphisms at positions 49 and 389. Here patients carrying the Gly389 allele required more concomitant medications for heart failure, regardless of their allele at position 49.[6] Thus, it may be necessary to treat heart failure patients based upon their β1-AR genotype. It is also important to note that this study was conducted during the initial titration phase of metoprolol and not in patients on chronic doses.

Similar work to the proposed study has been conducted by de Groote et al.[23] This study analyzed the association between β1,2-AR polymorphisms and the effects of the beta-blockers bisoprolol and carvedilol in patients with heart failure. This group reported a lack of association between the 4 previously mentioned SNPs and resting heart rate or change in LVEF following beta-blockade. A limitation of this study is that it only evaluated resting heart rate and did not include serum levels of the drugs to evaluate pharmacokinetic differences.

In contrast, Kaye et al. reported a positive association between β2-AR genotype and carriers of the Glu27 allele (both hetero and homozygotes) and improvement in LVEF following treatment with carvedilol. It is of further interest to note that this study found no associations to either genotype at position 16 and response to carvedilol.[24] This study opposes the findings of de Groote et al., and offers the first evidence that β2-AR genotype may be associated with the clinical response of heart failure patients receiving carvedilol.

In one of the most compelling studies to date, Liggett et al. genotyped patients who had participated in a previous clinical trial (BEST), testing the efficacy of the β-blocker bucindolol on mortality in heart failure patients. The initial results of the trial showed that bucindolol resulted in no favorable effects in heart failure patients when compared to placebo. However, when the data was reanalyzed and broken down by β1-AR genotype, numerous significant findings resulted. Based on the β1-AR genotype of the these patients, they found that the Arg389 homozygotes had an age, sex and race-adjusted 38% mortality reduction ($p=0.03$), while the Gly389 carriers had no clinical response compared to bucindolol compared with placebo.[25] This study is the first to demonstrate a difference in clinical response based solely upon genotype at position 389 of the β1-AR .

B6. Significance

Despite previous studies, no study has yet to characterize the role of the β1,2-AR SNPs in a heart failure patient population being treated with maximal doses of metoprolol and carvedilol. This novel study will not only clarify this role, but also take into account important pharmacokinetic and pharmacodynamic variables. This study will also be unique in that it will be comprised of a demographically diverse group of patients. The majority of the published studies have not included patients of diverse racial backgrounds. We are fortunate that our heart failure patient population here at UMMS includes a racially diverse background. This will help to account for any racial differences between the studies. Anecdotal evidence suggests that black patients do not respond as well to β-blockade. This may not be due to race, but rather the higher carrier frequency of the hyporesponsive Gly389 allele on the β1-AR in blacks. This study will examine the underlying role of genetics in governing this differential response. Furthermore, recent evidence by Rochais et al. has indicated that there is a difference in terms of β-blockade and downstream signaling between metoprolol and carvedilol. This study will be the first to investigate the clinical relevance of this by comparing the 2 β-blockers metoprolol and carvedilol and their degree of β-blockade in relation to β1-AR genotype at position 389.

D. Research Design and Methods:

Specific Aim #1: Determine the relationship between β1,2-AR and GRK5-41 genotype and clinical response in heart failure patients treated with β-blockers.

<u>Patient Population</u>. One-hundred eighty NYHA class II-III heart failure patients will be recruited from the heart failure clinics at the University of Maryland Medical Systems as well as the Veterans Administration.

Inclusion criteria. 1. Receiving a stable dose (unchanged in last 4 weeks) of either carvedilol or metoprolol extended-release. 2. Ambulatory and able to take 6 minute walk test.
Exclusion criteria. 1. Left-ventricular ejection fraction > 40% 2. Receiving any anti-arrhythmic drug therapy other than carvedilol or metoprolol extended-release 3. History of an ischemic incident (requiring emergency care) or myocardial infarction in the previous month 4. Requiring inotropic support other than digoxin 5. Changes in other heart failure medications such as ACEI's, ARB's, diuretics, digoxin, sprinolactone during the previous 2 weeks.
6. Patients having a resting heart rate greater than 90 bpm or less than 50 bpm.
Sample Size. Sample size was determined using the following parameter estimates: $\alpha = 0.05$, power = 0.80, between group difference (endpoint) of 6 bpm, with a between group standard deviation of ±10 bpm and a ratio of β1-AR Arg389 homozygotes to Gly389 carriers of 1:1. This will require 45 patients in each group (homozygote or variant carrier) or 90 patients per drug for a total of 180 patients. This will also require some patient's β1-AR genotype at position 389 to be identified *a priori* given a variant allele frequency at position 389 of 0.3 in Whites and Asians and 0.42 in Blacks (see table 1) to ensure equal numbers of both Arg homozygotes and Gly carriers. Carriers will be considered to have at least one copy of the variant Gly allele. Given the low frequency of the variant allele the Gly homozygotes will be grouped for analysis with the heterozygous carriers.
This protocol and consent forms were approved (04/19/2007, #H-28288) by the human subjects research committee and investigational research board (IRB) at both the University of Maryland as well as the Veterans Administration. This research will be conducted in compliance with all HIPPA laws regarding privacy.
Outcomes. The primary endpoint of this prospective study is change in exercise-induced heart rate in relation to β1,2-AR genotype. For this evaluation, the 6-minute walk test will be employed. The 6-minute walk test is a well known, safe and reliable measure of exercise capacity in patients with heart failure.[12] Negative chronotropic response on heart rate during exercise is considered the gold standard for assessing degree of β1-AR blockade.[12,13] The test will be performed at the heart failure clinic along an enclosed private corridor at least 30m in length. Turnaround points will be designated by orange cones. Patients will be instructed to begin walking and a timer will be started. Patients are permitted to slow down, stop and rest as necessary. No coaching or motivational talk will be provided. This test will be conducted in the manner outlined by the American Thoracic Society Guidelines.[26] Resting heart rate will be measured before the administration of the 6-minute walk test over 30 seconds using pulse oximetry as well as a commercially available heart-rate monitor. Patients will be seated for 5 minutes before a (resting) heart rate is taken. Patients will then complete the 6-minute walk test. Heart rate will be measured during the test via pulse oximetry along with a commercially available heart-rate monitor. The last heart rate recorded during the 6-minute walk test will be used as the endpoint to calculate the change in heart rate (resting – exercise induced heart rate). If at any point the patient is unable to complete the 6-minute walk test, a (seated) heart rate

will be recorded at the moment the patient deems they are no longer able to continue. This heart-rate will then be used as the endpoint to calculate change, but will also be duly noted that the patient was unable to complete the test. A secondary endpoint will be distance completed in the 6-minute walk test. Previous studies have shown significant difference in terms of distance covered on the 6-minute walk test in relation to β-AR genotype.[19] The results of this test will give us a clinical indication of how well the patient is beta-blocked. It is our hypothesis that patients carrying the variant Gly389 allele will have a larger increase in heart rate during the test as well as have lower distance covered during the test.

Genotyping. The β1,2-AR genotype is determined using polymerase chain reaction (PCR) and restriction enzyme digest analysis. Specific base pair sequences are amplified by PCR using appropriate primers and genotype is assigned based on genetic-bit analysis. Genotyping is carried out using the ABI Prism 7000 sequence detection system. Currently, we have determined genotype at the 4 positions for 99% of the patients. In the case that genotype cannot be determined they will not be counted towards the total number of patients required or used in any statistical analysis and be considered as dropout. Genotypes will be evaluated in accordance with Hardy-Weinberg equilibrium and previously reported allele frequencies. Each haplotype will also be tested for linkage disequilibrium.

Statistical Analysis. Between group comparisons will be performed using an unpaired t-test. Comparisons of multiple groups will be performed by analysis of variance (ANOVA). Comparison of categorical parameters will be performed using the Chi-square analysis. Relationships between genotype and outcome will be assessed by analysis of variance (ANOVA). Patient demographic information (age, sex, race, weight) along with heart failure medications (other than beta-blockers) and pertinent medical information (NYHA HF class, HF etiology) will be determined from hospital records and analyzed as covariates. A multivariate analysis with a general linear model will be performed to test for association between the 4 B-AR polymorphisms and different covariates (age, sex, race, NYHA HF class, HF etiology, LVEF, beta-blocker dose, change in exercise-induced heart-rate, and length of time on stable β-blocker dose). The data in this study will be analyzed using both SAS (Version 9.1 © 2003 Cary, NC) and GraphPadPrism (Version 4.03, © 2005 San Diego, CA). A p-value cutoff of 0.05 will be considered significant for all endpoints.

Specific Aim #2: Determine the relationship between β1,2-AR genotype and beta-blocker dose level.

Patient Population. Patient population will be the same as those defined in Aim #1.

Methods. For this aim, current dosages of metoprolol or carvedilol will be recorded from either patient charts or the computerized record system within the UMMS and VAMC. Stable dose is defined as a post titration dose that is unchanged in previous 4 weeks. β1,2-AR genotype will then be compared with these dosages. This documented dose will also be confirmed with each patient.

Results will be based upon the total daily dose of either carvedilol or metoprolol that the patient takes.

Outcomes. Daily beta blocker dose will be compared to β1,2-AR genotype at the 4 mentioned positions.

Statistical Analysis. The unpaired t-test will be used to compare groups in terms of β1,2-AR genotype and beta-blocker dose. This test will be done individually for each of the β1,2-AR SNPs previously mentioned. Mean daily dose of the Arg homozygotes will be compared to the mean daily dose of the Gly carriers at position 389. This should indicate if one of the β1,2-AR SNPs significantly affects the maximal stable dose the patient receives.

Specific Aim #3: Evaluate the role of pharmacokinetic variability in response to β-blockade.

Patient Population. Same patient cohort as described in aim #1.

Methods. Immediately following the 6-minute walk test, a 5cc blood sample will be obtained from each patient to determine plasma β-blocker concentrations. Plasma concentrations of metoprolol or carvedilol will be determined using a novel stereospecific LC/MS assay. The assay will be developed using a ThermoFinnigan TSQ triple quadrupole, with separation using a chiracel OD-R™ column to separate both enantiomers of carvedilol and metoprolol. This assay will be designed to detect total plasma levels of each drug along with specific enantiomers (R/S) present in each compound. The assay will be developed in such a manner that the minimal detectable quantity of each drug will be 1 ng/mL. The mobile phase will consist of acetonitrile and water. This novel assay will provide a simple accurate methodology to determine serum levels of both metoprolol and carvedilol along with their enantiomers. This assay will be developed in accordance the FDA Guidance on Bioanalytical Method Validation. If for some reason the novel assay is unable to accurately determine plasma beta-blocker concentrations then the HPLC method by Lanchote will be employed.

Outcomes. Plasma concentrations of both enantiomers (R/S) will be reported for each drug group. These plasma samples will be representative of the drug concentration at the time of the 6-minute walk test. This data will be used to account for any potential pharmacokinetic differences that could cause differences in clinical response to the drugs.

Statistical Analysis. Within group means and standard deviation will be compiled and between treatment group comparisons will be made using the unpaired t-test. Plasma β-blocker concentrations will be used to adjust for heart rate. This will be accomplished by using concentration corrected change in heart rate (% change in HR/plasma concentration of beta blocker). In addition concentration response curves will be modeled for linearity, where the slope of the concentration response curve will be compared between genotypes.

Specific Aim #4: Determine whether genotype at position 389 on the β1-AR is associated with the clinical degree of β-blockade between metoprolol and carvedilol.

Patient Population. Same patient cohort as described in Aim #1.

Methods. Same methodology described in Aim #1.
Outcomes. Clinical degree of beta blockade will be assessed by change in heart-rate from rest following the 6-minute walk test.
Statistical Analysis. Each drug arm will be divided into two groups (Arg homozygotes and Gly carriers). Groups will then be compared to the other drug group for the endpoints of change in heart-rate following 6-minute walk test, distance covered during the 6-minute walk test and resting heart-rate. Within group means and standard deviation will be compiled and between treatment group comparisons will be made using the unpaired t-test.

Timeline and Recruitment:

Patient enrollment began in April of 2007 following IRB approval. We expect to recruit approximately 2-3 patients per week for ~ 70 weeks with a goal of the estimated 180 patients needed to detect a between group difference of 6 beats per minute between the Arg389 homozygotes and the Gly389 carriers. Depending upon patient recruitment and the allele frequencies at position 389 some patients may have to be recruited by establishing their genotype a priori. Patients will be retrieved from a bank of approximately 300 heart failure patients who have been previously genotyped at position 389. This assessment will be completed once half (45 patients each drug arm) is reached and again at every additional 10 patients enrolled. Given the variant allele frequency of about (0.3-.4) based upon racial frequencies, it is expected that some variant allele patients will have to be identified a priori and enrolled to ensure equal representation of alleles in study group. Once patient enrollment is completed all plasma samples will be analyzed for their subsequent beta-blocker levels. Preliminary results will be presented at the 2008 AHA scientific sessions.

Ethical Aspects:

This study involves the collection and analysis of human DNA for the purpose of genotyping and phenotyping. Given this, we will employ special precautions to ensure privacy and confidentiality. All data is collected and stored according to HIPPA policies. It is unknown whether β1,2-AR or GRK5-41SNPs genotyped in this study are predictors of disease, therefore patients will not be given the results of their genotype during the study. If at any time the investigators believe this data can be beneficially used for the future management of the patient, then this data will be made available to the patient. All patients will be treated similarly despite race, gender, ethnicity or socioeconomic status.

Literature Cited:

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RESEARCH CONSENT FORM

Protocol Title: THE ROLE OF THE BETA-1,2-ADRENERGIC RECEPTOR SINGLE-NUCLEOTIDE POLYMORPHISMS ON THE TREATMENT OF HEART FAILURE WITH BETA-BLOCKERS.

Study No: 40624

Principal Investigator: Shawn Robinson M.D. (410)-328-3648

The following is a research study and participation on your part is voluntary. You may ask questions at any time. If you are consenting for someone else, note the word "you" is referring to the person you are consenting for.

PURPOSE OF STUDY

The purpose of this study is to understand why some medicines (beta-blockers) do not work as well in certain heart failure patients. Recent data suggest that this difference in response may be due to differences in your DNA. People have roughly 50,000 different genes in their chromosomes (DNA) that determine everything from eye color to making the heart work properly. Imagine if everyone's DNA was contained in a giant book with 50,000 chapters, and each chapter represented 1 gene. All the letters on the pages would be a code of instructions for making the different genes (for eye color, blood type, etc.). Everyone has the same 50,000 chapters and most of the letters on the pages are exactly the same from book to book. Some differences in this code would be expected in order to explain the differences that make person unique. However, by examining every single letter on every single page of this book, called the human DNA genome, scientists have determined that type-o's (one incorrect letter on a page) are quite common. Scientists have also shown, that many people can share the exact same type-o, on the exact same page of this book. Often these type-o's are harmless and do not change the meaning of the code and the chapter (i.e. gene) is unaltered. Other times, that one little type-o can change the whole code for that gene and create disease.

This research effort is to evaluate whether or not these type-o's are responsible for differences in response to certain medications (beta-blockers). This research will also identify if patients require different doses of beta-blockers due to their gene abnormalities. The medical name for these genetic type-o's is single nucleotide polymorphisms or mutations. For the purposes of this document, the genetic type-o's will referred to as "gene abnormalities".

You are being asked to participate in this study because your cardiologist identified you as having heart failure. You are also currently taking a beta-blocker (metoprolol or carvedilol), which we are investigating in this research study. You will be one of approximately 180 subjects to be asked to participate in this study.

The research will be conducted at the following location(s): University of Maryland Baltimore, University of Maryland Medical System, VAMHCS.

The information gathered from this study will attempt to answer the following questions.
1) Do these known gene abnormalities contribute to the difference in response to beta-blockade?
2) Are these same abnormalities responsible for how much beta-blocker a patient can tolerate without side effects?
3) Are differences in how much beta-blocker is in the blood stream the reason some patients respond differently?

PROCEDURES

You have been identified by your Doctor as having heart failure. Certain patients will be excluded from this study. If you are a woman of child-bearing potential, you will have a urine pregnancy test done. If you are pregnant, you will be excluded from this study because of unknown effects on the fetus. You will be excluded from the study if you have had any recent (last 2 weeks) changes in any of your heart failure medications.

In this study we will need to draw one tablespoon (5ml) of your blood and collect some important information about your cardiac medical history. We would also like to ask you to do a 6-minute walk test. You will walk as far as you can for six-minutes down a corridor with a cone at each end to designate a turnaround point. You can stop at anytime you feel uncomfortable.
The blood will be used to determine your beta-blocker drug level as well as isolate and study genes that are believed to play an important role in determining how the heart works. We will examine these genes for abnormalities, as well as for differences that may exist in the normal population. (For example, there is a genetic difference between people with brown eyes and those with blue eyes, but neither condition is abnormal.) If you have previously given your consent and blood sample for DNA analysis to Dr. Robinson then only a teaspoon of blood will be needed to perform the testing for beta blocker drug level.

Information about your cardiovascular health will be stored in a secure database. DNA extracted from your blood will be kept frozen indefinitely in the investigators' lab and will not have your name or other personal information. The test tube will be labeled with a unique code number only. The investigators named on this form, Dr. Shawn W. Robinson (The principal investigator, 410-328-3648), and Dr. Hobart L. Rogers will be the only ones with access to this code. In the future, as new research provides more information about the causes of heart disease, we may want to test your DNA samples for the presence of different gene abnormalities. If important information about how to treat heart disease is obtained from the study of your DNA, you can be contacted by one of the investigators listed above to determine if you are getting the best treatment for your condition. Total time required to participate in this study will take approximately 15 minutes.

You authorize the University of Maryland, School of Medicine and members of its professional staff to use your specimens for these purposes. You will not be entitled to any financial compensation if any profits are generated by such activities. If at any time you desire to have your sample withdrawn from further use, you may do so by contacting the principal investigator, Dr. Shawn W. Robinson.

POTENTIAL RISKS/DISCOMFORTS:

Potential Risks Associated with Blood Drawing: There is minimal risk of bruising, infection, and swelling associated with blood drawing. These risks will be minimized by using aseptic (clean) techniques and by having experienced research personnel draw all blood samples. There is also minimal pain associated with needle sticks and in rare instances, individuals have been known to faint during multiple needle sticks and blood drawing. The total amount of blood that will be drawn during the entire study is 1 tablespoon (15 mL). The amount of blood withdrawn when someone donates a unit of blood is approximately 30 tablespoons.

Potential Risk associated with the 6-minute Walk Test: During this test you will walk for 6-minutes to try and cover as much distance as possible. This may make you feel short of breath. The level of exercise you will be asked to perform is moderate and heart rate will be monitored during exercise. You will be allowed to adjust the pace of walking to a level that is tolerable to you. There is a small chance (<1%) that even moderate exercise can worsen any previous condition you may have. If any such event occurs you will be asked to stop exercising. In <1% of cases exercise could cause a heart rhythm irregularity, heart attack, or death. At all times emergency equipment and qualified personnel will provide emergency treatment should you have any events of this kind.

There is a risk of loss of confidentiality to subjects that participate in this trial. However all necessary precautions will be taken to assure that your confidentiality is maintained. Your information will be kept in secure locations. In any publication or presentation of research results, your identity will not be disclosed. Medical records that identify you may, however, be reviewed by authorized individuals such as: personnel and clinical research representatives or their representative and other employees of the University of Maryland, Division of Cardiology, regulatory authorities (such as US Food and Drug Administration), hospital auditors and Institutional Review Board members. Disclosure of your medical records to these authorized individuals may be protected by federal privacy rules and regulations. By signing this document, you consent to such inspections or reviews and to the copying of your records if required by any of these parties. You agree that data can be used for seeking, obtaining and maintaining regulatory approvals worldwide.

Please indicate your willingness to perform the 6-minute walk test by circling either "YES" or "NO"______(sign initials)

POTENTIAL BENEFITS

You will receive no direct benefit from participation in this study. However, your participation may help the investigators better understand why certain medicines may not be effective for certain patients based upon their DNA. It might also spare some patients from needless testing and medications that may not be beneficial for them. If important information is learned from the study of your DNA, you may become eligible for promising new treatments for your heart failure.

ALTERNATIVES TO PARTICIPATION

This is not a treatment study. Your alternative is to not take part. If you choose not to take part, your healthcare at University of Maryland, Baltimore will not be affected..

COSTS TO PARTICIPANTS

There are no costs to you as a consequence of your participation in this research study. You will not be paid to participate in this research study.

PAYMENT TO PARTICIPANTS

You will not be paid to participate in this study.
If you are injured because of study participation, you will receive emergency medical care if needed and you will receive assistance in getting other medical care as needed. You or your insurance carrier will be billed for the cost of care, just as you would be billed for any other medical care. The study staff can give you more information about this if you have a study injury.

As a participant, you are not waiving any of your legal rights. You can seek legal compensation for any injury that may occur to you during the study as a result of an error by a member of the research staff, the sponsor, or others.

CONFIDENTIALITY

The following study will involve confidential information. This information will be kept coded to help ensure your information is maintained in a confidential manner.

The data from the study may be published. However, you will not be identified by name. Your personal information will not be given out unless permitted by law. Everyone using study information will work to keep your personal information confidential. People designated from the institutions where the study is being conducted and the sponsor listed in the sections above, will be allowed to inspect sections of your medical and research records related to the study.

RIGHT TO WITHDRAW

Your participation in this study is voluntary. You may choose not to participate now or at any time without penalty or loss of benefits to which you are otherwise entitled. If you decide to stop participating in this study, if you have questions or concerns at any time, or if you need to report an injury related to the research, please contact Shawn Robinson (410-328-3648) or BART ROGERS (410-706-2460)

If you are a veteran wanting to confirm that this study is in fact IRB approved and is being conducted at the VAMHCS, you may contact Shawn Robinson at (410-328-3648) or (410-605-5463). If this is a "clinical trial", additional information can be found at the clinical trials web site (www.clinicaltrials.gov).

In addition to the risks described in this form, there may be unknown risks/discomforts involved in participating in the study. Study staff will update you in a timely way on any new information that may affect your health, welfare, or decision to stay in this study. The investigator SHAWN ROBINSON or sponsor can decide to withdraw you from the study at any time. You could be removed from the study for reasons related solely to you (for example, not following study-related directions from the Investigator, a treatment becomes available for you which may be better for you than the care available in the study, or you have a serious reaction during the study). Also, the entire study

may be stopped by the sponsor, the Investigator, the Institutional Review Board, the facility where the study is being carried out, or the University. The sponsor may also decide to stop the Investigator's participation in the study. In that case, your participation will end unless another investigator is identified and approved by the sponsor and the Institutional Review Board.

If you are injured because of study participation, you will receive emergency medical care if needed and you will receive assistance in getting other medical care as needed. You or your insurance carrier will be billed for the cost of care, just as you would be billed for any other medical care. If you incur uninsured medical costs, they are your responsibility. The study staff can give you more information about this if you have a study injury.

As a participant, you are not waiving any of your legal rights. You can seek legal compensation for any injury that may occur to you during the study as a result of an error by a member of the research staff, the sponsor, or others.

UNIVERSITY STATEMENT CONCERNING RESEARCH RISKS

The University is committed to providing participants in its research all rights due them under State and federal law. You give up none of your legal rights by signing this consent form or by participating in the research project. Please call the Institutional Review Board (IRB) if you have questions about your rights as a research participant.

The research described in this consent form has been classified as minimal risk by the IRB of the University of Maryland, Baltimore (UMB). The IRB is a group of scientists, physicians, experts, and other persons. The IRB's membership includes persons who are not affiliated with UMB and persons who do not conduct research projects. The IRB's decision that the research is minimal risk does not mean that the research is risk-free. You are assuming risks of injury as a result of research participation, as discussed in the consent form.

If you are harmed as a result of the negligence of a researcher, you can make a claim for compensation. If you have questions, concerns, complaints, or believe you have been harmed through participation in this research study as a result of researcher negligence, you can contact members of the IRB or the staff of the Human Research Protections Office (HRPO) to ask questions, discuss problems or concerns, obtain information, or offer input about your rights as a research participant. The contact information for the IRB and the HRPO is:

University of Maryland School of Medicine
Human Research Protections Office
BioPark I
800 W. Baltimore Street, Suite 100
Baltimore, MD 21201
410-706-5037

By signing this Consent Form, you are not giving up any legal rights. If this research project is conducted in a negligent manner and you are injured as a direct result, you may be able to recover the costs of care and other damages from the individuals or organizations responsible for your injury.

If you have questions, concerns, complaints, or believe you have been harmed through participation in this research study as a result of researcher negligence, you can contact members of the IRB or the Human Research Protections Office (HRPO) to ask questions, discuss problems or concerns, obtain information, or offer input about your rights as a research participant. The contact information for the IRB and the HRPO is:

University of Maryland School of Medicine
Human Research Protections Office
BioPark I
800 W. Baltimore Street, Suite 100
Baltimore, MD 21201
410-706-5037

____
Participant's Signature

Date:_______________________________

____
Investigator or Designee Obtaining Consent Signature
Date:_______________________________

____
Witness*
Date:_______________________________

EVALUATION TO SIGN CONSENT FORM

Subject I.D. #: ___________ Subject Initials: _________ Subject Source: ___________

Date of Rating: _____/_____/_____

Protocol (Optional): ___000189______________

PROCEDURE:
Make a subjective judgment regarding item 1 below. Ask the patient questions 2-6. The evaluator may select the language to use in asking the questions in order to help the patient understand them.

19.1 ITEMS:

1. Is the patient alert and able to communicate with the examiner?
   ________ yes _______ no

2. Ask the patient to name at least two (2) potential risks incurred as a result of participating in the study.
   ____
   ____

3. Ask the patient to name at least two (2 things that will be expected of him/her in terms of patient cooperation during the study.
   ____
   ____

4. Ask the patient to explain what he/she would do if he/she decides that they no longer wish to participate in the study.
   ____
   ____

5. Ask the patient to explain what he/she would do if he/she is experiencing side effects or discomfort.
   ____
   ____

6. Ask the patient to explain how medications (or treatments) are assigned during the study.
   ____

SIGNATURES:
I hereby certify that the above patient is alert, able to communicate and able to give acceptable answer to items 2, 3, 4, 5, and 6 above.

Evaluator Date Witness

HIPAA form

Name of Study Volunteer: ____

Date of Birth: _______________

Medical Record Number: __________________________

NAME OF THIS RESEARCH STUDY:	The role of the B1,2-adrenergic receptors in the treatment of heart failure with beta-blockers
UMB IRB APPROVAL NUMBER:	**H-*28288***
Researcher's Name:	**Dr. Shawn Robinson**
Researcher's Contact Information:	

Department of Medicine/Division of Cardiology

University of Maryland School of Medicine (UMSOM)

22 S. Greene St. Rm N3W77
410-328-3648

This research study will use health information that identifies you. If you agree to participate, this researcher will use just the health information listed below.

The Specific Health Information To Be Used or Shared:

- B1,2-adrenergic receptor genotype
- Distance completed on 6-minute walk test
- Resting heart rate and heart rate during and following the 6-minute walk
- Current names and dosages of all medications the patient is currently taking

Federal laws require this researcher to protect the privacy of this health information. He will share it only with the people and groups described here.

PEOPLE AND ORGANIZATIONS WHO WILL USE OR SHARE THIS INFORMATION:

- Dr. Shawn Robinson and Dr. Bart Rogers and his research team.
- The sponsor of the study, or its agents, such as data repositories or contract research organizations
- Organization that will coordinate health care billing or compliance such as offices within UMSOM; the University of Maryland, Baltimore (UMB); University Physicians, Inc. (UPI) and the faculty practices of the UMB; University of Maryland Medical System (UMMS) and the Veterans Affairs Maryland Health Care System (VAMHCS).
- Your health insurer to pay for covered treatments

THIS AUTHORIZATION WILL NOT EXPIRE. BUT YOU CAN REVOKE IT AT ANY TIME.

To revoke this Authorization, send a letter to this researcher stating your decision. He will stop collecting health information about you. This researcher might not allow you might to continue in this study. He can use or share health information already gathered. The data will be destroyed at the time indicated below:

- Immediately at the end of this study

ADDITIONAL INFORMATION:

- You can refuse to sign this form. If you do not sign it, you cannot participate in this study. This will not affect the care you receive at:
  - University Physicians, Inc. (UPI)
  - University of Maryland Medical System (UMMS)
  - Veteran Affairs Maryland Health Care System (VAMHCS)

  It will not cause any loss of benefits to which you/your child are otherwise entitled.
- Sometimes, government agencies such as the Food and Drug Administration or the Department of Social Services request copies of health information. The law may require this researcher, the UMSOM, UPI, UMMS or VAMHCS to give it to them.
- This researcher will take reasonable steps to protect your health information. However, federal protection laws may not apply to people or groups outside the UMSOM, UMB, UPI, UMMS or VAMHCS.
- Except for certain special cases, you/your child have the right to a copy of your health information created during this research study. You may have to wait until the study ends. Ask this research how to get a copy of this information from him/her.

My signature indicates that I authorize the use and sharing of my protected health information for the purposes described above. I also permit my doctors and other health care providers to share my protected health information with this researcher for the purposes described above.

Signature: ____

Date: ________________________

Name (printed) ____

Privacy Questions? Call the UMSOM Privacy Official (410-706-0337) with questions about your/your child's rights and protections under privacy rules.

Appendix II – Table I. Patient Demographics

									Meds					**Genotype**
Pt ID	Age	Sex	Race	Wt	Etiology	LVEF	NYHA Class	Drug	Diuretic	ACE/ARB	Digoxin	Aldactone	Hydralazine	Position 389
1	67	M	2	188	1	15	3	C	0	1	0	0	0	22
2	56	M	1	194	0	30	3	M	1	1	1	1	0	22
3	38	M	2	200	0	10	3	M	1	1	1	1	0	23
4	40	M	2	280	0	20	2	M	1	1	1	1	1	23
5	60	M	1	148	1	20	2	C	0	1	1	1	0	22
6	50	M	1	152	0	30	2	C	1	1	1	1	0	22
7	60	M	1	146	0	25	2	M	0	1	0	1	0	22
8	52	F	2	237	1	20	2	M	1	1	0	0	0	23
9	82	M	1	177	1	20	2	C	1	1	1	0	0	22
10	57	M	2	282	0	35	3	C	1	1	0	0	1	33
11	55	M	1	292	1	30	2	M	0	1	1	1	0	33
12	47	F	2	205	1	40	3	M	0	1	0	1	0	23
13	59	M	1	179	1	30	2	C	0	1	0	0	0	22
14	66	M	1	124	1	25	2	M	0	1	1	1	0	22
15	50	M	1	200	1	20	3	M	1	1	1	0	0	22
16	49	M	2	350	1	20	3	C	1	0	0	1	1	23
17	76	M	2	116	1	20	3	M	1	1	1	0	1	22
18	43	F	2	166	0	25	2	C	1	1	0	0	0	23
19	47	M	2	237	1	20	2	M	0	1	1	0	0	23
20	53	M	1	159	0	40	3	M	1	1	1	0	0	22

Race: 1 = Caucasian, 2 = African American; Etiology: 0 = nonischemic, 1= ischemic; Meds: 0 = no, 1 = yes; β1-389: 2 = Arg allele; 3 = Gly allele; ND genotype not determined

Table I. (continued)

									Meds					**Genotype**
Pt ID	Age	Sex	Race	Wt	Etiology	LVEF	NYHA Class	Drug	Diuretic	ACE/ARB	Digoxin	Aldactone	Hydralazine	Position 389
21	55	F	1	200	0	40	3	M	0	1	0	0	0	22
22	49	F	2	285	0	15	2	C	1	1	0	0	0	22
23	46	M	2	159	1	35	2	M	1	1	0	1	0	22
24	51	M	1	294	1	15	3	M	1	0	0	0	0	22
25	63	M	1	132	1	30	3	M	0	1	0	0	0	22
26	62	M	1	174	1	10	3	C	1	1	1	1	0	22
27	73	M	1	185	1	15	2	M	1	1	1	1	0	33
28	57	M	2	321	0	32	2	M	1	1	0	0	0	23
29	67	F	2	180	0	35	2	C	0	1	1	1	0	23
30	57	M	2	268	0	30	2	M	1	1	0	0	0	23
31	74	F	2	160	0	25	2	M	1	0	1	0	1	33
32	75	M	2	157	1	40	2	M	1	0	0	0	1	23
33	46	M	2	164	1	15	3	C	1	1	0	0	1	22
34	24	M	1	252	0	35	2	C	0	1	0	0	0	22
35	64	F	1	119	0	35	2	M	0	1	0	0	1	23
36	70	M	1	168	1	25	2	C	0	1	1	0	0	23
37	61	M	2	165	0	15	2	C	1	1	1	0	0	23
38	44	M	2	328	1	20	3	C	1	1	0	0	0	23
39	45	M	2	159	0	15	2	M	1	1	1	0	0	22
40	59	M	1	191	1	15	2	M	1	1	0	1	0	ND

Race: 1 = Caucasian, 2 = African American; Etiology: 0 = nonischemic, 1 = ischemic; Meds: 0 = no, 1 = yes; β1-389: 2 = Arg allele; 3 = Gly allele; ND genotype not determined

Table I. (continued)

											Meds			**Genotype**
Pt ID	Age	Sex	Race	Wt	Etiology	LVEF	NYHA Class	Drug	Diuretic	ACE/ARB	Digoxin	Aldactone	Hydralazine	Position 389
41	60	M	2	199	1	30	2	M	1	1	0	1	0	23
42	37	F	2	274	1	25	2	M	1	1	0	1	0	23
43	42	M	2	277	0	25	3	M	1	1	1	1	0	22
44	51	M	2	182	0	35	2	M	0	1	0	0	0	23
45	31	M	2	214	0	20	2	C	1	1	1	1	0	33
46	54	M	1	219	1	20	3	M	1	1	1	1	0	22
47	63	M	1	218	1	30	3	M	1	1	0	1	0	22
48	59	F	1	235	0	35	2	C	1	1	0	0	0	23
49	57	M	2	143	0	30	2	C	0	1	0	0	0	22
50	62	M	1	190	1	20	2	C	1	0	1	0	0	22
51	75	F	2	120	0	15	3	M	1	1	1	1	0	22
52	48	M	2	177	0	10	2	C	1	1	0	0	1	22
53	23	M	1	200	0	25	2	C	1	1	1	1	0	23
54	73	M	1	225	1	25	3	C	1	1	1	0	0	23
55	59	M	2	257	1	25	3	C	0	1	0	1	0	23
56	38	F	2	187	0	20	2	C	1	1	1	1	0	22
57	60	M	1	224	1	15	3	C	0	1	0	0	0	22
58	69	M	1	206	1	10	2	M	0	1	1	0	0	22
59	60	M	1	215	1	25	2	M	1	1	0	0	0	22
60	77	M	1	164	1	30	3	M	0	1	0	1	0	23

Race: 1 = Caucasian, 2 = African American; Etiology: 0 = nonischemic, 1 = ischemic; Meds: 0 = no, 1 = yes; β1-389: 2 = Arg allele; 3 = Gly allele; ND = genotype not determined

Table I. (continued)

Pt ID	Age	Sex	Race	Wt	Etiology	LVEF	NYHA Class	Drug	Diuretic	ACE/ARB	**Meds** Digoxin	Aldactone	Hydralazine	**Genotype** Position 389
61	71	M	1	184	0	20	3	M	1	1	0	1	0	22
62	46	M	2	162	0	37	2	M	0	1	1	0	0	23
63	68	M	1	242	1	25	2	C	1	1	1	0	0	23
64	72	M	2	217	1	20	2	C	1	0	0	1	0	22
65	56	M	1	182	1	15	3	M	1	0	0	0	1	23
66	70	M	2	212	2	30	2	C	1	1	0	1	0	22
67	49	M	2	211	2	25	2	M	0	1	0	0	0	23
68	62	M	2	212	1	15	3	M	1	1	1	0	1	23
69	59	M	2	304	0	30	2	M	1	1	0	0	1	23
70	68	M	2	172	0	20	3	M	1	1	1	0	0	23
71	61	M	1	223	0	30	3	M	1	1	1	0	0	22
72	80	M	1	165	1	32	2	M	1	1	0	0	0	22
73	82	M	1	186	1	20	2	M	1	1	0	0	0	22
74	84	M	1	195	0	30	3	M	1	1	1	1	0	22
75	62	M	2	130	0	20	2	M	1	1	1	1	0	22
76	63	M	1	243	1	25	3	M	1	1	0	1	0	33
77	60	M	1	179	1	35	2	M	0	1	1	1	0	33
78	54	M	2	215	0	10	2	M	1	0	1	1	0	22
79	67	M	2	232	0	27	2	M	1	1	0	0	0	23
80	52	M	2	224	0	35	2	C	1	1	1	1	1	23

Race: 1 = Caucasian, 2 = African American; Etiology: 0 = nonischemic, 1 = ischemic; Meds: 0 = no, 1 = yes; β1-389: 2 = Arg allele; 3 = Gly allele; ND = genotype not determined

Table I. (continued)

											Meds			**Genotype**
Pt ID	Age	Sex	Race	Wt	Etiology	LVEF	NYHA Class	Drug	Diuretic	ACE/ARB	Digoxin	Aldactone	Hydralazine	Position 389
81	62	M	2	197	0	35	2	C	1	1	1	1	0	33
82	62	M	1	199	1	20	2	C	1	1	1	1	0	22
83	63	M	1	246	0	29	2	C	0	0	0	0	0	22
84	75	M	2	239	0	20	3	C	1	1	0	0	0	33
85	62	M	1	236	0	30	2	C	0	1	0	0	0	23
86	54	M	2	234	1	30	2	C	1	1	0	0	0	33

Race: 1 = Caucasian, 2 = African American; Etiology: 0 = nonischemic, 1 = ischemic; Meds: 0 = no, 1 = yes; β1-389: 2 = Arg allele; 3 = Gly allele; ND = genotype not determined

Table II. Patient Results from Clinical Trial

Pt ID	Drug	Dose (mg/day)	Serum Level (ng/ml)	**β1-AR Position** **49**	**389**	**β2-Ar Position** **16**	**27**	**GRK5** **41**	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	ΔHR (bpm)	Walk Distance (ft)
1	C	50	10.3	11	22	11	22	11	140	80	80	11	1102
2	M	200	92.2	11	22	13	22	11	98	68	67	18	840
3	M	200	3.1	13	23	33	23	14	96	60	81	9	1088
4	M	100	2.5	13	23	33	23	44	98	58	80	40	1188
5	C	50	19.4	11	22	13	22	11	96	58	73	13	1094
6	C	50	12.6	11	22	33	33	11	86	60	87	24	1090
7	M	150	60.2	11	22	13	22	11	76	44	71	46	1570
8	M	200	126.4	13	23	13	22	14	108	64	70	25	868
9	C	25	4.5	11	22	13	23	11	101	56	63	31	1258
10	C	50	12.9	11	33	33	23	11	108	54	79	16	1232
11	M	200	23	11	33	13	23	11	112	68	70	25	874
12	M	150	27.4	13	23	33	23	11	106	60	75	22	1224
13	C	25	4.3	11	22	11	22	11	116	70	51	47	1886
14	M	25	13.6	11	22	11	23	11	120	70	48	8	1524
15	M	100	21.8	11	22	33	33	11	102	60	58	40	1410
16	C	50	60	13	23	11	22	11	128	76	78	21	948
17	M	100	143.0	13	22	33	23	44	110	60	64	14	852
18	C	25	3.9	11	23	13	23	14	118	70	73	12	1360
19	M	300	50.0	11	23	13	23	14	120	60	78	20	1416
20	M	200	165.0	11	22	33	23	11	102	60	54	40	1456

ND = Genotype not determined; Genotype 49: 1 = Ser allele; 4 = Gly allele; Genotype 389: 2 = Arg allele, 3 = Gly allele; Genotype 16: 1 = Arg allele, 3 = Gly; Genotype 27: 2 = Gln allele, 3 = Gly allele; GRK 41: 1 = Gln allele, 4 = Leu allele.

Table II. (continued)

				β1-AR Position		**β2-Ar Position**		**GRK5**					
Pt ID	Drug	Dose (mg/day)	Serum Level (ng/ml)	**49**	**389**	**16**	**27**	**41**	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	ΔHR (bpm)	Walk Distance (ft)
21	M	50	18.9	11	22	33	33	11	102	62	54	16	1342
22	C	80	28.9	11	22	13	22	11	138	87	66	39	1162
23	M	200	66.0	11	22	33	33	14	118	80	65	24	1696
24	M	100	59.0	11	22	33	23	ND	90	70	72	33	676
25	M	200	10.30	11	22	33	23	11	132	74	56	29	840
26	C	50	22.6	11	22	11	22	11	110	80	60	11	924
27	M	50	2.5	11	33	33	23	11	120	70	61	43	1342
28	M	200	38.8	13	23	33	22	14	122	84	67	27	1166
29	C	50	19.3	11	23	33	33	14	92	54	83	24	1222
30	M	100	2.5	11	23	11	22	11	112	76	62	50	1262
31	M	50	2.5	11	33	13	22	14	118	56	79	15	950
32	M	200	81.2	11	23	13	22	14	128	60	65	26	902
33	C	25	85.6	13	22	13	22	11	128	70	70	24	868
34	C	50	6.0	13	22	13	23	11	120	68	65	45	1714
35	M	200	58.8	11	23	13	23	11	138	76	70	22	1270
36	C	50	7.1	11	23	33	23	11	125	65	50	17	1200
37	C	50	10.1	11	23	13	22	14	136	94	59	34	1346
38	C	100	4.3	13	23	13	22	11	148	106	83	46	1178
39	M	100	11.7	13	22	11	22	11	80	50	62	50	1636
40	M	125	4.6	ND	ND	ND	22	11	74	46	64	18	1400

ND = Genotype not determined; Genotype 49: 1 = Ser allele; 4 = Gly allele; Genotype 389: 2 = Arg allele, 3 = Gly allele; Genotype 16: 1 = Arg allele, 3 = Gly; Genotype 27: 2 = Gln allele, 3 = Gly allele; GRK 41: 1 = Gln allele, 4 = Leu allele.

Table II. (continued)

				β1-AR Position		β2-Ar Position		GRK5					
Pt ID	Drug	Dose (mg/day)	Serum Level (ng/ml)	**49**	**389**	**16**	**27**	**41**	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	ΔHR (bpm)	Walk Distance (ft)
41	M	200	18.9	11	23	33	23	ND	100	58	66	15	1168
42	M	150	2.5	13	23	11	22	44	96	64	60	48	1086
43	M	200	2.5	13	22	13	22	11	110	80	81	9	870
44	M	100	2.5	13	23	13	23	14	122	76	64	77	1142
45	C	50	7.8	11	33	33	23	11	110	70	84	24	754
46	M	200	328.0	13	22	33	33	11	100	60	65	44	1608
47	M	100	32.4	11	22	11	22	11	116	80	53	31	904
48	C	50	30.4	11	23	13	23	11	152	78	64	27	922
49	C	50	17.5	11	22	11	22	14	101	62	77	37	1622
50	C	50	14.9	11	22	11	22	11	132	68	55	43	1212
51	M	100	14.3	11	22	13	22	44	130	68	75	45	720
52	C	12.5	3.4	13	22	13	22	11	140	80	56	26	1558
53	C	50	8.4	11	23	33	23	11	105	60	75	24	1298
54	C	50	27.5	11	23	33	23	11	122	60	50	15	844
55	C	25	1.2	13	23	11	22	11	129	77	56	35	1162
56	C	50	13.8	13	22	13	22	14	143	94	76	23	1318
57	C	25	4.2	11	22	13	23	11	83	53	51	15	1102
58	M	200	51.2	11	22	13	22	11	103	63	63	36	1140
59	M	200	141.2	11	22	13	22	11	87	63	69	16	1170
60	M	200	266	11	23	13	23	11	117	62	50	16	840

ND = Genotype not determined; Genotype 49: 1 = Ser allele; 4 = Gly allele; Genotype 389: 2 = Arg allele, 3 = Gly allele; Genotype 16: 1 = Arg allele, 3 = Gly; Genotype 27: 2 = Gln allele, 3 = Gly allele; GRK 41: 1 = Gln allele, 4 = Leu allele.

Table II. (continued)

Pt ID	Drug	Dose (mg/day)	Serum Level (ng/ml)	**β1-AR Position** **49**	**389**	**β2-Ar Position** **16**	**27**	**GRK5** **41**	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	ΔHR (bpm)	Walk Distance (ft)
61	M	50	13.1	11	22	33	33	11	112	60	61	25	946
62	M	200	56	11	23	13	22	14	131	77	78	8	950
63	C	50	2.6	11	23	33	33	11	90	60	61	29	840
64	C	50	10.7	11	22	13	22	14	115	58	57	19	952
65	M	100	45.2	11	23	13	22	11	137	68	65	7	804
66	C	50	12	13	22	11	22	11	116	67	90	23	1192
67	M	200	27.4	11	23	33	22	11	135	86	65	26	1408
68	M	200	209.2	11	23	13	22	14	130	62	47	20	788
69	M	200	2.5	11	23	33	22	14	152	91	79	32	956
70	M	100	14.94	11	23	13	23	14	131	94	61	31	898
71	M	300	7.88	11	22	13	22	11	104	66	83	29	958
72	M	100	37.6	11	22	13	23	11	82	49	55	24	1080
73	M	100	10.78	13	22	33	23	11	128	66	60	17	900
74	M	50	2.5	11	22	11	22	11	123	56	45	32	904
75	M	200	149.8	13	22	11	22	14	112	78	61	30	1400
76	M	200	3.9	11	33	13	23	11	154	76	62	27	662
77	M	100	25	11	33	13	23	11	123	74	59	19	1216
78	M	100	31.4	11	22	13	22	11	116	71	55	13	1208
79	M	200	29.4	11	23	11	22	11	128	86	58	60	1020
80	C	50	11.4	11	23	11	22	14	136	69	64	35	1766

ND = Genotype not determined; Genotype 49: 1 = Ser allele; 4 = Gly allele; Genotype 389: 2 = Arg allele, 3 = Gly allele; Genotype 16: 1 = Arg allele, 3 = Gly; Genotype 27: 2 = Gln allele, 3 = Gly allele; GRK 41: 1 = Gln allele, 4 = Leu allele.

Table II. (continued)

Pt ID	Drug	Dose (mg/day)	Serum Level (ng/ml)	**β1-AR Position** **49**	**389**	**β2-Ar Position** **16**	**27**	**GRK5** **41**	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	ΔHR (bpm)	Walk Distance (ft)
81	C	50	10.5	11	33	11	22	14	159	75	61	29	1264
82	C	50	9.4	11	22	13	23	11	133	65	52	30	878
83	C	50	19.1	11	22	33	33	11	131	67	60	16	1256
84	C	50	2.26	11	33	33	22	44	134	60	70	40	1378
85	C	50	7.9	11	23	13	23	11	104	76	88	14	1268
86	C	50	41.3	11	33	13	23	11	107	61	84	30	1260

ND = Genotype not determined; Genotype 49: 1 = Ser allele; 4 = Gly allele; Genotype 389: 2 = Arg allele, 3 = Gly allele; Genotype 16: 1 = Arg allele, 3 = Gly; Genotype 27: 2 = Gln allele, 3 = Gly allele; GRK 41: 1 = Gln allele, 4 = Leu allele.

Table III. Metoprolol Group – Arg 389 Homozygotes

Pt ID	Dose (mg/day)	Dose (mg/kg)	*S* – Metoprolol (ng/ml)	389 Genotype	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	RHR/S-Met (bpm/ng/ml)	Δ HR (bpm)	Δ HR/S-met (bpm/ng/ml)	Walk Distance (ft)
1	200	2.27	92.2	22	98	68	67	0.73	18	0.20	840
2	150	2.26	60.2	22	76	44	71	1.18	46	0.76	1570
3	25	0.44	13.6	22	120	70	48	3.53	8	0.59	1524
4	100	1.10	21.8	22	102	60	58	2.66	40	1.83	1410
5	100	1.90	143.0	22	110	60	64	0.45	14	0.10	852
6	200	2.77	165.0	22	102	60	54	0.33	40	0.24	1456
7	50	0.55	18.9	22	102	62	54	2.85	16	0.85	1342
8	200	2.77	66.0	22	118	80	65	0.98	24	0.36	1696
9	100	0.75	59.0	22	90	70	72	1.22	33	0.56	676
10	200	3.33	10.30	22	132	74	56	5.44	29	2.82	840
11	100	1.38	11.7	22	80	50	62	5.31	50	4.28	1636
12	200	2.01	328.0	22	100	60	65	0.20	44	0.13	1608
13	100	1.01	32.4	22	116	80	53	1.64	31	0.96	904
14	100	1.83	14.3	22	130	68	75	5.23	45	3.14	720
15	200	2.14	51.2	22	103	63	63	1.23	36	0.70	1140
16	200	2.05	141.2	22	87	63	69	0.49	16	0.11	1170
17	50	0.60	13.1	22	112	60	61	4.64	25	1.90	946
18	300	2.96	7.88	22	104	66	83	10.53	29	3.68	958
19	100	1.33	37.6	22	82	49	55	1.46	24	0.64	1080
20	100	1.18	10.78	22	128	66	60	5.57	17	1.58	900

Table III. (continued)

Pt ID	Dose (mg/day)	Dose (mg/kg)	*S* – Metoprolol (ng/ml)	389 Genotype	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	RHR/S-Met (bpm/ng/ml)	Δ HR (bpm)	Δ HR/S-met (bpm/ng/ml)	Walk Distance (ft)
21	200	3.38	149.8	22	112	78	61	0.41	30	0.20	1400
22	100	1.02	31.4	22	116	71	55	1.75	13	0.41	1208
23	200	1.59	2.5	22	110	80	81	32.40	9	3.60	870
24	50	0.56	2.5	22	123	56	45	18.00	32	12.80	904
Mean.	139	1.72	61.9		106	65	62	4.51	28	1.77	1152
s.d.	69	0.90	76.2		16	10	10	7.15	12	2.68	320

Table IV. Metoprolol – Gly 389 Carriers

Pt ID	Dose (mg/day)	Dose (mg/kg)	*S* –Metoprolol (ng/ml)	389 Genotype	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	RHR/S-Met (bpm/ng/ml)	Δ HR (bpm)	Δ HR/S-met (bpm/ng/ml)	Walk Distance (ft)
25	200	2.20	3.1	23	96	60	81	26.13	9	2.90	1088
26	200	1.86	126.4	23	108	64	70	0.55	25	0.20	868
27	150	1.61	27.4	23	106	60	75	2.74	22	0.80	1224
28	300	2.78	50.0	23	120	60	78	1.56	20	0.40	1416
29	200	1.37	38.8	23	122	84	67	1.73	27	0.70	1166
30	200	2.80	81.2	23	128	60	65	0.80	26	0.32	902
31	200	3.70	58.8	23	138	76	70	1.19	22	0.37	1270
32	200	2.21	18.9	23	100	58	66	3.49	15	0.79	1168
33	200	2.68	266	23	117	62	50	0.19	16	0.06	840
34	200	2.72	56	23	131	77	78	1.39	8	0.14	950
35	100	1.21	45.2	23	137	68	65	1.44	7	0.15	804
36	200	2.09	27.4	23	135	86	65	2.37	26	0.95	1408
37	200	2.08	209.2	23	130	62	47	0.22	20	0.10	788
38	100	1.28	14.94	23	131	94	61	4.08	31	2.07	898
39	200	1.90	29.4	23	128	86	58	1.97	60	2.04	1020
40	100	0.79	2.5	23	98	58	80	32.00	40	16.00	1188
41	100	0.82	2.5	23	112	76	62	24.80	50	20.00	1262
42	150	1.20	2.5	23	96	64	60	24.00	48	19.20	1086
43	100	1.21	2.5	23	122	76	64	25.60	77	30.80	1142
44	200	1.45	2.5	23	152	91	79	31.60	32	12.80	956
45	200	1.51	23	33	112	68	70	3.04	25	1.09	874

Table IV. (continued)

Pt ID	Dose (mg/day)	Dose (mg/kg)	S –Metoprolol (ng/ml)	389 Genotype	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	RHR/S-Met (bpm/ng/ml)	Δ HR (bpm)	Δ HR/S-met (bpm/ng/ml)	Walk Distance (ft)
46	200	1.81	3.9	33	154	76	62	15.90	27	6.92	662
47	100	1.23	25	33	123	74	59	2.36	19	0.76	1216
48	50	0.59	2.5	33	120	70	61	24.40	43	17.20	1342
49	50	0.69	2.5	33	118	56	79	31.60	15	6.00	950
Mean	164	1.75	44.9		121	71	67	10.61	28	5.71	1060
s.d.	60	0.77	65.7		16	11	9	12.35	17	8.47	206

Table V. Carvedilol – Arg 389 Homozygotes

Pt ID	Dose (mg/day)	Dose (mg/kg)	*S* – Carvedilol (ng/ml)	389 Genotype	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	RHR/S-Car (bpm/ng/ml)	ΔHR (bpm)	ΔHR/S-Car (bpm/ng/ml)	Walk Distance (ft)
1	50	0.59	10.3	22	140	80	80	7.77	11	1.07	1102
2	50	0.74	19.4	22	96	58	73	3.76	13	0.67	1094
3	50	0.72	12.6	22	86	60	87	6.90	24	1.90	1090
4	25	0.31	4.5	22	101	56	63	14.00	31	6.89	1258
5	25	0.31	4.3	22	116	70	51	11.86	47	10.93	1886
6	80	0.62	28.9	22	138	87	66	2.28	39	1.35	1162
7	50	0.63	22.6	22	110	80	60	2.65	11	0.49	924
8	25	0.34	85.6	22	128	70	70	0.82	24	0.28	868
9	50	0.44	6.0	22	120	68	65	10.83	45	7.50	1714
10	50	0.77	17.5	22	101	62	77	4.40	37	2.11	1622
11	50	0.58	14.9	22	132	68	55	3.69	43	2.89	1212
12	12.5	0.16	3.4	22	140	80	56	16.47	26	7.65	1558
13	50	0.59	13.8	22	143	94	76	5.51	23	1.67	1318
14	25	0.25	4.2	22	83	53	51	12.14	15	3.57	1102
15	50	0.51	10.7	22	115	58	57	5.33	19	1.78	952
16	50	0.52	12	22	116	67	90	7.50	23	1.92	1192
17	50	0.55	9.4	22	133	65	52	5.53	30	3.19	878
18	50	0.45	19.1	22	131	67	60	3.14	16	0.84	1256
Mean	44	0.50	16.6		118	69	66	6.92	27	3.15	1233
s.d.	16	0.18	18.6		19	11	12	4.43	12	3.03	291

Table VI. Carvedilol – Gly 389 Carriers

Pt ID	Dose (mg/day)	Dose (mg/kg)	*S* – Carvedilol (ng/ml)	389 Genotype	Sys. BP (mmHg)	Dia. BP (mmHg)	Resting HR (bpm)	RHR/S-Car (bpm/ng/ml)	ΔHR (bpm)	ΔHR/S-Car (bpm/ng/ml)	Walk Distance (ft)
19	50	0.31	60	23	128	76	78	1.30	21	0.35	948
20	25	0.33	3.9	23	118	70	73	18.72	12	3.08	1360
21	50	0.61	19.3	23	92	54	83	4.30	24	1.24	1222
22	50	0.65	7.1	23	125	65	50	7.04	17	2.39	1200
23	50	0.67	10.1	23	136	94	59	5.84	34	3.37	1346
24	100	0.67	4.3	23	148	106	83	19.30	46	10.70	1178
25	50	0.47	30.4	23	152	78	64	2.11	27	0.89	922
26	50	0.55	8.4	23	105	60	75	8.93	24	2.86	1298
27	50	0.49	27.5	23	122	60	50	1.82	15	0.55	844
28	25	0.21	1.2	23	129	77	56	45.90	35	28.69	1162
29	50	0.45	2.6	23	90	60	61	23.46	29	11.15	840
30	50	0.49	11.4	23	136	69	64	5.61	35	3.07	1766
31	50	0.47	7.9	23	104	76	88	11.14	14	1.77	1268
32	50	0.39	12.9	33	108	54	79	6.12	16	1.24	1232
33	50	0.51	7.8	33	110	70	84	10.77	24	3.08	754
34	50	0.56	10.5	33	159	75	61	5.81	29	2.76	1264
35	50	0.46	2.26	33	134	60	70	30.97	40	17.70	1378
36	50	0.47	41.3	33	107	61	84	2.03	30	0.73	1260
Mean	50	0.49	14.9		122	70	70	11.73	26	5.31	1180
s.d.	15	0.12	15.6		20	14	12	11.88	10	7.42	244

Appendix III – Power Calculations

Table I. Power for each endpoint measured at β1-AR position 389

Overall (n = 85) Endpoint	Power
Systolic B.P. (mmHg)	0.82
Diastolic B.P. (mmHg)	0.22
Resting HR (bpm)	0.38
ΔHR (bpm)	< 0.01
6-min Walk (ft)	0.26

Metoprolol Arm (n = 49)	Power
Endpoint	
Systolic B.P. (mmHg)	0.90
Diastolic B.P. (mmHg)	0.50
Resting HR (bpm)	0.43
ΔHR (bpm)	< 0.01
6-min Walk (ft)	0.21

Carvedilol Arm (n = 36)	Power
Variable	
Systolic B.P. (mmHg)	0.09
Diastolic B.P. (mmHg)	0.06
Resting HR (bpm)	0.16
ΔHR (bpm)	0.06
6-min Walk (ft)	0.09

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