

Mechanisms of Pharmacogenomic Effects of Genetic Variation
of the Cardiac Adrenergic Network in Heart Failure

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Abbreviations: β AR, β -adrenergic receptor; α_2 AR, α_2 -adrenergic receptor; GRK, G-protein coupled receptor kinase; β ARK, β -adrenergic receptor kinase; SNP, single nucleotide polymorphism; VO_2 , oxygen consumption; PKA, protein kinase A; cAMP, cyclic adenosine 3'-5' monophosphate.

ABSTRACT

One of the goals of pharmacogenomics is the use of genetic variants to predict an individual's response to treatment. While numerous candidate and genome-wide associations have been made for cardiovascular response-outcomes, little is known about how a given polymorphism imposes the phenotype. Such mechanisms are important, as they tie the observed human response to specific signaling alterations and thus provide cause-and-effect relationships, aid in the design of hypothesis-based clinical studies, can help to devise work-around drugs, and can reveal new aspects of the pathophysiology of the disease. Here we discuss polymorphisms within the adrenergic receptor network in the context of heart failure and β -blocker therapy where multiple approaches to understand mechanism have been undertaken. We propose a comprehensive series of studies, ranging from transfected cells, transgenic mice, and *ex vivo* and *in vitro* human studies as a model approach to explore mechanisms of action of pharmacogenomic effects and extend the field beyond observational associations.

Chronic heart failure represents a significant treatment challenge, and although various pharmacologic therapies have been introduced over the past 2 decades, mortality remains high, with ~40-50% of individuals dying within 5 years of diagnosis, and ~25% dying within the first year. Well-recognized within the field is the high degree of interindividual variability in the response to drugs in the treatment of heart failure, that is not readily attributed to clinical, demographic or environmental factors (van Campen et al., 1998). This variability has led to investigation of potential genetic factors that influence drug responsiveness in heart failure, a field termed pharmacogenomics (or pharmacogenetics). In this article we review the population genomics, molecular properties, and the results of clinical studies of common polymorphisms within the adrenergic receptor signaling network in heart failure, with an emphasis on linking potential molecular mechanisms to the human phenotypes. Here we refer to a polymorphism as a variation in a sequence, compared to a common reference sequence, that is found to occur with an allele frequency of 1% or greater in any population.

In acute loss of organ perfusion from virtually any cause, or when an increase in perfusion is required such as during exercise or stress, the body responds by activation of the sympathetic nervous system, with increases in cardiac output due to activation of cardiomyocyte β -adrenergic receptors (β AR). This system is well adapted for acute (short-term) needs for enhanced cardiac performance, consistent with the “fight or flight” nature of the sympathetic nervous system. However, chronic stimulation represents a less than optimal response to this physiologic need, and can ultimately contribute to pathogenic effects. In chronic heart failure, a persistently activated sympathetic nervous system is primarily manifested by increased plasma norepinephrine, a result of enhanced release of the neurotransmitter from presynaptic nerve terminals such as those that innervate the heart. Such persistent β AR activation in the failing

heart, which has limited metabolic and physiologic reserves, leads to worsening heart failure. β -blockers appear to exert their therapeutic effect in heart failure by breaking this cycle, via antagonizing the effects of norepinephrine at cardiomyocyte β AR (Fig. 1). With proper titration, cardiac energetics improve, the cardiotoxic effects of the β_1 AR subtype are diminished, and “reverse remodeling” of heart failure associated gene-expression patterns occurs, leading to enhanced cardiac output and survival (Bristow, 2003; Mann and Bristow, 2005). With improved cardiac function and systemic perfusion, sympathetic activation is reduced as reflected in lower plasma norepinephrine levels. Cardiac β AR function, which is reduced in the failing heart, also improves with β -blocker treatment (Brodde, 2007). Indeed, the expression and function of β AR in chronic heart failure is in constant flux, based on hemodynamic status and treatment effects. β_1 - and β_2 AR are expressed on human cardiomyocytes, with β_1 AR being the primary mediator of catecholamine mediated increases in inotropy and chronotropy. In chronic heart failure, β_1 AR expression is decreased, while β_2 AR expression is relatively stable; both β_1 - and β_2 AR functional coupling is desensitized (Bristow et al., 1990). The downregulation and desensitization of β_1 AR is thought to be due to chronically elevated catecholamines, and may be enhanced due to increased expression of G-protein coupled receptor kinases (GRKs), which is also observed in human heart failure (Hata et al., 2004). GRKs phosphorylate β_1 - and β_2 AR, resulting in the binding of β -arrestin to the receptor, which sterically interdicts between receptor and G_s and promotes uncoupling. The decreased β AR function in heart failure is not sufficient, though, to protect the heart from the aforementioned vicious cycle. An intriguing conundrum surrounds how best to break the cycle. Several studies have shown that inhibiting the increase in GRK expression has a salutary effect in animal models of heart failure (Hata et al., 2004). This is accompanied by restored β AR function. Since β -arrestin also acts as a signaling molecule

(DeWire et al., 2007), it is not entirely clear whether decreasing these other effects of altered GRK expression improves cardiac function, which results in restoration of β AR signaling, or whether improved β AR signaling is the primary event that results in chronic restoration of cardiac function.

Presynaptic cardiac nerve terminals express two adrenergic receptors which act in a negative feedback manner to limit NE release. These receptors, the α_{2A} - and α_{2C} AR subtypes, appear to control release of NE from high frequency and low frequency stimuli, respectively (Hein et al., 1999). Double knockout α_{2A}/α_{2C} mice develop heart failure presumably due to unregulated NE release (Hein et al., 1999). Even heterozygous ($\alpha_{2C}^{+/-}$) knockout mice, under the conditions of pressure overload from transaortic constriction, develop heart failure, suggesting that one or both of these α_2 AR subtypes may be potential drug targets for heart failure (Gilsbach et al., 2007).

A Mechanistic Approach for Pharmacogenomic Studies. Our initial investigation of potential pharmacogenomic loci for β -blocker treatment in heart failure was focused on the primary therapeutic target, the β_1 AR. This candidate gene approach (as opposed to unbiased genomic-wide scans) is appropriate, in our opinion, for pharmacogenomic studies where the drug target and the downstream signaling events, are well-established. This approach is outlined in Fig. 2. Initial polymorphism discovery is carried out using a reference set of genomic DNA from a collection of ethnically diverse individuals, such as the Human Variation Panel of the Coriell Institute (<http://ccr.coriell.org/nigms/cells/humdiv.html>). Typically, samples from 40 European-Americans (Caucasians), 40 African-Americans, and 40 Asians are utilized. This provides for a 95% probability of detecting (at least once) polymorphisms with allele frequencies of ~ 0.03 (3%)

if the polymorphism is completely confined to one racial group, and ~0.01 if found in all three racial groups. While it might be desirable to use samples derived from patients with the disease of interest, the prevailing notion is that common polymorphisms will be present in the “normal” population, perhaps at a different allele frequency than the diseased population, but nevertheless present, and thus a reference group such as indicated above is acceptable for polymorphism discovery. In the approach discussed here (Fig. 2), any nonsynonymous polymorphisms, as well as those in the promoter, or other untranslated regions, are studied *in vitro* in transfected cell systems. From these results, the generation of transgenic mice is considered, which provides an opportunity to explore the variants in the cell-type of interest, in the organ of interest, under the relevant physiologic conditions. And of course, physiologic readouts can be obtained which can be correlated with signaling events. In tandem with mouse studies, human tissue studies can be utilized for physiologic measurements if freshly obtained, or signaling and expression studies if any banked tissue is available. Also, small cohorts of genotyped patients can be studied for short-term physiologic phenotypes, such as the response exercise or to infusions of agonists or antagonists. At this juncture in these studies, a phenotype may well be established at the molecular and physiologic levels using these multiple approaches. Based on this information a *hypothesis-based* clinical trial can be designed to ascertain potential effects in humans of the variant(s) in question on drug response, or a suitable previously conducted trial with archived DNA can be utilized. In this article we will focus on two genes, the β_1 AR and GRK5, since the multiple investigative strategies shown in Fig. 2 have been completed and represent examples of the approach.

Variations in the β_1 AR gene

Cell-based studies

Using this targeted approach to defining the genetic determinants of β -blocker efficacy, we began by examining the coding region of the intronless β_1 AR gene. Two common non-synonymous SNPs were found (Table 1) at nucleotides 145 (A/G) which results in a Ser (major allele) or Gly at amino acid 49 of the extracellular amino-terminus of the receptor, and at nucleotide 1165 (G/C) which results in an Arg (major allele) or Gly at amino acid 389. Of note, Gly at position 389 was found when the human β_1 AR was first cloned, and has been termed the “wild-type” receptor for the majority of structure-function studies, yet it is the less common allele in most populations. However, in African-Americans Gly has about the same prevalence as Arg. We have thus refrained from using the term wild-type for either variant but simply refer to them by allele, such as β_1 Arg389 or β_1 Gly389.

β_1 Arg/Gly389

The variation at amino acid 389 lies within a predicted fourth intracellular loop, formed by a stretch of ~12 amino acids from the distal seventh transmembrane spanning-domain to the membrane-anchoring palmitoylated cysteine(s). By analogy with other GPCRs this fourth loop is an α -helix. As shown in Fig. 3, this region of the β_1 AR is highly conserved amongst many diverse species. And, Arg is found in the analogous position in every species where sequence data is available, except for humans where Arg or Gly is found. Given the nature of these two amino acids, this homology analysis suggested that the variation at position 389 may have functional relevance. Competition binding studies (Mason et al., 1999) in partially purified cell membranes from stably transfected Chinese hamster fibroblasts (CHW cells) expressing both

receptors at equivalent levels revealed high-affinity agonist binding that was readily detected with Arg389, but rarely found with Gly389 (Fig. 4A, B). In addition, the Arg389 curves shifted to the right and became monophasic with the addition of GppNHp, while the Gly389 curves were unaffected by guanine nucleotide. This indicated a larger proportion of receptors that could attain the high-affinity active state (% R_H) for Arg389 compared to Gly389, and consequently a greater change in free energy upon agonist binding and subsequent coupling. In the presence of GppNHp, the low affinity binding constant (K_L) was the same for both receptors. Consistent with these results, [3S]GTP γ S binding with transfected COS-7 cell membranes was greater with the Arg389 variant compared to the Gly389 variant. Adenylyl cyclase activities in membranes revealed slightly greater basal activities with the Arg389 receptor and ~3-fold greater isoproterenol-stimulated activation for Arg389 compared to Gly389 (Fig. 4C). Similar results were found with the agonists epinephrine and norepinephrine. Another group (Joseph et al., 2004) performed similar studies in transfected CHO cells at low expression levels and also found that Arg389 had greater G_s coupling confirming our studies in CHW cells. In their studies, the isoproterenol-stimulated cAMP increase with β_1 Arg389 was ~30-fold greater than β_1 Gly389. These investigators also determined binding affinities for a number of antagonists and partial agonists, including metoprolol, carvedilol, bisoprolol, and propranolol, and found no differences in affinity between the Arg and Gly receptors. Intramolecular fluorescence resonant energy transfer (FRET) has been used to assess the inverse agonist activities of bisoprolol, metoprolol, and carvedilol (Rochais et al., 2007). In these studies, all three antagonists evoked a FRET change (consistent with a conformational change in the receptor) for both the Arg and Gly forms of the receptor, but carvedilol acting at β_1 Arg389 displayed the greatest change. The locations of the energy donor and energy acceptor moieties in the intramolecular domains of GPCRs

markedly affects resonant energy transfer ratios (Swift et al., 2007), and thus it is difficult to fully interpret these studies in relation to a physiologic outcome. Nevertheless, they do suggest that there may be compound-specific phenotypes for the position 389 variants.

The more favorable conformation for agonist-promoted G_s-coupling of the Arg389 receptor vs. the Gly389 receptor suggested that GRK-promoted desensitization might also be greater for Arg389, since phosphorylation is conformation-dependent. The aforementioned transfected CHW cells were treated with vehicle or vehicle with 10 μM isoproterenol for 20 min, washed, membranes prepared, and agonist-stimulated adenylyl cyclase activation determined. The results revealed ~50% greater desensitization for Arg389 compared to Gly389 (Rathz et al., 2003). Interestingly, when one examines the absolute values of the activities, genetic variation has an impact on signaling of the same magnitude as homologous desensitization. As can be seen in Fig. 4D, the desensitized Arg389 receptor signals equivalently to the control (not desensitized) Gly389 receptor.

β₁Ser/Gly49

The Ser49 and Gly49 β₁ARs were stably expressed in CHW as well as HEK-293 cells, and in studies by our laboratory we found no differences in agonist-promoted coupling to adenylyl cyclase in either cell line (Rathz et al., 2002). Another group, though, has reported an increased basal and agonist-stimulated adenylyl cyclase activities with the Gly49 receptor in transfected HEK-293 cells (Levin et al., 2002). However, both groups found that these recombinantly expressed β₁ARs underwent little agonist-promoted downregulation (loss of net receptor expression) after 24-hours of exposure to high concentrations of isoproterenol under cell culture conditions. Indeed, in some instances expression increased. Both groups utilized

incubations with cyclohexamide to block new receptor synthesis, and under these conditions agonist-promoted downregulation of both Ser and Gly49 forms of the receptor was noted. And, both groups reported an increase in agonist-promoted downregulation of the Gly49 receptor compared to the Ser49 receptor (55% vs. 36%, respectively, in our studies). This polymorphism is localized to the extracellular amino-terminus of the β_1 AR, ~33 bases 3' to a glycosylation site. On SDS-PAGE, the Ser49 receptor migrated at two molecular weights suggesting a homodimer or altered glycosylation compared to Gly49. While a homodimer cannot be excluded, the high molecular weight species of Ser49 was sensitive *in vivo*, and *in vitro*, to inhibitors of N-glycosylation (Rathz et al., 2002). Since glycosylation can affect receptor trafficking, these observations may represent the mechanism of altered downregulation, although it is unclear based on its position how altered glycosylation is evoked by the polymorphism. Taken together, while we recognize that these studies are dependent on highly reductionist model systems, we have considered that the β_1 Gly49 receptor may alter cardiac phenotypes in heart failure, potentially due to enhanced downregulation.

Transgenic mouse studies

To assess the relevance of the β_1 Arg389 and β_1 Gly389 receptors to cardiac function, transgenic mice (FVB/N strain) overexpressing the two human receptors on myocytes were generated using the α -myosin heavy chain promoter (Mialet-Perez et al., 2003). We had previously generated β_2 AR overexpressing cardiac mice and had delineated the appropriate expression levels over background β AR expression that provide for differentiation between two β_2 ARs with known differences in G_s coupling (Turki et al., 1996). We thus chose two β_1 AR overexpressing mouse lines expressing either the Arg389 or Gly389 β_1 AR at matched levels of

~1000 fmol/mg for most of the longitudinal studies. The salient features of their physiologic function are shown in Fig. 5. Both baseline and maximal dobutamine-stimulated contractility (the first derivative of pressure by time) were greater for Arg389 hearts compared to Gly389 at 3-months of age (Fig. 5A). These results were consistent with the transfected cell studies and revealed the relevance of the polymorphisms in the cell-type of interest, and, at the level of intact organ function. Interestingly, at 6-months of age, Arg389 mice continued to have enhanced baseline contractility, but were not responsive to dobutamine (Fig. 5B). We found that this desensitization was due to a decreased affinity for receptor- G_s coupling, and a decrease in protein expression of G_{os} and AC5/6, which paralleled a loss of ventricular function and failure by 9-months of age. This suggested a change in the coupling of the Arg389 receptor that occurred during the development of β_1 AR-mediated cardiomyopathy, a term that we (unfortunately) termed a “phenotypic switch.” But, subsequent studies in human hearts (see below) showed only a modest change in the magnitude of the contractile differences between Arg and Gly hearts in end-stage heart failure compared to normal hearts, so in humans there does not appear to be a reversal of the phenotypes. Additional experiments with the transgenic mice revealed the first evidence that there may be a differential response to β -blockers based on genotype (Mialet-Perez et al., 2003). Acute infusion of propranolol into hearts in the *ex vivo* work-performing model (Fig. 6A) revealed that Arg389 hearts had a dose-dependent decrease in contractility, while Gly389 hearts had a smaller decrease that was only found at the highest concentration. *In vivo* studies were carried out by administering propranolol in the drinking water of 4-month old mice for one month and monitoring heart rate by echocardiography. As shown in Fig. 6B, only Arg389 mice displayed a decrease in heart rate.

As per Fig. 2, ancillary studies have been carried out in young transgenic mouse hearts examining gene transcript expression to begin to understand potential unique signaling properties of the Arg389 and Gly389 receptors. Given that these mice had overexpressed receptors, and that the Arg389 mice develop heart failure by 9-months of age, we utilized 3-month old mice, whose hearts showed no anatomic, histologic, biochemical, or physiologic evidence of heart failure or other pathologic features. Nevertheless, the limitations of the model call for careful interpretation. We were particularly concerned that the enhanced inotropy/chronotropy in the resting state of the Arg389 vs. Gly389 hearts might simply result in regulation of energy-related genes proportional to the physiologic enhancement. To provide a “filter” for such events, we utilized the type V adenylyl cyclase cardiomyocyte-specific overexpressing mouse that we had previously generated (Tepe et al., 1999). We had shown that the hearts from these mice had persistently elevated resting and agonist-stimulated contractility throughout their lives, with no evidence of pathologic consequences. The extent of the enhanced inotropy and chronotropy (using non-transgenic mice as the reference) was the same as what we observed for the hyperfunctional Arg389 mice. Thus we could separate AC/PKA-dependent events from Arg (or Gly)-specific events by comparing these with the events found with the ACV hearts. Gene expression was ascertained from six hearts in each of the four groups (NTG, Arg389, Gly389, and ACV) using a complete mouse genome array representing 39,000 transcripts. These results are depicted in the Venn diagrams of Fig. 7. As can be seen, 1041 genes were uniquely regulated by the Arg389 transgene (i.e., not found with Gly389 or ACV). In contrast, 245 genes were uniquely regulated in the Gly389 hearts. And, as expected there were relatively large overlap groups of genes that we co-regulated by the two β_1 ARs or ACV. Pathway analysis algorithms (such as Ingenuity and ToppGene) revealed a number of networks that were uniquely regulated

by Arg389. The most statistically significant network involved regulation of extracellular matrix-associated genes known to be activated directly or indirectly by TGF β (Swift et al., 2008). These data could benefit from pathway-building exercises by other groups, and the raw data is provided in the supplement to the aforementioned paper. In general, we have considered the scheme depicted in Fig. 7 as a way to identify new pharmacogenomic or disease-risk genes within the context of the Arg389 polymorphism. The expression of genes in the nontransgenic mice is set as the “reference.” And the perturbations imposed by the transgenes considered as four possibilities, initially stratified by cAMP-dependent and –independent events. Within the cAMP-dependent mechanism, a number of genes are altered in the β_1 -Arg and β_1 -Gly hearts, and by definition this group includes all transcripts altered in AC5 hearts. Not surprisingly, the genes in this group are dominated by those of respiration and energy metabolism, given that cAMP/PKA activation is a major mechanism by which cardiac inotropy and chronotropy are increased. In the non-cAMP-dependent set of genes, some are common to both β_1 -Arg389 and β_1 -Gly389. These represent, then, pathways that are activated by these receptors in a non-allele-specific manner, which does not involve cAMP signaling, and are unlikely to be allele-specific pathogenic or pharmacogenomic loci. The genes whose transcripts were altered in a non-cAMP/PKA, allele-specific manner represent unique signaling that is apparently dependent on the single amino acid difference in the β_1 AR at position 389, and may represent genes or pathways that might provide insight into heart failure pathogenesis, or, novel therapies directed towards heart failure in those with the Arg389 genotype.

Human studies: physiological outcomes

To further assess the roles of the position 389 alleles in the context of human heart failure, a number of studies with physiologic endpoints have been carried out with relatively small cohorts. In one such study, graded exercise testing in 263 patients with class III/IV heart failure was performed with peak oxygen consumption ($\dot{V} O_2$) as the major outcome (Wagoner et al., 2002). There was a readily apparent difference in $\dot{V} O_2$ between β_1 Arg389 homozygotes and Gly389 homozygotes (17.7 ± 0.4 vs. 14.5 ± 0.6 ml/kg/min, $P = 0.006$). Heterozygotes had a $\dot{V} O_2$ that was in-between the homozygous subjects at 16.9 ± 0.6 . There was no confoundment by etiology of heart failure, baseline LVEF or β -blocker use. When stratified by the position 49 polymorphism, there were few homozygous β_1 Gly49 subjects, but homozygous Ser49 patients had higher $\dot{V} O_2$ than Gly49 carriers (homozygotes and heterozygotes). However, this appears to be driven by Arg389, due to high linkage disequilibrium between the 49 and 389 alleles. This study, then, confirmed the hyperdynamic nature of the β_1 Arg389 heart in humans. These results of exercise responsiveness have been replicated recently by another group using heart failure subjects (Sandilands et al., 2005), but the association does not appear to hold in normal healthy subjects (Leineweber et al., 2006). Interestingly, though, direct assessment of cardiac β_1 AR function by dobutamine infusion in normal subjects has shown differential responses by the β_1 -389 genotype (Bruck et al., 2005). These investigators showed not only enhanced heart rate and contractility in Arg389 vs. Gly389 homozygous subjects, but found that dobutamine-stimulated plasma renin activity was markedly greater in the Arg389 individuals, indicating that renal β_1 AR function is also affected by this polymorphism. Finally, they report that the β -blocker bisoprolol decreased dobutamine-promoted cardiac and renin responses more potently in Arg389 vs. Gly389 individuals. These studies, then, are consistent with our findings in the transgenic mouse

(Mialet-Perez et al., 2003) and human exercise studies (Wagoner et al., 2002) as well as isolated human hearts (see below). In another study, the effects on left ventricular ejection fraction of β -blockade with carvedilol in 224 heart failure patients was assessed after >6 months of treatment. After titration, the dose of carvedilol was the same in β_1 Arg389 vs. Gly389 patients. Arg389 homozygotes showed a greater improvement in LVEF than Gly389 homozygotes ($8.7 \pm 1.1\%$ vs. $0.93 \pm 1.7\%$, $P < 0.02$). Heterozygotes appeared to show improvement similar to the Arg homozygotes ($7.02 \pm 1.5\%$). Similar results with the β -blocker metoprolol were reported in another study of 61 heart failure patients (Terra et al., 2005). These studies have recently been replicated in 135 patients treated with carvedilol (Molenaar et al., 2007). Here the respective improvements for the two homozygous states were 18% vs. 6%, with heterozygotes being 11%. In our view, these types of studies, which are highly focused and hypothesis-driven with an emphasis on human physiology, provide an important link between cell- or animal-based studies, and longitudinal studies of patients with heart failure. And, they provide for a refinement of the hypothesis, or the study design, for such large patient studies.

Human studies: *ex vivo* cardiac mechanics

The effects of β_1 AR genotype on human responses have been assessed using right ventricular trabeculae from human non-failing and failing hearts, studied in organ-bath preparations (Liggett et al., 2006). Non-failing (normal) hearts were donor hearts that were not ultimately utilized for transplant due to ABO incompatibility, or other non-cardiac issues. Failing hearts were obtained at the time of cardiac transplantation for end-stage heart failure. These results are shown stratified by genotype in Fig. 9. In non-failing hearts from individuals homozygous for Arg389, the maximal contractile force was greater than that of hearts from

Gly389 carriers (Fig. 9A). The absolute difference in force between Arg389 and Gly389 responses in these non-failing trabeculae amounted to ~ 8 mN/mm² greater for Arg389. In failing hearts, this phenotype was maintained, but the absolute differences were not as great (Fig. 9B, note scale change), amounting to 3.5 mN/mm². So, while there is no “switch” or “reversal” in the phenotypes, as was suggested in the mice, there is an attenuation of the phenotypic differences by genotype between non-failing and failing hearts. This may be due to the Arg389 receptor undergoing greater signal desensitization during the catecholamine excess that accompanies end-stage heart failure, which is consistent with the previously discussed cell-based studies (Rathz et al., 2003). Of note, by radioligand binding, there were no differences in β_1 - or β_2 AR expression levels between Arg389 and Gly389 hearts. Additional experiments in failing hearts have also been carried-out with carvedilol and the atypical β -blocker bucindolol (Fig. 9C, D). Here trabeculae were pre-stimulated with forskolin which provides conditions for detecting weak partial agonist or inverse agonist effects. Bucindolol caused a dose-dependent decrease in force generation in trabeculae from Arg389 homozygous hearts, but not from Gly389 carrier hearts. In contrast, carvedilol acted as a neutral antagonist in trabeculae with either genotype.

Human studies: heart failure survival

Taken together, the cell, transgenic mouse, *ex vivo* human heart, and human physiological studies all indicated that the Arg389 form of the β_1 AR achieves a greater signaling potential than the Gly389 form and that those with the Arg389 form have greater short-term responses to β -blockers. These differences could have physiological or pharmacologic relevance in heart failure. To ascertain the consequences of this polymorphic variation for heart failure survival, we genotyped archived DNA from a prospective, double-blinded, placebo-controlled trial of the β -

blocker bucindolol in class III-IV heart failure. The trial, the Beta-Blocker Evaluation of Survival Trial (BEST), had been terminated prior to achieving its enrollment goals because of an interim analysis indicating little efficacy as per the predefined endpoints, and a lack of investigator equipoise (BEST Trial Investigators, 2001).

One-thousand forty patients from BEST consented to the Substudy. The clinical characteristics of the placebo and bucindolol groups were well matched, as were these groups when further stratified by homozygous β_1 Arg389 or -Gly389 carriers (see ref (Liggett et al., 2006), Supporting Information Table 4). Of particular note, there were no differences in age, sex, race, LVEF, etiology of heart failure, NYHA class, or baseline heart rates and blood pressures, amongst the groups. The primary outcome was all cause mortality, adjudicated heart failure hospitalizations, and the combined endpoint. The statistical comparisons were with these outcomes comparing the placebo and bucindolol groups in Arg389, and, in Gly389 patients. Because the study had the placebo arms, the potential effects of the polymorphism on heart failure progression in the absence of β -blocker could be assessed, and because comparisons were between bucindolol and placebo by genotype, any subtle differences evoked by a polymorphism on an endpoint could be detected and attributed to a drug-specific effect. Cumulative survival curves were constructed by Kaplan-Meier methods and the Cox proportional-hazards regression model was used to examine the effects of treatment stratified by the indicated genotype. Results were adjusted for age, sex, and race. Because of the limited number of comparisons and a hypothesis that was based on the results we found in the cell-based, mouse-based, and human ventricle studies, we considered P values <0.05 as significant, without adjustments for multiple comparisons. The main results of this study are summarized in Fig. 10. As can be readily observed, one group had improved survival, which was the one with patients having the

β_1 Arg389 homozygous genotype who were receiving the active drug (bucindolol). In contrast, Gly389 carriers had identical survival whether they were on placebo or bucindolol. Of note, by looking at the two placebo groups, we can conclude that the polymorphism does not appear to significantly alter survival in the absence of β -blocker. For Arg389 patients treated with bucindolol compared to placebo, the hazard ratio (HR) = 0.62, 95% confidence interval (C.I.) = 0.40–0.96, $P = 0.03$, indicating an improvement in survival with bucindolol in those with this genotype. This same comparison in Gly carriers revealed no difference in survival (HR = 0.90, 95% C.I. = 0.62–1.30, $P = 0.57$), indicative of no treatment response to bucindolol. There was also an apparent influence of β_1 AR genotype on heart failure exacerbations during bucindolol treatment, as measured by hospitalization due to heart failure. With this outcome, Arg389 patients compared to placebo had HR = 0.64, 95% C.I. = 0.46–0.88, $P = 0.006$. In contrast, Gly389 carriers showed no benefit of the drug compared with placebo in terms of hospitalizations (HR = 0.86, 95% C.I. = 0.64–1.15, $P = 0.30$). For the combined outcome of time to first heart failure hospitalization or death, a bucindolol-associated favorable treatment effect was evident for Arg patients compared with placebo (HR = 0.66, 95% C.I. = 0.50–0.88, $P = 0.004$), but it was not apparent in bucindolol-treated Gly389 carriers vs. placebo (HR = 0.87, 95% C.I. = 0.67–1.11, $P = 0.250$). The β_{149} polymorphisms provided no additional predictive value. An issue for this study was the known small, but significant, difference in the frequency of the Gly allele between blacks and nonblacks (Table 1) and the fact that, in the entire BEST cohort (BEST Trial Investigators, 2001), bucindolol's mortality effect in blacks appeared to be less favorable than in nonblacks. We considered whether our findings were based simply on being able to proportionately identify blacks which would place them into the Gly group (the non-responders). If the effect was due to some other African gene(s), then it is conceivable that

the 389 allele was being used only as an ancestral marker. However, based on the number of blacks in the study ($\approx 20\%$), the allele frequency difference would have needed to be >10 -fold in order for Gly to be a meaningful surrogate for identification of blacks-only and to affect overall outcome. Furthermore, the HRs were adjusted for race, and nevertheless, an advantage was observed for Arg but not Gly patients. We also carried out this same analysis excluding the black subjects, thus removing any potential for confounding by race. For mortality, Arg patients treated with bucindolol had an HR = 0.56, 95% C.I. = 0.34–0.90, $P = 0.017$, vs. placebo. The bucindolol treatment HR for Gly = 0.81, 95% C.I. = 0.53–1.24, $P = 0.34$, vs. placebo.

There are several unique components of the BEST Substudy that are noteworthy when considering comparisons to other studies of β -blocker efficacy and β_1 AR polymorphisms. BEST utilized the β -blocker bucindolol, which has some distinct pharmacologic properties. Of all β -blockers that have been studied in the treatment of heart failure, bucindolol shows the greatest sympatholytic effect (i.e., norepinephrine lowering) which are similar in magnitude to the effects of the central imadazoline receptor agonist moxonidine (Cohn et al., 2003). In addition, bucindolol acts as an inverse agonist in human heart preparations at the β_1 Arg389, but not the β_1 Gly389 (Liggett et al., 2006). On the other hand, metoprolol and carvedilol act as neutral antagonists without inverse activity in these isolated human hearts. And finally, bucindolol has similar binding affinities for both β_1 AR and β_2 AR, and thus is a nonselective β -blocker. Concerning trial design, BEST was a multicenter, prospective, randomized and placebo-controlled trial, with stringent entry criteria and extensive patient phenotyping. Given the above, and the $\sim 38\%$ improvement in outcomes observed in Arg389 patients taking bucindolol vs. placebo, it is important to consider whether these findings are also true for the β -blockers currently available and in common use for heart failure treatment in the U.S., metoprolol and

carvedilol. To our knowledge there is only one β_1 AR polymorphism study in heart failure (White et al., 2003) with a placebo arm, which is a substudy of MERIT-HF. Of note, ~45% of these patients had mild heart failure (class II), mean follow-up period was only 12 months, and there were few deaths, so the combined outcome of hospitalization and death was utilized. The analysis compared outcome by genotype in the combined cohort of placebo- and metoprolol-treated patients, and the major conclusion was that Gly389 patients did not have improved outcomes. It is not possible from this study to assess whether there was a pharmacogenetic effect since comparisons within the two treatment arms, by genotype, were not performed. Shin et al. (Shin et al., 2007) reported the results of a longitudinal observational study of 227 heart failure patients with survival as the endpoint, where 81% were receiving an unspecified β -blocker. No association with β_1 AR polymorphisms was noted, but a 2-locus haplotype of the β_2 AR subtype was associated with poor survival. de Groote et al. (de Groote et al., 2005), in a study of 444 Caucasian heart failure patients, all of whom were treated with either bisoprolol or carvedilol, found a different β_2 AR 2-locus haplotype associated with survival (univariate analysis only), and no association with a β_1 AR polymorphism. Finally, a retrospective 2-center catheterization-laboratory registry study of 637 heart failure patients, who were being treated with either carvedilol or metoprolol, has been recently reported examining the coding β_1 AR polymorphism, those of the β_2 AR, and a surrogate for an α_2C AR polymorphism (Sehnert et al., 2008). No associations between any of these polymorphisms and survival were found. In contrast to the BEST Substudy, this study was retrospective, patient enrollment was based on having a cardiac catheterization (a potential selection bias), a formal β -blocker titration protocol was not in place, and compliance was not monitored past 6 months. So, any differences between this study (or the

aforementioned other studies) and the BEST Substudy may be due to the “noise” from their designs, or due to the effects of the β_1 AR polymorphism being specific for bucindolol.

A β AR-desensitizing GRK5 polymorphism is protective in experimental and clinical heart failure and mimics β -blockers.

Whereas minute-by-minute cardiac function is critically regulated by β AR signaling stimulated by systemically circulating or locally released sympathetic catecholamines, these responses are contextually modulated by G-protein receptor kinases, or GRKs. As described above, under normal conditions, this acute stress response transiently activates downstream β AR signaling pathways that increase ventricular ejection performance and heart rate. After the exertion is over or stress is relieved, cardiac output and catecholamine levels normalize. However, chronically depressed cardiac output in systolic heart failure persistently activates these same catecholaminergic- β AR signaling pathways, resulting in cardiomyocyte toxicity that creates a downward functional spiral of worsening heart failure that stimulates more catecholamines, which further injure the heart, etc. (Fig. 1). As discussed earlier, an important mechanism that could potentially protect myocardium from the pathological consequences of uninterrupted β AR signaling is GRK-mediated phosphorylation of myocardial β AR (Ferguson, 2001; Pitcher et al., 1998), which recruits β -arrestins that displace bound G-proteins, thus partially uncoupling β ARs from signaling to G_s . β -arrestins also target β ARs to clathrin-coated pits for endocytic receptor internalization, resulting in β AR downregulation, and serve as “signals” themselves, by way of their capacity to chaperone other molecules (DeWire et al., 2007).

The prototypical cardiovascular GRK is GRK2, originally designated β -adrenergic

receptor kinase (β -ARK) (Benovic et al., 1986; Benovic et al., 1987). Of seven mammalian GRKs (Benovic et al., 1989), the most highly abundant in myocardium are GRK2 and GRK5 (Kunapuli and Benovic, 1993; Premont et al., 1994), both of which can desensitize agonist-occupied β AR. Despite these similarities, GRK2 and GRK5 are structurally distinct and are members of separate GRK sub-families (Premont et al., 1995), which are: GRKs 1 and 7 (retinal opsin kinases), GRKs 2 and 3 (a.k.a. β -ARK1 and 2), and GRKs 4, 5, and 6.

In clinical heart failure, the cycle of sympathetic stimulation leading to β AR dysfunction can be interrupted with pharmacological β AR blockade, which prolongs group mean survival in heart failure (see Fig. 1) (MERIT-HF Study Group, 1999; Packer et al., 1996). An experiment of nature suggests that increased activity of GRK5 can provide some of the benefits associated with pharmacological β -blockers (“genetic- β -blockade”), and thus act as a pharmacogenomic locus by indicating which individuals would not benefit from “exogenous” β -blockade. A comprehensive screening for coding polymorphisms of GRK2 and GRK5 found no common non-synonymous polymorphisms in GRK2, but identified four allelic variants of GRK5: cDNA nucleotide position 122 A/T changes glutamine (Gln) to leucine (Leu) at amino acid 41 in the amino terminus, adjacent to a calmodulin binding domain; nucleotide 840 G/A changes arginine to histidine at amino acid 304 within the catalytic domain; nucleotide 1274 C/T changes threonine to methionine at amino acid 425 in the carboxyl terminus, not corresponding to any known functional domain; and nucleotide 1624 C/G changes proline to alanine at amino acid 542 within the carboxyl terminus calmodulin-binding domain (Fig. 11A) (Liggett et al., 2008). The amino acids encoded by three of the four major GRK alleles are completely conserved within members of the same GRK subfamily, and glutamine at amino acid 41 is conserved in all non-retinal opsin human GRKs (including each of the splice variants of GRK4 and GRK6) (Fig. 11B), and across

mammalian species in GRK5 (Liggett et al., 2008). GRK5 variation at amino acids 304, 425, and 542 was infrequent (<2% allele frequency) in a diverse human cohort. In contrast, the GRK5 Leu41 allele, while rare in Caucasians (allele frequency 0.01-0.02), was common among African Americans (allele frequency 0.20, prevalence of heterozygous carriers of 0.35, and homozygous 0.05).

As might be expected for a polymorphism within a putative regulatory domain, rather than within the catalytic domain, the Leu41 substitution did not affect intrinsic *in vitro* GRK kinase activity measured by *in vitro* rhodopsin phosphorylation. However, when the pharmacological properties of the GRK5 Leu41 minor allele were compared to those of “wild-type” GRK5 Gln41 in the intact cell setting, a differential effect on β AR signaling was found. Each variant was co-expressed at equivalent levels with either human β_1 AR (Liggett et al., 2008) or β_2 AR (Wang et al., 2008) in cultured CHO cells, cells expressing GRK5 Leu41 showed enhanced agonist-promoted desensitization of β AR-stimulated adenylyl cyclase activity for both β AR subtypes (Fig. 12). In the case of β_2 AR, studies of intact cells also revealed increased agonist-mediated receptor phosphorylation and internalization. Improved GRK5 Leu41-mediated desensitization of a protein-kinase A (PKA) phosphorylation site β_2 AR mutant further demonstrated that PKA was not involved, implicating GRK phosphorylation sites in enhanced β AR receptor uncoupling. Together, these studies show that β AR phosphorylation, desensitization, and internalization from GRK5 Leu41 encoded by the variant allele accelerates uncoupling from adenylyl cyclase and more efficiently attenuates β AR signaling. In other words, the *gain* of GRK5 desensitization function in the variant manifests as a *more rapid loss* of β AR signaling in cell-based systems.

In considering the functional impact of the GRK5 Glu to Leu substitution at amino acid 41, one must consider the important calmodulin binding domain spanning amino acids 20-39 (Pronin et al., 1997). Binding of calcium-bound calmodulin inhibits GRK5 catalytic activity, alters its membrane-binding properties, thereby increasing its ability to phosphorylate soluble (non-receptor) substrates, and alters the pattern of GRK5 autophosphorylation (Freeman et al., 1998; Sallese et al., 2000). GRK5 has a much greater affinity for calcium-bound calmodulin than GRK2 (IC₅₀ of ~40 nM vs. 2 μ M), suggesting that increases in intracellular calcium may specifically inhibit GRK5. Currently, these interactions must be considered speculative as the specific impact of the variant allele on GRK5-calmodulin interactions has not yet been examined.

Both GRK5 and GRK2 desensitize myocardial β AR. Since previous studies in mice had demonstrated that genetic ablation of GRK2 in the heart exacerbated catecholamine cardiomyopathy produced by chronic isoproterenol infusion (Matkovich et al., 2006), we predicted that improved β AR desensitization and more rapid attenuation of toxic β AR signaling by GRK5 Leu41 would have the reciprocal effects, i.e., would protect against heart failure in the same model. To test this hypothesis, cardiac-specific transgenic mice expressing equivalent levels of either human GRK5 Gln41 or GRK5 Leu41 were created and subjected to comparative detailed analyses of cardiac phenotypes at baseline and after chronic isoproterenol infusion (Liggett et al., 2008). Overexpression of human GRK5 at low levels (~5-fold endogenous levels) in these studies produced no baseline phenotype, which contrasts with a previous report of diminished basal and catecholamine-stimulated cardiac function in transgenic mice expressing comparatively high (30-fold endogenous levels) expression of bovine GRK5 (Chen et al., 2001). Both wild-type GRK5 and the Leu41 variant shifted the concentration-response curve for

isoproterenol-stimulation of contractility in isolated perfused hearts significantly and equally to the right, consistent with decreased sensitivity of cardiac β AR to agonist. Most importantly, sub-acute desensitization of isoproterenol (β AR)-stimulated cardiac contractility was significantly accelerated in the GRK5 Leu41 transgenics, compared to wild-type GRK5 overexpressors, in perfused mouse hearts (Fig. 13A). Moreover, when left ventricular dilation and contractile function were examined at progressive time intervals after isoproterenol mini-pump implantation, GRK5 Leu41-expressing mice were protected from the adverse consequences of chronic β AR stimulation, similar to treatment with the non-selective β AR antagonist, propranolol (Fig. 13B). In contrast, mice overexpressing wild-type GRK5 Gln41 developed the characteristic dilated catecholamine cardiomyopathy in response to isoproterenol, although they could still be protected by pharmacological β AR blockade (Liggett et al., 2008).

The results of cell-based and transgenic mouse studies suggested that both the pharmacological and physiological consequences of expressing GRK5 Leu41 are similar to being treated with β -blockers, i.e., to decrease β AR signaling under conditions of chronic agonist stimulation. Since heart failure is the medical condition in which treatment with pharmacological β -blockers most dramatically affects the disease (β -blockade improves left ventricular function in failing hearts and decreases mortality rates by approximately half in those with heart failure (Fauchier et al., 2007)), we explored the effects of GRK5 genotype on clinical outcome in human heart failure. Since the minor allele shows significant prevalence in African Americans (>40% carry one or two alleles), but not Caucasians (<2% carry any allele) we genotyped the GRK5 Gln/Leu41 locus in 375 African Americans that had been recruited into a longitudinal NIH/NHLBI-funded study of heart failure. Since GRK5 Leu41 mimics β -blocker treatment in experimental heart failure, we measured how disease outcome (time from heart failure onset to

death or cardiac transplantation) was modified by the interaction of GRK5 genotype and β -blocker treatment status. There was no difference in GRK5 Leu41 allele frequency in non-affected African Americans versus those with dilated or ischemic cardiomyopathy, showing that the GRK5 Leu41 polymorphism does not modify the risk for developing heart failure. However, functional allelic variants that are not risk factors for a disease may nevertheless change disease outcome or response to specific therapies if the pathological pathway they modify is activated only after onset of the disease, such as hyper-activation of cardiac catecholaminergic signaling in heart failure. (For this reason, β -blockers are used to treat heart failure rather than to prevent it.) Indeed, we observed that GRK5 Leu41 carriers not treated with β -blockers had longer transplant-free survival times than their wild-type GRK5 counterparts ($P = 0.013$). Cox proportional hazards modeling with adjustment for age and sex described a protective effect of GRK5 Leu41 in β -blocker untreated subjects (Hazard ratio, 0.28; 95% confidence interval, 0.12-0.66; $P = 0.004$) that was comparable to protection afforded by pharmacological β -blockade in wild-type GRK5 subjects (Hazard ratio, 0.19; 95% confidence interval, 0.10-0.34; $P < 0.001$).

As a clinical study, the GRK5 heart failure has significant limitations: It was performed in a relatively small number of subjects recruited from a single referral center, and with a limited number of endpoints. The decision to treat or not with β -blockers was not randomized (nor could it ethically have been), and the results are therefore subject to unknown factors possibly related to intolerance of the drug on one hand (e.g., asthma or hemodynamic instability), or for indications for β -blocker therapy other than heart failure (e.g., ischemic heart disease) on the other. Thus, these human findings need to be replicated in larger, multi-center trials, and randomized prospective trials will be required before this gene-drug interaction should be considered to justify changing in the current class 1 indication for β -blockers in heart failure for

even a sub-group of heart failure patients. In the meantime, the strong concordance between GRK5 Leu41 effects in recombinant cell culture systems, in transgenic mice, and in the initial study of human heart failure is sufficiently striking to suggest that gene-drug interactions such as this may have more impact on interindividual variability in treatment response than is now recognized.

Beyond individual polymorphisms – the challenge of haplotypes

The β_1 AR (Small et al., 2008), β_2 AR (Drysdale et al., 2000), α_{2A} AR (Small et al., 2006), and α_{2C} AR (Small et al., 2004) genes have polymorphisms in their promoter, 5'UTR, and 3'UTR regions, as well as their coding regions. While individual analysis of a promoter SNP, for example, could reveal a phenotype using reporter assays, it is difficult to know the relevance of this individual SNP within the context of all the other variations in these intronless genes. This situation is somewhat different than a coding SNP that has a marked phenotype in terms of ligand binding, G-protein coupling, or desensitization, where such functions are clearly relevant to drug response. We have therefore proposed that these SNPs should be studied within the context of the other SNPs as they occur in nature, i.e., in the context of the haplotype. The number of theoretically possible haplotypes is 2^N , where N is the number of SNPs. However, the human population is fairly young, and thus there has not been enough time for so many recombination events to have the number of haplotypes approach this theoretical number. Interestingly, we (Small et al., 2003) and others (Stephens et al., 2001) have found that the number of haplotypes for any gene is approximately equal to the number of SNPs x 1.1 (SNP and haplotype frequency 0.01 or greater). Thus for the α_{2C} AR, we found 20 SNPs (thus a theoretical 1.04×10^6 haplotypes), but only 24 haplotypes (Small et al., 2004). The α_{2C} AR

haplotypes represent an informative example of the complexity that this next step in genetic variation-phenotype association studies presents. Table 2 shows the SNPs and the haplotypes of the α_{2C} AR in African-Americans, Caucasians, and Asians. It is readily appreciated that certain haplotypes are cosmopolitan (i.e., 1, 2) while others are overrepresented in certain racial groups (i.e., 8, 14). Secondly, it is clear that only 7 haplotypes occur at an allele frequency ≥ 0.05 in at least one racial group. However, genotyping to only identify these 7 leaves ~10-15% of subjects with incorrect haplotypes (if a limited haplotype-tagged SNP approach is used for identification) or are grouped as “other.” Also, in the 3’UTR (position r) there is a 21 bp deletion. In the insertion form, there is a SNP within this stretch of nucleotides (position s). Depending on the genotyping technique, the s polymorphism might be called in the presence of the r deletion, since the wild-type allele would not be found. Another issue revolves around the 12 nucleotide in-frame deletion polymorphism (position q) that occurs in the third intracellular loop of the receptor, deleting amino acids 322-325. The α_{2C} Del322-325 has been studied in transfected cells and found to be markedly dysfunctional (Small et al., 2000), and has been associated with several heart failure phenotypes (Gerson et al., 2003; Lobmeyer et al., 2007; Small et al., 2002). However, Del322-325 is found in 9 different α_{2C} AR haplotypes. What if promoter polymorphisms within a haplotype increased expression? Would that move the cellular or clinical phenotype to an intermediate phenotype? Or conversely, SNPs that lower expression could accentuate the Del322-325 effect, again adding heterogeneity to the phenotypes. Indeed, using whole-gene transfections we have shown that haplotype does affect receptor expression (Small et al., 2004). Thus in a clinical study, genotyping only at Del322-325 doesn’t give one all the pertinent information that may affect drug response or other traits. The need to genotype more SNPs, and to impute haplotypes, would appear to require greater numbers of subjects for

clinical trials since there are more genotypic bins. And, some would argue, additional power considerations must be made due to multiple comparisons. However, in candidate gene studies, it seems inappropriate to genotype at one SNP position, when there are multiple SNPs, particularly when molecular studies have shown haplotype effects. Of note, reliance on linkage disequilibrium between a SNP within a gene, and several other SNPs in the gene, must be shown to be valid prior to studies. For the $\alpha_{2C}AR$, even over a small number of bases (~4,300), low levels of linkage disequilibrium were found between some SNPs, and the degree of linkage disequilibrium varied by race (Small et al., 2004). This represents a major weakness of unbiased genome-wide association studies. Multiple SNPs of the $\alpha_{2C}AR$ could be on a chip for such study, and due to low linkage disequilibrium, none would have identified (tracked with) the Del322-325 polymorphism.

CONCLUSIONS

The β_1AR and GRK5 polymorphisms discussed appear to play a role in the response to β -blocker therapy, and a mechanistic basis for the effects have arisen from studies using multiple approaches. These types of studies, using transfected cells, transgenic mouse, *ex vivo* human hearts, and human physiological outcomes, each have limitations. But taken together, build a case for cause-and-effect for a polymorphism and a drug-response phenotype. They also provide new insights into alternative therapies and the pathophysiology of heart failure. Nevertheless, questions remain as to their roles in individualizing heart failure therapy, and there may be other polymorphisms that have an even greater impact that are yet to be discovered. Nevertheless, we propose that effort be expended in both clinical studies as well as mechanistic studies so that pharmacogenomics can include well-conducted clinical trials and a linking of these results to

mechanism of action.

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Footnote

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Figure Legends

Fig. 1. The vicious cycle of chronic catecholamine stimulation in heart failure. With an acute event and a normal heart, β AR activation by catecholamines increases cardiac output without deleterious effects (within limits). With prolonged stimulation of a pathologic heart which has less capacity to respond, continued forced contractility has extensive maladaptive consequences leading to worsening cardiac output. The cycle can be attenuated by β -blockers or GRK activity. See text for discussion.

Fig. 2. An approach to exploring relevance of candidate-gene polymorphisms. Dashed lines represent ancillary studies that utilize reagents or cohorts collected within the main sequence of events (solid lines).

Fig. 3. Conservation of amino acid sequences within the fourth intracellular loop of the β_1 AR for various species.

Fig. 4. Properties of the β_1 AR Arg389 and Gly389 variants in transfected cell membranes. A, B), results from agonist competition studies. C), results from agonist-promoted adenylyl cyclase studies. D), results from short-term agonist-promoted desensitization studies. C = control, D = desensitized by pre-exposure to isoproterenol.

Fig. 5. Physiologic effects of human Arg389 and Gly389 β_1 ARs expressed on myocytes of transgenic mice. Mice were studied in the *ex vivo*, work-performing model. The first derivative

of pressure per unit time ($+dP/dt_{\max}$) is a measure of contraction. A, 3-month old mice; B, 6-month old mice.

Fig. 6. Differential cardiac responses to β -blockade in Arg389 and Gly389 β_1 AR transgenic hearts. A, results from acute infusion of propranolol in the *ex-vivo* work-performing model. B), results from a 1-month oral administration of propranolol with heart rates determined in intact mice by echocardiography.

Fig. 7. Distribution of transcripts that are regulated in a unique, or common, manner in the hearts from the three indicated transgenic mice. The numbers in each region represent the number of genes significantly up- or downregulated compared to non-transgenic littermates. The partition sizes are not to scale.

Fig. 8. Potential mechanistic implications of filtered gene expressions from β_1 -Arg389, -Gly389, and ACV transgenic mouse hearts. The sizes of the circles are proportional to the number of genes in each pool. P = pathogenic, DR = drug response.

Fig. 9. Contractile responses to ligands in right ventricular trabeculae from human failing and non-failing hearts. Data are shown stratified by Arg389 homozygous and Gly389 carrier genotypes.

Fig. 10. Kaplan-Meyer survival curves for patients in the BEST trial, stratified by β_1 AR genotype and drug/placebo. Those with the Arg389 homozygous genotype had a significantly

decreased mortality compared to those with the same genotype receiving placebo. In contrast, Gly389 carriers showed no apparent response to bucindolol compared to placebo (see text for hazard ratios).

Fig. 11. Sequence comparison of human GRKs. The non-retinal GRKs (2, 3, 4, 5, 6) all have Q in position 41 analogous to GRK5 (yellow bar). The most common polymorphism of GRK5 is this Q>L variation.

Fig. 12. Enhanced β AR desensitization evoked by the GRK5-L41 polymorphism. A) β_1 AR were co-expressed with GRK5-Q41 or -L41 and the kinetics of isoproterenol promoted cAMP accumulation determined. The L41 responses are quenched to a greater extent, and more rapidly, than those from cells expressing Q41. B) In a different model of desensitization, β_2 AR was expressed with the two GRKs and exposed to vehicle or isoproterenol for 30 min, washed and membranes prepared. Adenylyl cyclase activities were then performed with the indicated concentrations. The β_2 AR with Leu41 co-expressed underwent a greater degree of desensitization compared to Gln41.

Fig. 13. GRK5-L41 protects against catecholamine-mediated left-ventricular dilatation in mice. A) Isoproterenol administered by mini-pump evokes an increase in the left ventricular end diastolic dimension (LVEDD) in non-transgenic (NTG) mice that is blocked by propranolol. B) Expression of GRK5-L41 results in no increase in LVEDD during isoproterenol infusion and propranolol has no effect. In contrast, GRK5-Q41 mice displayed equivalent levels of increased LVEDD, and the same response to propranolol, as do NTG mice.

Fig. 14. Association of GRK5 polymorphisms, β -blocker usage, and survival in human heart failure. Shown are Kaplan-Meier curves with the proportion of patients surviving as the Y-axis. In homozygous GRK5-Q41 patients, β -blockers (BB) have a characteristic effect, with an improvement in survival. In GRK5-L41 carrier patients, survival in the absence of β -blocker treatment is similar to that of GRK5-Q41 patients taking β -blockers (green line). In the -L41 patients, there was no major improvement in survival in those receiving β -blockers over those who were not.

Table 1. Coding region variations of the β_1 AR and GRK5 genes. MAF, minor allele frequency.

Gene name	Common	Nucleotide Variability [†]	Amino Acid Variability [*]	MAF (%)	
				White	Black
<i>ADRB1</i>	β_1 AR	145 (A/G)	49 (Ser/Gly)	15	13
<i>ADRB1</i>	β_1 AR	1165 (C/G)	389 (Arg/Gly)	27	42
<i>ADRB1</i>	β_1 AR	1166 (G/T)	389 (Arg/Leu)	<0.1	0.9
<i>GRK5</i>	GRK5	122 (A/T)	41 (Gln/Leu)	1.3	23
<i>GRK5</i>	GRK5	840 (G/A)	304 (Arg/His)	<0.01	0
<i>GRK5</i>	GRK5	1274 (C/T)	425 (Thr/Met)	0	0.02
<i>GRK5</i>	GRK5	1624 (C/G)	542 (Pro/Ala)	<0.01	0

* the most common allele in a general U.S. population (composed of 12% African-Americans) is the first provided

[†] nucleotide position is relative to the AUG initiator codon

Table 2. Haplotypes of the α_{2C} -adrenergic receptor gene.

location cde:	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	AA	Ca	As
HAP #																							
1	T	C	C	G	C	C	G	T	T	C	G	G	C	C	C	T	Ins	Ins	T	G	23.8	58.8	39.6
2	T	C	C	G	C	C	G	T	T	C	G	G	C	C	C	T	Ins	Ins	C	C	13.8	13.8	12.5
3	T	C	C	G	C	C	G	G	T	C	G	G	C	C	C	T	Del	Ins	C	C	21.3	0	8.3
4	T	C	C	G	C	C	G	G	T	C	G	G	C	C	G	T	Del	Ins	C	C	7.5	0	0
5	T	G	T	G	C	C	G	T	T	A	C	G	C	C	C	T	Ins	Ins	C	C	7.5	0	0
6	T	C	C	G	C	C	G	T	T	C	G	G	C	C	C	T	Del	Ins	C	C	5	0	0
7	T	C	C	T	C	C	G	G	T	C	G	G	C	C	C	T	Del	Ins	C	C	5	0	0
8	C	C	C	G	T	C	G	T	T	C	G	A	C	C	C	T	Ins	Del	*	G	2.5	7.5	27.1
9	T	C	C	G	C	C	G	G	T	C	G	G	C	C	C	C	Del	Ins	C	C	2.5	3.75	0
10	T	C	C	G	C	C	G	T	T	C	G	G	C	C	C	T	Ins	Del	*	G	1.25	0	2.1
11	T	C	C	G	C	C	G	G	T	C	G	G	C	C	C	T	Ins	Ins	T	G	2.5	0	0
12	T	G	T	G	C	C	G	T	T	C	C	G	C	C	C	T	Ins	Ins	C	C	2.5	0	0
13	T	C	C	G	C	C	G	T	G	C	G	G	C	C	C	T	Ins	Ins	T	G	0	2.5	0
14	T	C	C	G	C	C	G	T	T	C	G	G	C	G	C	T	Ins	Ins	C	C	0	11.3	0
15	C	C	C	G	C	C	G	G	T	C	G	G	C	C	C	T	Del	Ins	C	C	1.25	0	0
16	C	C	C	G	C	C	G	T	T	C	G	G	C	C	C	T	Ins	Ins	C	C	1.25	0	0
17	C	C	C	G	C	C	G	T	T	C	G	G	C	C	C	T	Ins	Del	*	G	1.25	0	0
18	T	C	C	G	C	C	A	T	T	C	G	G	A	C	C	T	Del	Ins	T	G	1.25	0	0
19	C	C	C	G	C	C	G	G	T	C	G	G	C	C	C	C	Del	Del	*	C	0	1.25	0
20	T	C	C	G	C	C	A	G	T	C	G	G	A	C	C	T	Del	Ins	C	C	0	1.25	0
21	C	C	C	G	T	C	G	T	T	C	G	A	C	C	C	C	Ins	Del	*	G	0	0	4.2
22	C	C	C	G	T	C	A	T	T	C	G	A	A	C	C	T	Ins	Del	*	G	0	0	2.1
23	C	C	C	G	T	T	G	T	T	C	G	A	C	C	C	T	Ins	Del	*	G	0	0	2.1
24	T	C	C	G	C	C	G	T	T	C	G	G	C	C	C	C	Ins	Ins	T	G	0	0	2.1

Shown are the allele frequencies (in %) of the haplotypes for each ethnic group. See Ref (Small et al., 2004) for the position in the gene relative to the location code. The colors indicate the position in the gene (red, promoter; blue, 5'UTR; purple, coding; green, 3'UTR. AA, African-American; Ca, Caucasian; As, Asian; *, not applicable due to deletion at r.

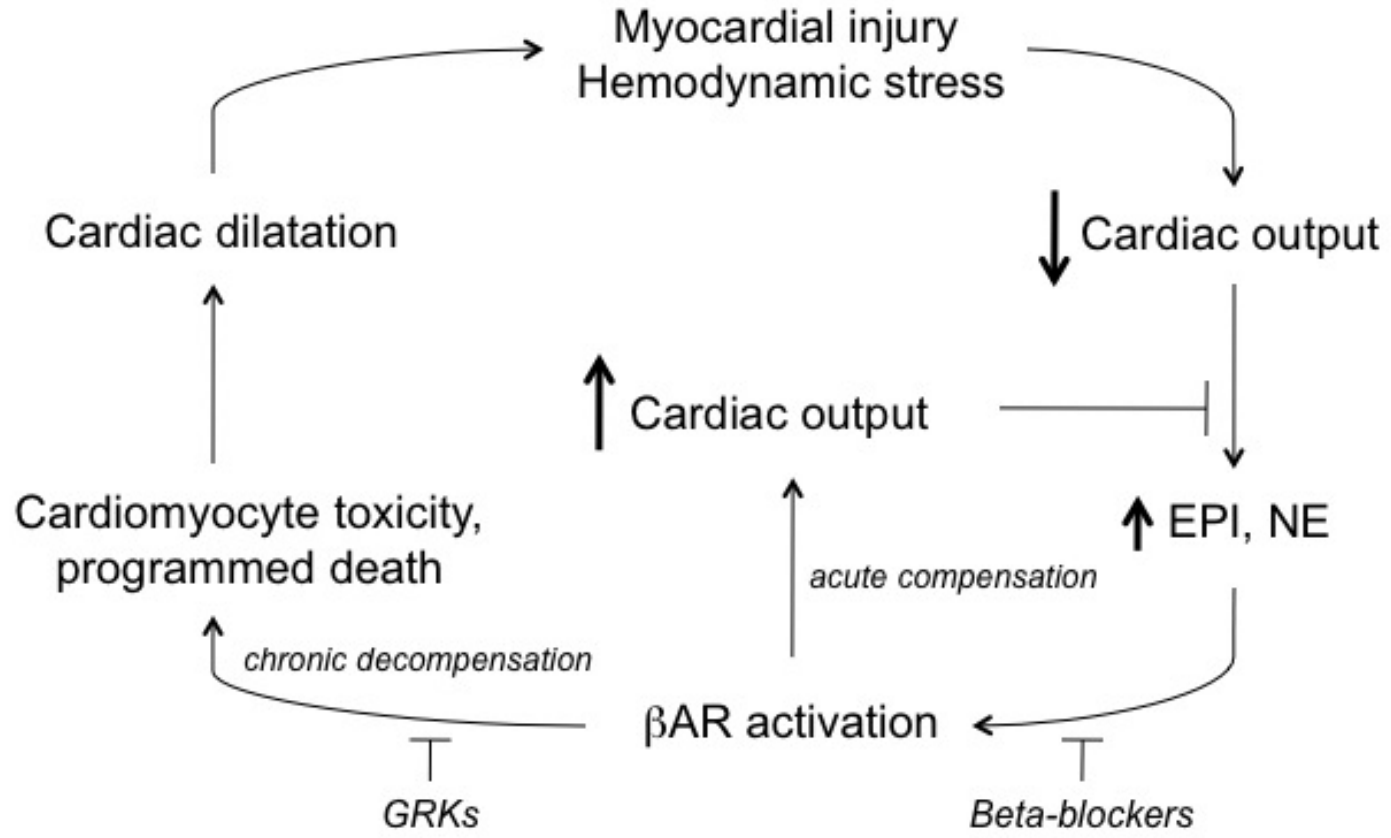


Figure 1

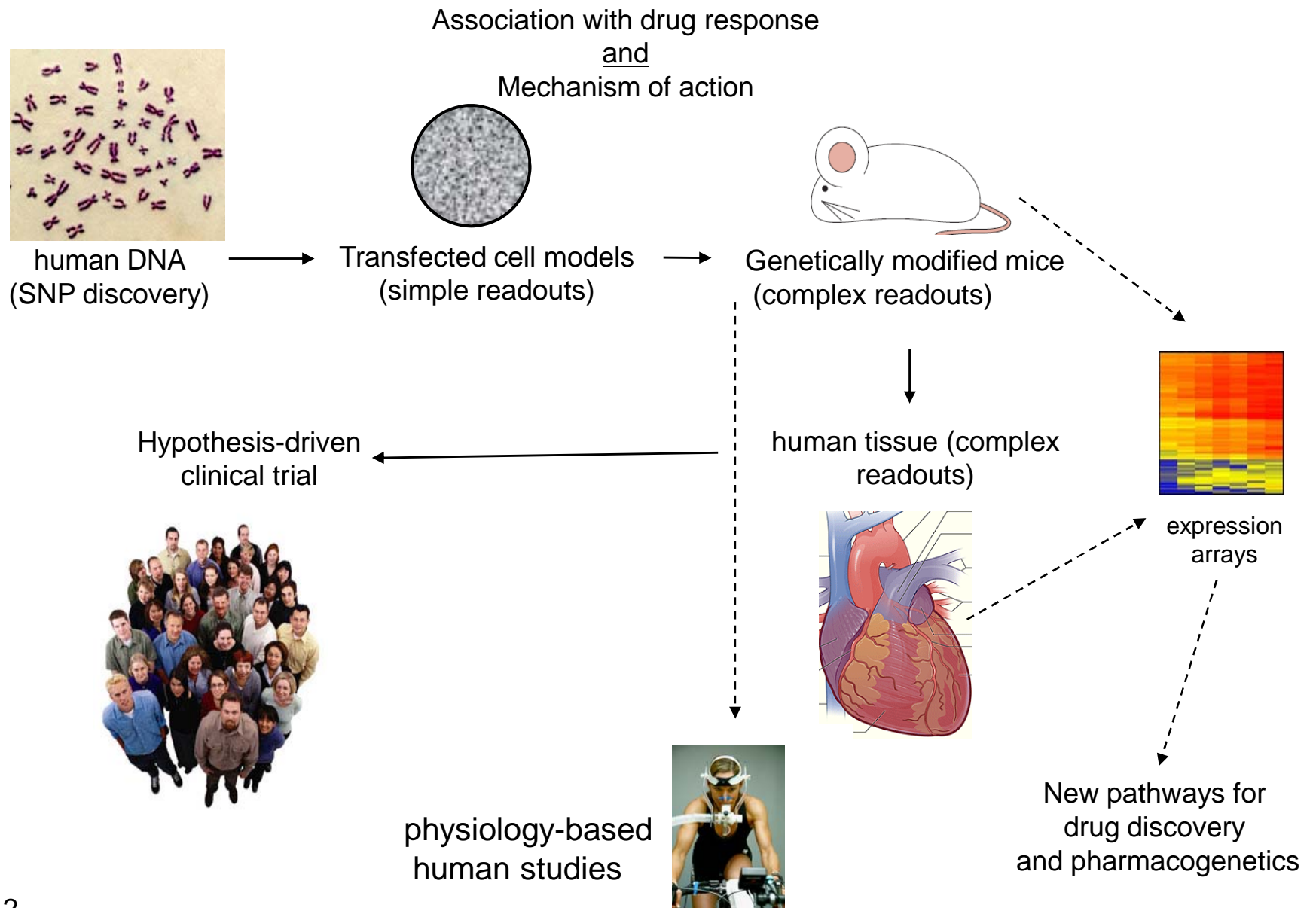


Figure 2

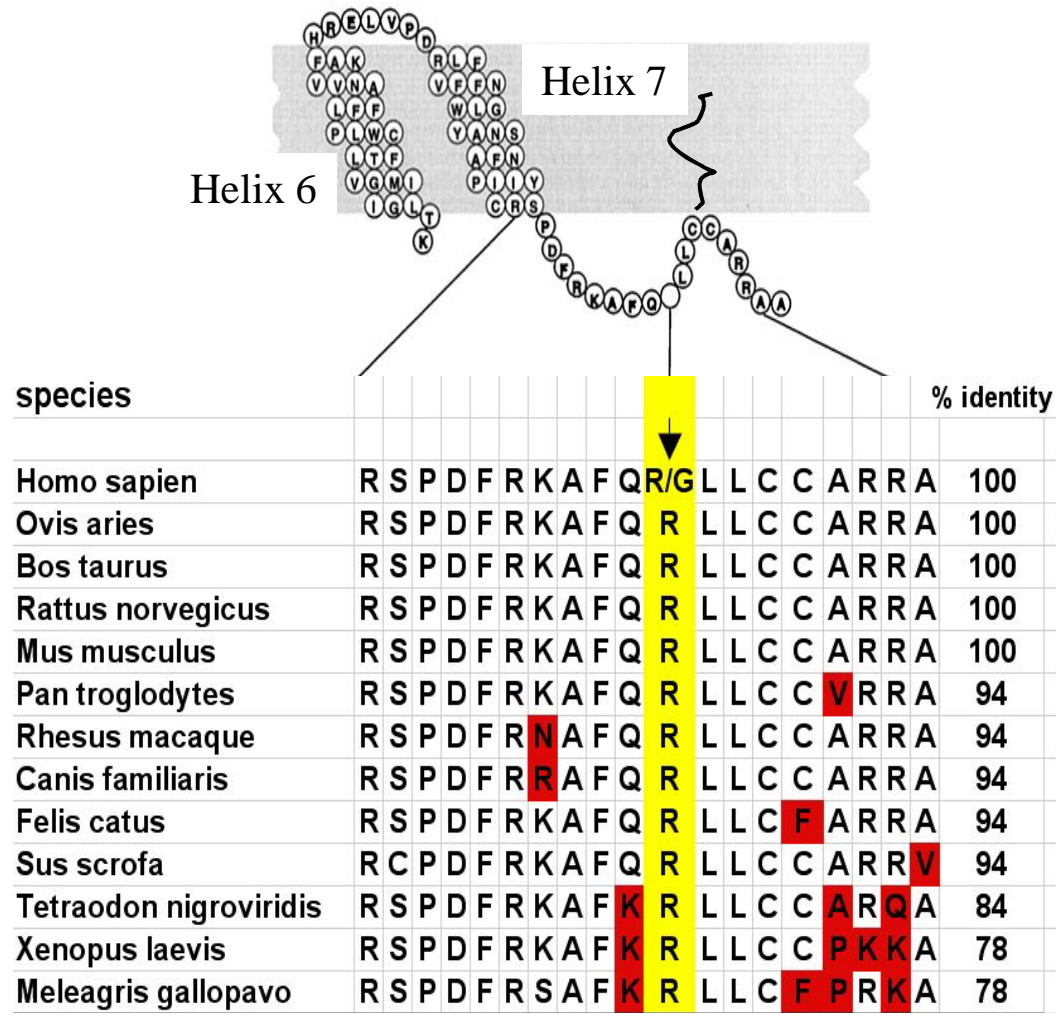


Figure 3

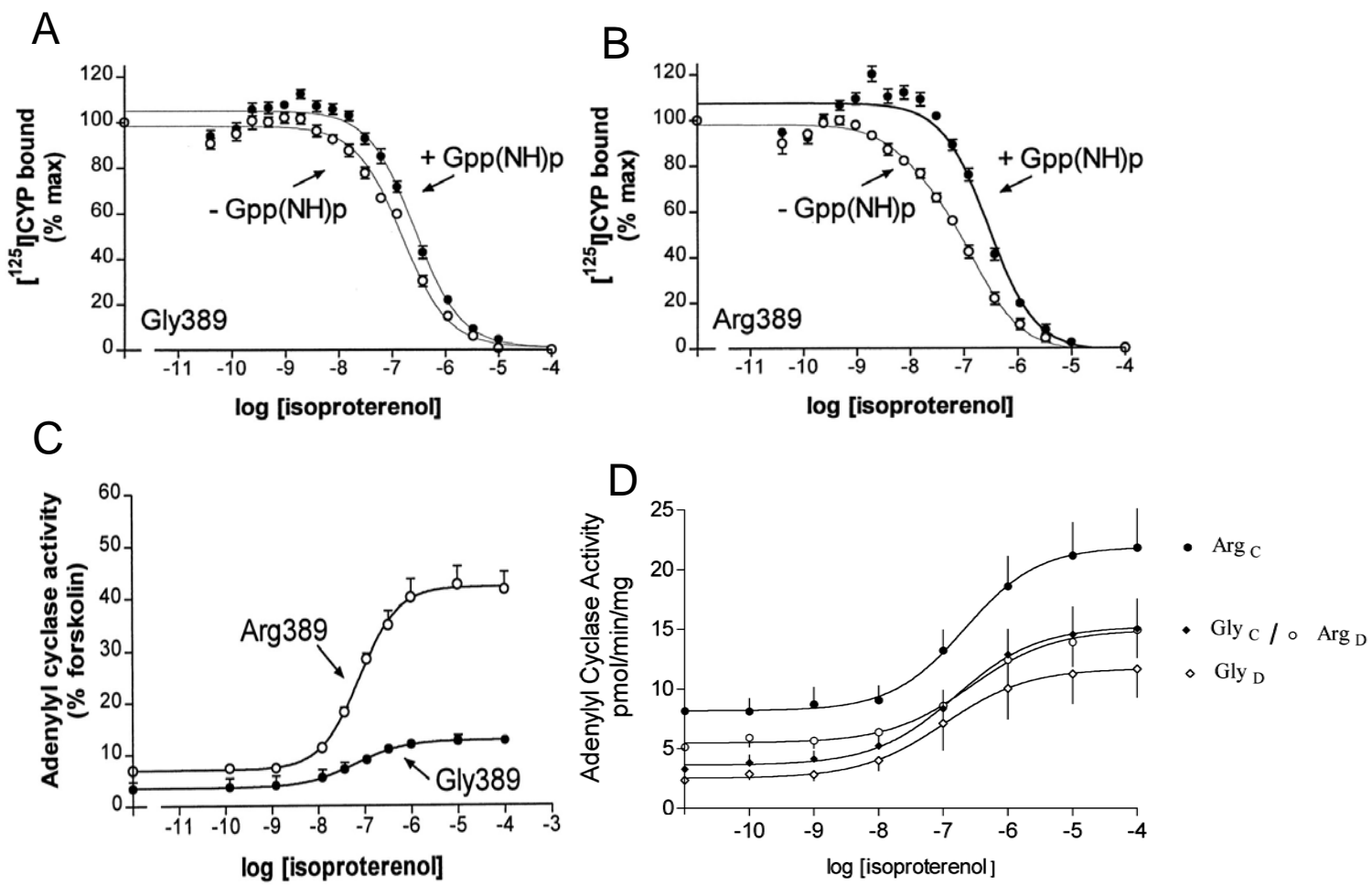


Figure 4

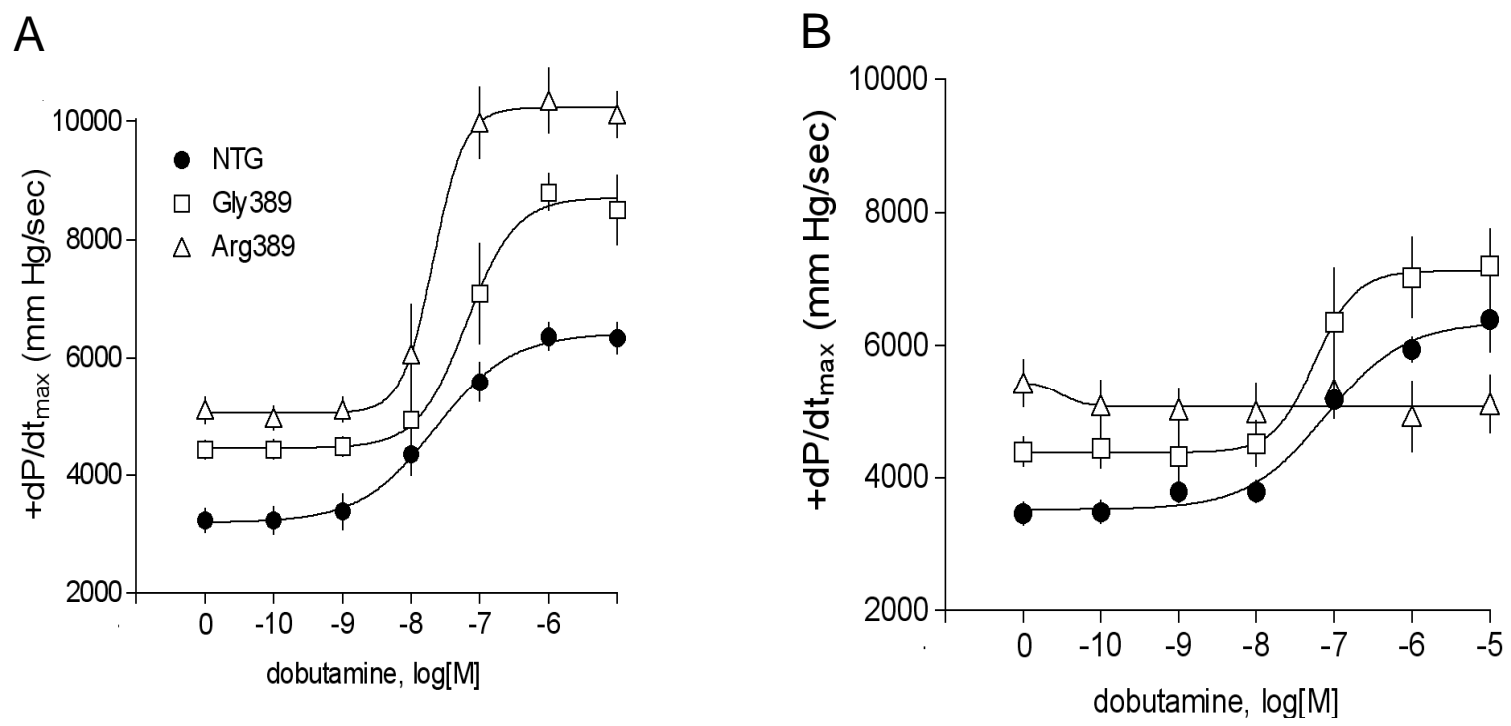


Figure 5

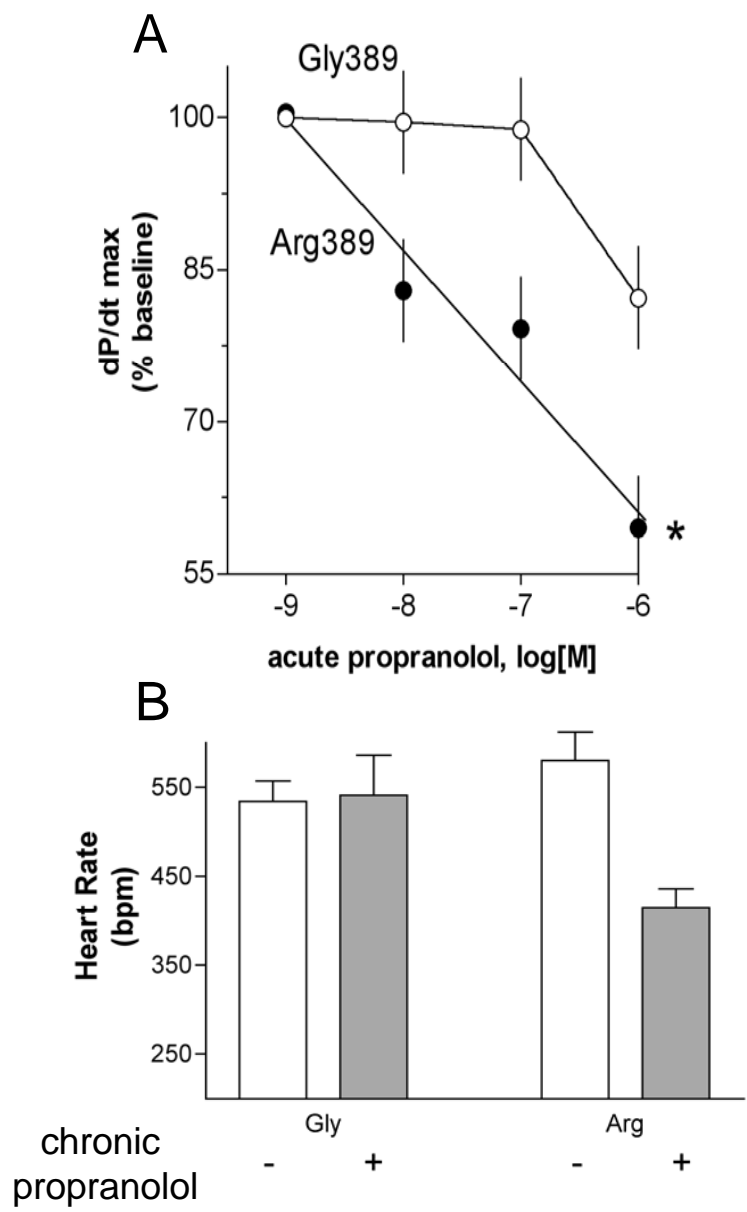


Figure 6

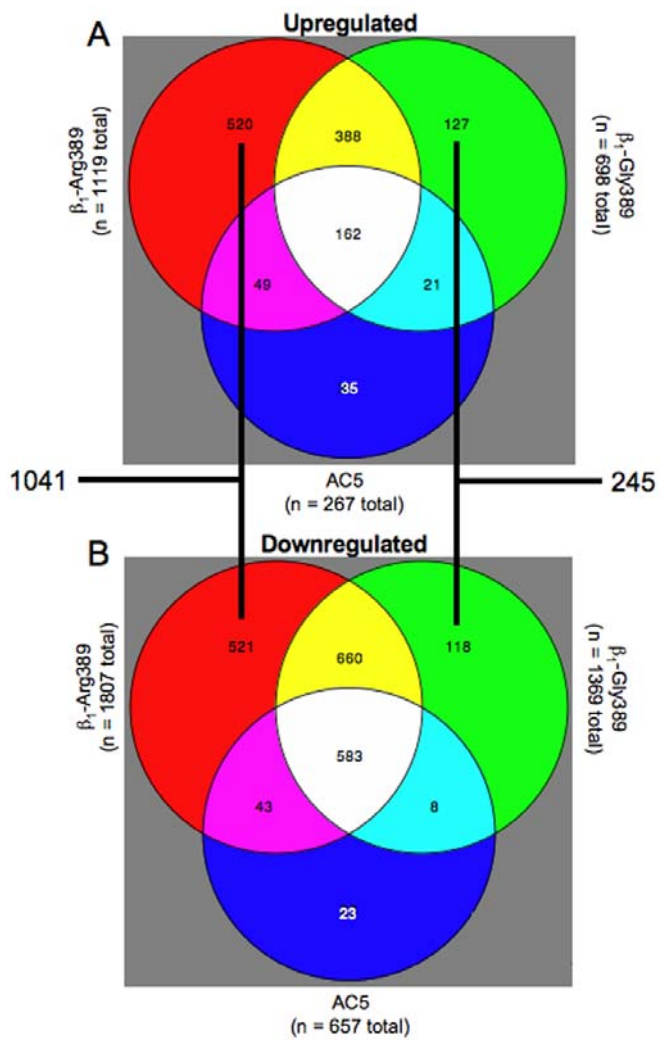


Figure 7

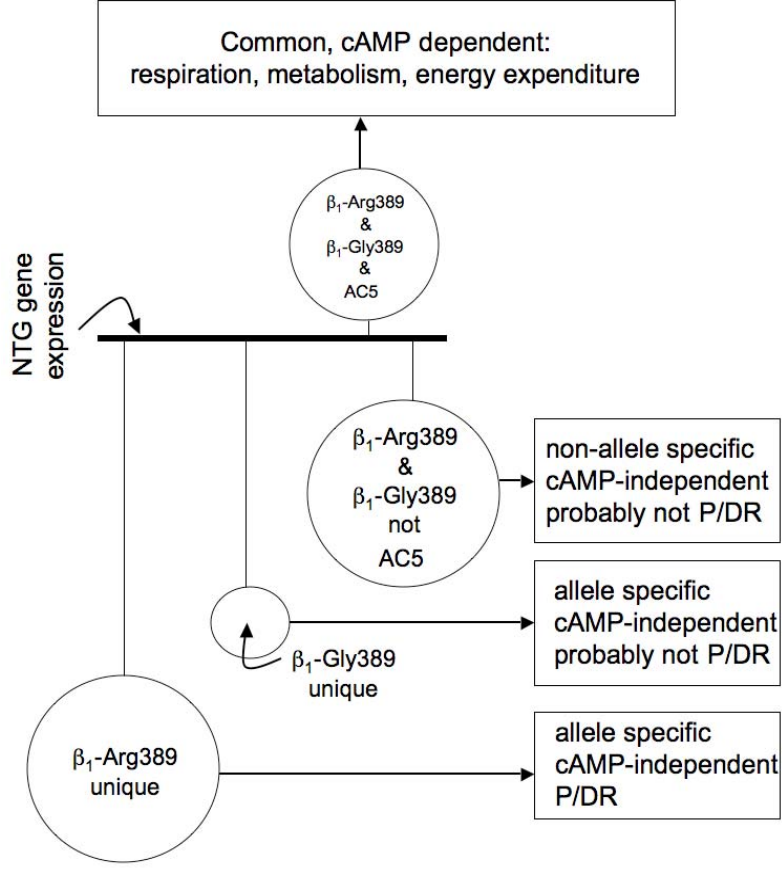


Figure 8

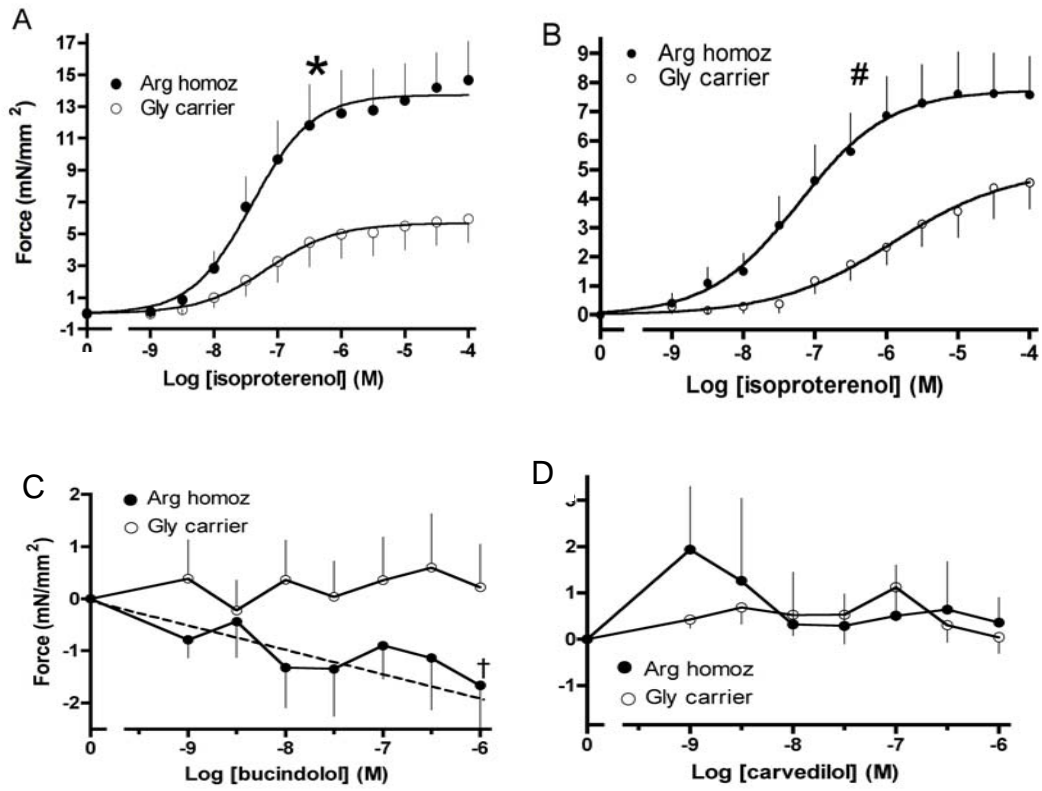


Figure 9

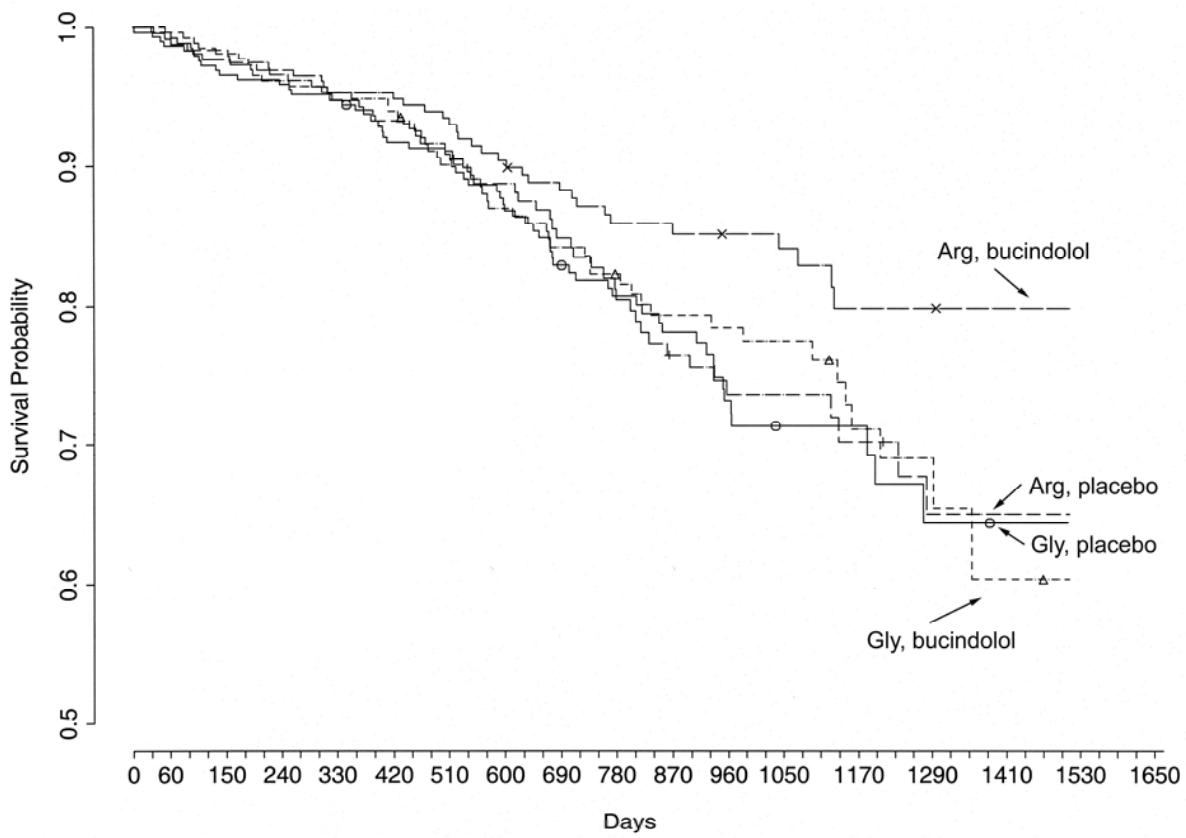


Figure 10

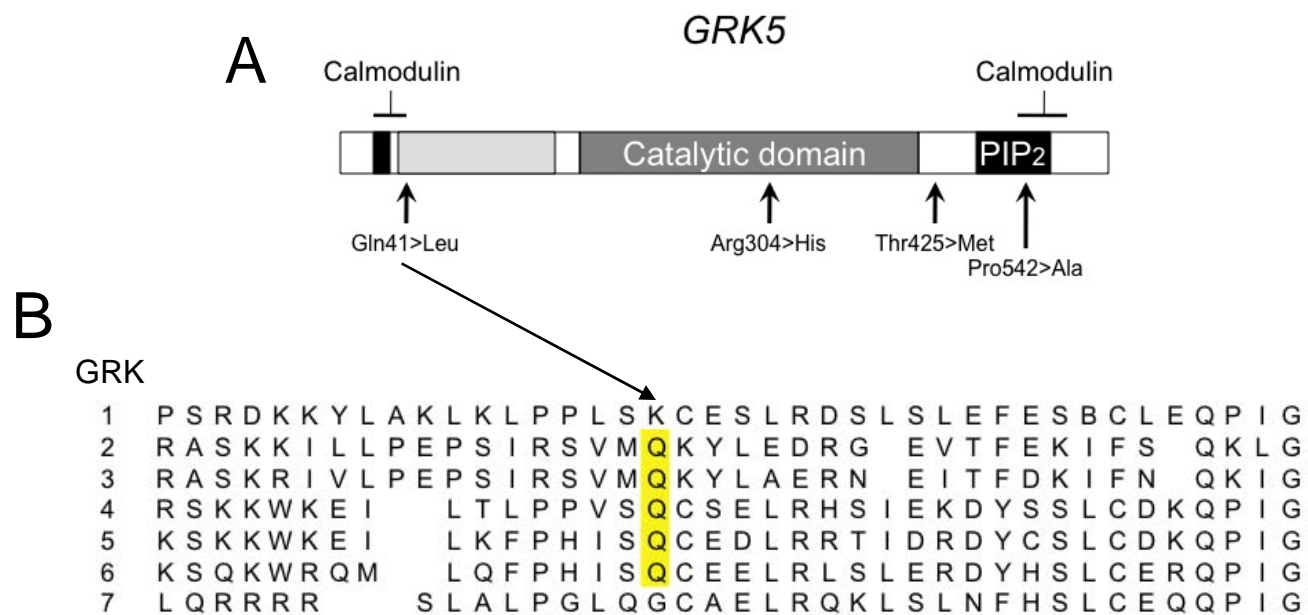


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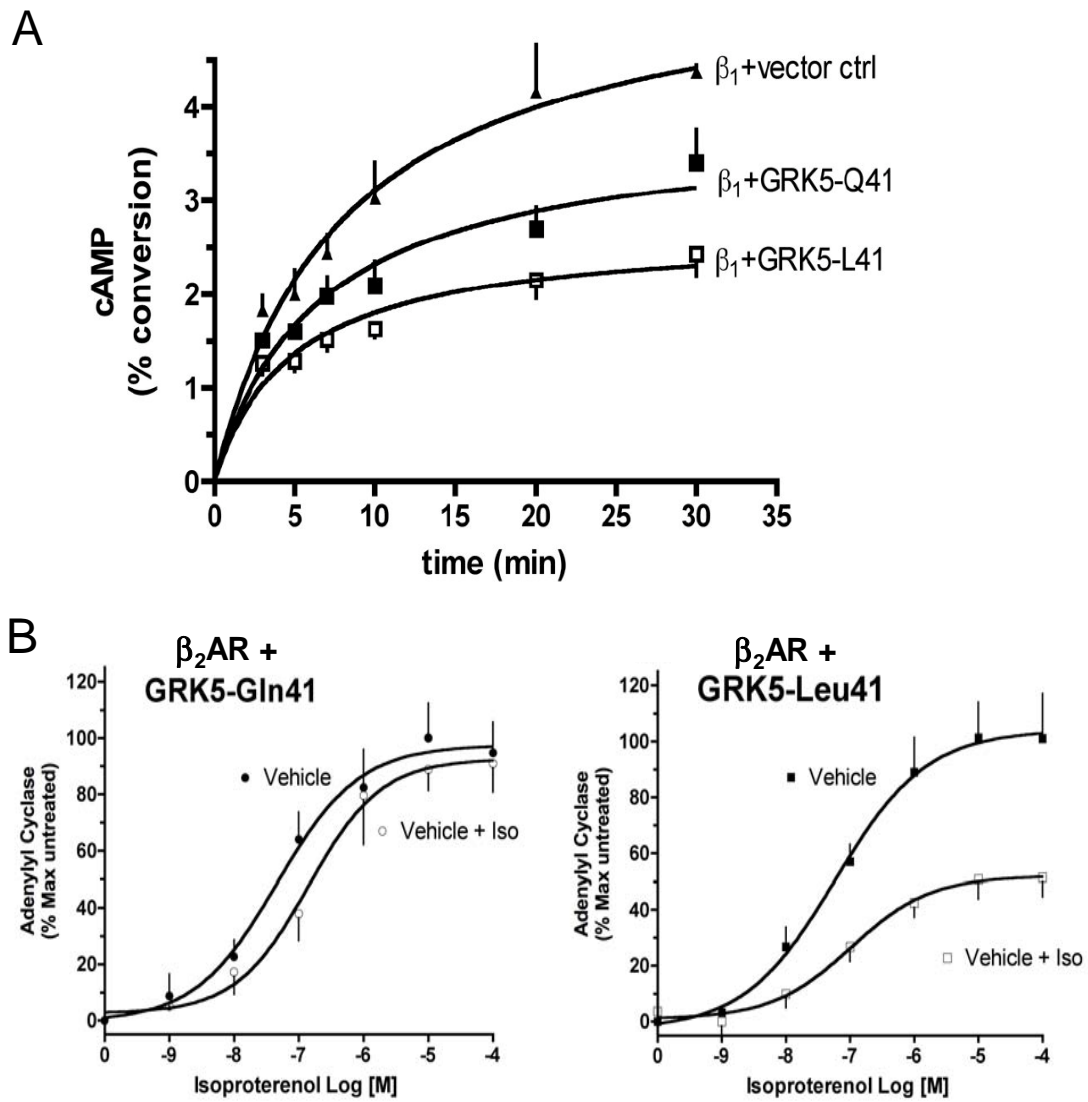


Figure 12

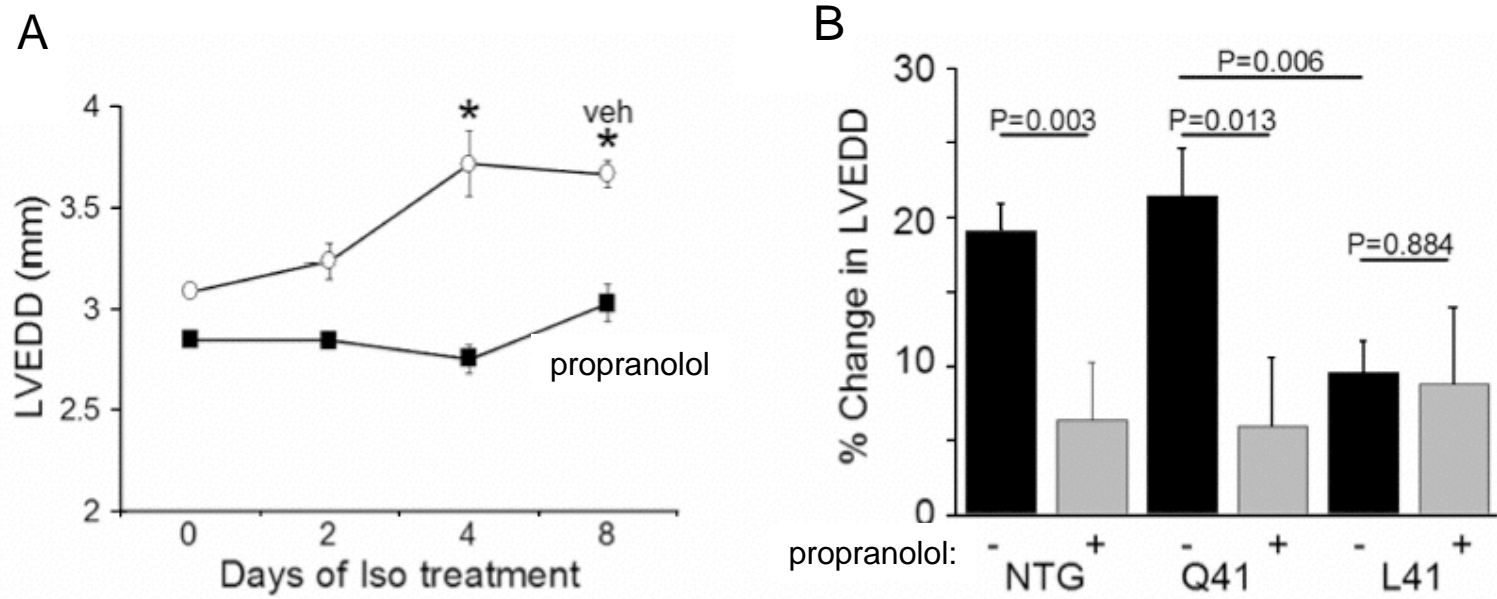


Figure 13

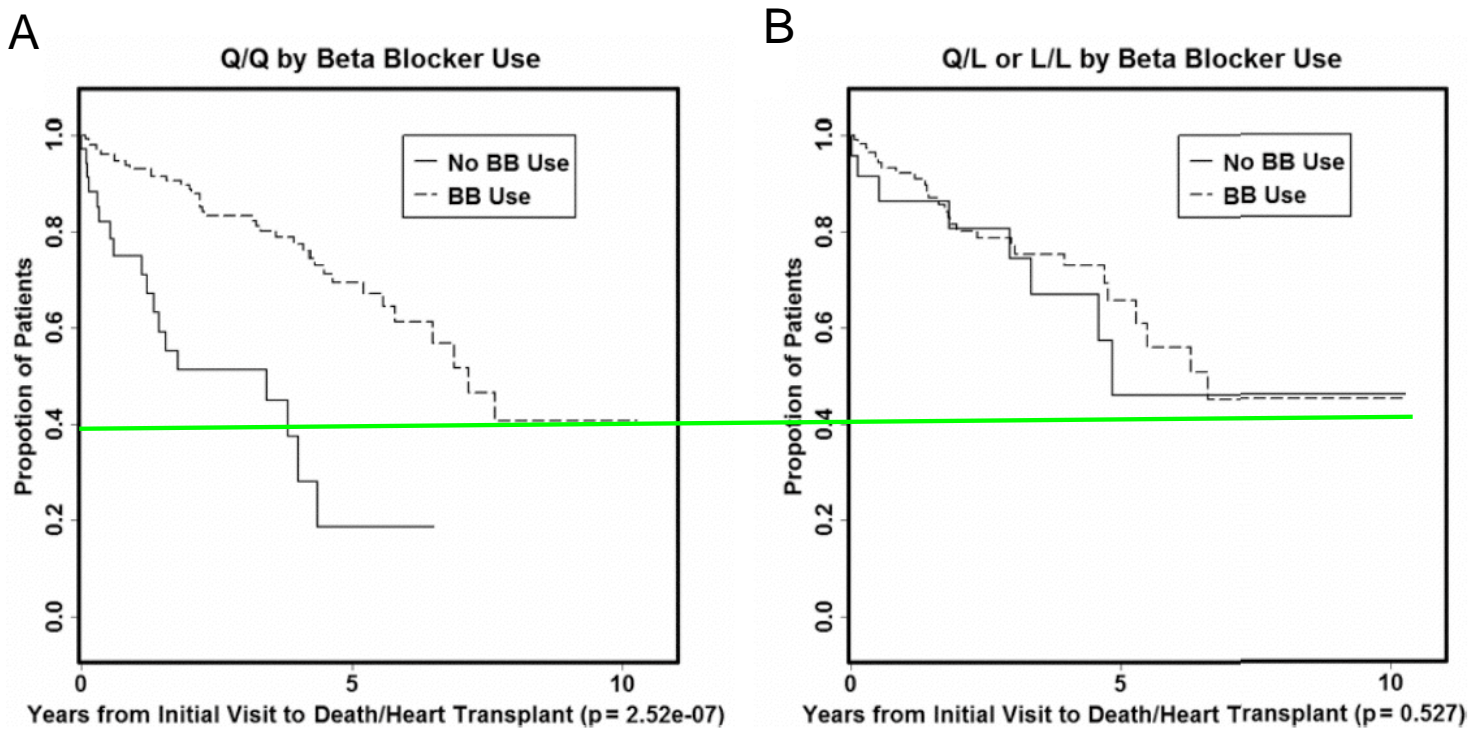


Figure 14