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Cardiovascular Pharmacogenomics of Adrenergic Receptor Signaling: Clinical Implications and Future Directions

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Abstract

G-protein-coupled receptors (GPCRs) are the targets for many drugs, and genetic variation in coding and noncoding regions is apparent in many such receptors. In this superfamily, adrenergic receptors (ARs) were among the first in which single-nucleotide polymorphisms (SNPs) were discovered, and studies including *in vitro* mutagenesis, genetically modified mouse models, human *ex vivo* and *in vitro* studies and pharmacogenetic association studies were conducted. The signal transduction in these receptors includes amplification steps, desensitization, crosstalk, and redundancies, enabling potential mitigation of the size of the clinical effect for a single variant in a single gene. Nevertheless, convincing evidence has emerged that several variants have an impact on therapy, with certain caveats as to how the results are to be interpreted. Here we review these results for selected ARs and associated regulatory kinases relative to the pharmacogenomics of β -blocker treatment for hypertension and heart failure. We emphasize the linking of clinical results to molecular mechanisms, discuss study design limitations, and offer some recommendations for future directions.

Pharmacogenetics had its birth in the late 1950s; through the late 1990s, it centered almost exclusively on loss-of-function mutations in drug-metabolizing enzymes, which lead to dramatic differences in drug pharmacokinetics corresponding to observable clinical phenotypes. These loss-of-function mutations are characterized by the absence of functional protein and a consequent substantial impact on drug metabolism, particularly in the case of drugs for which the metabolism is predominantly dependent on the affected enzyme. These examples have some analogies to monogenic diseases, in which mutations in a single gene have a major impact. Even today, the most clinically advanced pharmacogenetic examples are those for which there is a loss-of-function mutation that dramatically affects drug pharmacokinetics.

However, just as most diseases are not monogenic, the genetic contributors to variability in drug response also do not predominantly arise from a single gene with a loss-of-function mutation. The current challenge in pharmacogenomics is to unravel the array of genes whose variability contributes to differences in drug efficacy or toxicity.

G-protein-coupled receptors (GPCRs) are the most common protein target among currently marketed drugs. As a result, they were among the first group of proteins whose genetic

variability was studied in order to extend pharmacogenomics beyond drug-metabolizing enzymes. Among the best studied of the GPCRs are the adrenergic receptors (ARs). Among the most commonly used drugs that target a GPCR are the β -adrenergic receptor blockers.

Cardiovascular diseases are the most common cause of death in the United States and in other Western countries, and they exact substantial toll in terms of premature deaths, morbidity, lost productivity, and health-care costs. Among the cardiovascular drug classes, few have a wider range of indications in cardiovascular disease than the β -blockers. Specifically, the use of β -blockers is the preferred or strongly recommended therapy in patients with heart failure, ST-segment elevation myocardial infarction (acute and chronic), unstable angina or non-ST-segment elevation myocardial infarction, chronic stable angina, atrial fibrillation (rate control), and symptomatic control of premature ventricular beats; β -blockers are also among the acceptable first-line therapies in hypertension. Within these disease states, β -blockers have primary indications because of their ability to alter the course of the disease and/or reduce mortality (e.g., for heart failure, acute coronary syndromes, and hypertension) or to control symptoms or address a measurable phenotype (e.g., chronic stable angina, atrial fibrillation rate control, hypertension, and premature ventricular beats). The wide range of conditions for which they are useful and the fact that their protein target is affected by genetic variation, with documented *in vitro* and cellular effects, make the β -blockers logical targets for pharmacogenomics research.

Here we describe the pharmacogenomic discoveries pertaining to these ARs in the context of the use of β -blockers for the treatment of hypertension and heart failure. We also highlight the additional insights from studies of genes that encode GPCR signaling or regulatory proteins and discuss current implications and future challenges. These examples were selected because they are among the strongest examples of GPCR pharmacogenomics in the literature, with potential future clinical implications. Additionally, lessons learned from the studies highlighted here are likely to be relevant across the broader GPCR family.

ARs AND ASSOCIATED PROTEINS

GPCRs comprise the largest superfamily of signaling molecules in the genome. Their topology consists of an extracellular amino terminus, seven transmembrane-spanning α -helical regions, three extracellular loops, three intracellular loops, and a carboxy-terminal intracellular tail. Many GPCRs also have a fourth intracellular loop formed by an attachment of part of the intracellular tail to the membrane through palmitoylated cysteines. This loop is predicted to be an α -helix (sometimes denoted as the eighth helix).

The ARs are GPCRs that represent principal components of the sympathetic nervous system and use the endogenous hormone/neurotransmitters epinephrine and norepinephrine as agonists. Almost every cell type or organ expresses one or more of the nine AR subtypes, and ARs are critical for the maintenance of cellular, organ, and whole-body homeostasis at “rest,” during normal physiological stress such as exercise, and during pathologic stress such as during decreased end-organ perfusion. The distribution of the three α_1 -AR subtypes, the three α_2 -AR subtypes, and the three β -AR subtypes have been described elsewhere.¹ As highlighted in the schematic in Figure 1, α_1 -ARs couple to the heterotrimeric G protein G_q , with $G_{\alpha q}$ activating phospholipase C, thereby converting phosphatidylinositol 4,5-bisphosphate to the second messengers inositol triphosphate and diacylglycerol. β -ARs couple primarily to G_s , with $G_{\alpha s}$ activating adenylyl cyclase, which converts adenosine-5'-triphosphate to the second messenger cyclic adenosine monophosphate. α_2 -ARs couple to G_i , with $G_{\alpha i}$ inhibiting adenylyl cyclase and cyclic adenosine monophosphate production. The $\beta\gamma$ -subunits of these heterotrimer G proteins also act to evoke signals that can be highly dependent on cell type. Furthermore, these receptors also appear to signal via other

mechanisms independent of G-protein interaction. In this review, we concentrate on the β_1 -AR, β_2 -AR, and α_2 C-AR, in view of the nature of their polymorphisms, and the results from clinical studies to date.

Of additional interest are a group of kinases that phosphorylate most GPCRs when activated by agonists. These kinases, termed G-protein-coupled receptor kinases (GRKs), act to quench, or desensitize, agonist-promoted signaling.² This process requires the binding of β -arrestins to the GRK-phosphorylated receptor, which interdicts the receptor-G protein interaction, partially “uncoupling” the receptor from signaling. GRK-mediated desensitization is thought to be a homeostatic mechanism that integrates and regulates the myriad signals received by the cell. In other words, GRK-mediated signaling fine-tunes second-messenger generation, and may protect “overstimulation” by high concentrations of endogenous or exogenously administered agonists. Seven GRKs have been identified: GRK1 and GRK7 (which are restricted to the rods and cones of the eye), GRK2 (also known as β -ARK1), GRK3 (also known as β -ARK2), GRK4, GRK5, and GRK6. Although GRKs 2–6 can phosphorylate multiple GPCRs, there is substrate specificity that is established by the presence of Ser or Thr in the third intracellular loop of the GPCR and/or cytoplasmic tail, as well as the conformation of the receptor in the agonist-bound state, which is in part dictated by the transmembrane-spanning domain sequence.³

ARs IN HEART FAILURE AND HYPERTENSION

Both β_1 -AR and β_2 -AR are expressed on human cardiomyocytes (Figure 1) and promote increased cardiac inotropy and chronotropy, with the β_1 -AR serving a dominant role in this regard.⁴ In heart failure arising from almost any cause, catecholamines (particularly norepinephrine) are elevated, representing the system’s attempt to increase cardiac output via cardiac β_1 -ARs. Although such chronic stimulation is highly effective in an acute setting such as traumatic hypovolemia, in the case of a heart with limited metabolic and physiological reserves, it appears to worsen ventricular function, leading to a vicious circle. Therefore, β -blockers, judiciously titrated to partially antagonize the “toxic” effects of norepinephrine at the cardiomyocyte, can promote cellular remodeling of the heart toward improved hemodynamics. The β_2 -ARs are expressed to a lesser extent than the β_1 -ARs and are distributed at the cell surface in discrete regions that are thought to facilitate specialized signaling, such as signaling to G_{ai} .⁵ Both β -AR subtypes appear ultimately to signal to certain common events, as well as to events that are distinct to a given subtype. For example, prolonged β_1 -AR stimulation is associated with a more pro-apoptotic state, whereas β_2 -AR has several antiapoptotic signaling consequences.⁶ Although these properties might suggest that, in the high-catecholamine state of heart failure “ β_1 -AR-specific” antagonists might have a better treatment effect, this has not been evident in clinical trials. Nevertheless, trials are under way to explore the benefits of administering a β_1 -AR antagonist with a β_2 -AR agonist in heart failure, in an attempt to produce the most favorable β -AR status.

The antihypertensive effect of β -blockers results primarily from antagonism of β_1 -ARs expressed on juxtaglomerular cells of the kidney, thereby decreasing the release of renin.¹ Additionally, there is typically a lowering of heart rate during therapy, which, in some individuals, may contribute to the overall antihypertensive effect. α_2 A-ARs expressed in the central nervous system provide important regulation of peripheral sympathetic output, and α_2 -AR agonists such as clonidine decrease blood pressure (BP) through these central α_2 A-ARs. α_2 -ARs are also expressed on presynaptic sympathetic peripheral nerve endings, and norepinephrine released within the cleft acts through a negative feedback mechanism to inhibit subsequent release.⁷ Consequently, the status of these cardiac presynaptic nerve α_2 -ARs (α_2 A- and α_2 C-AR subtypes) indirectly affects responsiveness to β -blockers, given that

these α_2 -ARs partially control the release of the endogenous agonist for β_1 -AR. Presynaptic β_2 -ARs have also been described, and these appear to promote the release of norepinephrine.⁸

GENETIC POLYMORPHISMS OF THE ARS

The first discrete, nonsynonymous polymorphism of any GPCR was described in 1993, when the β_2 -AR was shown to have several single-nucleotide polymorphisms (SNPs).⁹ Subsequent studies showing that these SNPs have functional effects were published in 1993 and 1994, spurring the publication of a substantial number of reports over the years related to variations in this receptor and other GPCRs and clinical association studies related to disease risk, disease modification, or predictors of drug response.¹⁰ Since then, nonsynonymous polymorphisms have been defined for genes encoding most of the ARs.

The common polymorphisms of the ARs are shown in Table 1, with approximate allele frequencies in persons of European descent and African descent in the United States. A concerted effort has been made by a number of groups to study the molecular consequences of these polymorphisms, using various techniques including recombinant expression, generation of genetically modified mice, and function or expression experiments in human tissues.¹⁰ Besides providing additional support for the association studies, these molecular studies provide insight into how one may potentially circumvent a genetics-based differential response by using other available drugs and point to new approaches to therapy that may be less vulnerable to genetic variations.

Genetic polymorphisms of the β_1 -AR

The β_1 -AR has two commonly variable sites, at positions 49 and 389. At the latter position, Arg or Gly may be found, with Arg being more common in individuals of European descent, and Gly occurring with approximately the same frequency as Arg in those of African descent. This polymorphism is within the intracellular eighth helix, which is known to be involved in coupling to $G_{\alpha s}$. In membrane adenylyl cyclase assays from recombinantly expressing cells, Arg389 had greater basal and agonist-stimulated activity as compared with Gly389, consistent with the concept of enhanced coupling (Figure 2a).¹¹ This phenotype gave us the initial idea that, in a clinical setting, those with the Arg389 receptor may be more responsive to β -blockers, given that there is more “room” to move from the higher activity of Arg389 to some lower level. Several additional studies in recombinant cells, as well as in transgenic mouse and human hearts, showed that this interaction is more complex. First, we showed that Arg389 underwent a greater degree of agonist-promoted desensitization relative to Gly389 in recombinant cells.¹² These results indicated that the conformation of Arg389 is more favorable for phosphorylation by GRKs and is consistent with the notion that the more “active” coupling conformation is also the most favorable for GRK-mediated desensitization. Therefore, the phenotype of Arg389 appeared to have the capacity to undergo greater modification under conditions of elevated levels of catecholamines.

In order to ascertain the potential physiologic significance in relation to the heart, transgenic mice were generated using the α -myosin heavy chain promoter overexpressing the human Arg or Gly389 receptor on cardiomyocytes to an equal extent.¹³ Using a perfused *ex vivo* model, hearts were studied at baseline and in response to the agonist isoproterenol. Contractility (+dP/dt) and heart rate were the primary outcomes. In 3-month-old mice, baseline contractility was slightly elevated as compared with Gly389, and the maximal stimulated contractility was also increased by ~30% (Figure 2b). Heart rate at baseline and for several concentrations of the agonist were also higher in Arg389 mice but, at maximally tolerated doses, appeared to converge with those in Gly389 mice. In the same model,

Arg389 mice showed a marked decrease in $+dP/dt$ with infusion of propranolol, which was not observed in Gly389 hearts (Figure 2c).¹³ With chronic dosing of propranolol in the drinking water, only Arg389 mice showed a decrease in heart rate as determined by echocardiography (Figure 2d).

These data were the first to suggest that these β_1 -AR polymorphisms may alter the effectiveness of β -blocker therapy in a physiologic setting. In normal hearts from Arg389 individuals, a substantially greater contractile force was generated by agonists as compared with hearts from Gly389 carrier individuals (Figure 2e). The same phenotype was also observed in failing hearts, although the difference between the force generated by hearts from Arg389 individuals vs. those from Gly389 was less than in normal hearts (Figure 2f). This is consistent with a dampening of the effect size of the Arg389, but there was no evidence of an actual switch between the phenotypes of Arg389 and Gly389 in progressive heart failure such as has been observed in transgenic mouse models.^{13,14}

In the amino terminus of the β_1 -AR lies a polymorphism at amino acid position 49, where Ser (the major allele) or Gly can be found.¹⁵ This region of the receptor is not involved with agonist binding or coupling to G_s ; however, in analogy with some other GPCRs, it may play a role in membrane insertion, expression, trafficking, and agonist-promoted downregulation. In our laboratory, we expressed the two polymorphic versions of the human β_1 -AR on the basis of the position 49 SNPs in human embryonic kidney 293 cells and Chinese hamster fibroblasts and found no differences in agonist-promoted stimulation of adenylyl cyclase activity. Levin *et al.*¹⁶ performed similar experiments in human embryonic kidney 293 cells, and reported increased basal and agonist-promoted adenylyl cyclase activities in the Gly49 variant, although the mechanism for this unexpected finding remains unclear. Both groups noted an enhanced agonist-promoted downregulation of the Gly49 receptor.^{15,16} Given that the downregulation phenotype has been demonstrated by two independent groups, it is probable that the Gly49 receptor will alter cardiovascular responses to β -blockers because of its enhanced downregulation status.

Polymorphisms of the α_{2C} -AR

Although β_1 -ARs are the primary targets of β -blockers in the treatment of heart failure, cardiac ischemia, and hypertension, the source of catecholamines for stimulating these receptors is the cardiac presynaptic sympathetic nerve. As mentioned, α_{2A} -ARs, α_{2C} -ARs, and β_2 -ARs are expressed on the cardiac presynaptic nerve endings, with the α_2 -ARs acting to inhibit norepinephrine release.⁷ The most extreme phenotype¹⁷ of the polymorphisms within these receptor genes is found to carry the α_{2C} -Del322–325. This receptor has an in-frame deletion of 12 nucleic acids, with a loss of Gly-Ala-Gly-Pro within the third intracellular loop of the receptor. In recombinant cells expressing wild-type α_{2C} -Del322–325 at equivalent levels, agonist-promoted coupling to inhibition of adenylate cyclase was severely blunted with the deletion mutant (Figure 3).¹⁷ Two other intracellular consequences of α_{2C} -AR activation—inositol triphosphate production and extracellular signal-regulated kinases 1/2 phosphorylation—were also severely impaired in the presence of the α_{2C} -Del322–325 receptor.¹⁷ Taken together, this phenotype points toward a potential loss of regulation of norepinephrine release in humans with this α_{2C} -AR polymorphism, leading to enhanced norepinephrine release in these individuals and a greater potential for β_1 -AR stimulation. Because of these findings, the α_{2C} -Del322–325 polymorphism was considered to be a potential pharmacogenomic locus for predicting the response to β -blockers.

Polymorphisms of the β_2 -AR

The β_2 -AR polymorphisms may be more applicable to β -agonist treatment such as for asthma,¹⁸ but these receptors are expressed on virtually all cell types including

cardiomyocytes and vascular smooth muscle, and certain β -blockers are not selective for the β_1 -AR subtype. Three nonsynonymous polymorphisms have been shown to exist in the coding region of the β_2 -AR, at amino acid positions 16, 27, and 164.¹⁰ The latter polymorphism is rare (Ile164, heterozygous frequency ~0.03), but agonist-promoted coupling to stimulation of adenylate cyclase is markedly lower in Ile164-expressing cells as compared with wild-type (Thr) ones.¹³ This uncoupling was also demonstrated in transgenic mice in the form of a depressed agonist-promoted cardiac contractility in Ile vs. Thr164 hearts.¹⁰ Therefore, this severe phenotype may have importance in a small number of subjects. The common β_2 -AR polymorphisms (Arg or Gly at 16; Gln or Glu at 27) are in the amino terminus and appear to affect agonist-promoted loss of receptor expression (downregulation).¹⁹ In the original report,¹⁹ all four possible haplotypes were studied in recombinant cells: Gly16/Glu27, Gly16/Gln27, Arg16/Gln27, and Arg16/Glu27. However, the latter haplotype is rarely, if ever, found.²⁰ Of the three main haplotypes, the two receptors with the Gly16 allele (regardless of the position 27 genotype) were found to undergo enhanced agonist-promoted downregulation as compared with Arg16/Gln27.¹⁰ For this genotype, (under the conditions of a stably transfected cell line, and using an artificial promoter), downregulation is associated with the allele at position 16, with the allele at position 27 being irrelevant. However, SNPs in the 5' promoter region and the 3' untranslated region of the intronless β_2 -AR gene also have an impact on baseline expression or agonist-promoted downregulation.²¹ Therefore, genotyping with haplotype-tagging SNPs (which might include position 27) may be useful to further refine predictive value. Indeed, Glu at position 27 uniquely identifies β_2 -AR haplotype II-1, which includes several unique SNPs in the 5'-flanking region and 7Cs in the poly-C tract of the 3' untranslated region.²¹ This haplotype displays a relatively higher baseline expression and less agonist-promoted downregulation than other haplotypes when studied using a whole-gene transfection system.²¹ Taken together, β_2 -AR SNPs at amino acid positions 16 and 27 (or nucleotide positions outside the open reading frame), have potential direct or indirect predictive power with regard to variability in receptor function or response to therapy.

Polymorphisms of the GRKs

As mentioned, GRKs act to "quench" agonist-promoted GPCR signaling; thus, a gain-of-function phenotype for GRK activity results in a loss of function for a given GPCR (and *vice versa*). The gene encoding the prototypic and widely expressed GRK isoform GRK2 has undergone extensive resequencing, and no common nonsynonymous polymorphisms have been found in its coding region.²² GRK4 was initially found to be expressed exclusively in the testis; subsequent studies have suggested that it may also be expressed in other tissues such as renal proximal tubules.²³ However, the absence of GRK4-specific antibodies and the presence of multiple splice variants have made it difficult to localize the expression of this kinase as compared with the more widely distributed GRKs 2, 3, 5, and 6. Three nonsynonymous coding polymorphisms have been identified in GRK4 (Table 1). Functional characterization of these polymorphic forms has been limited to potential interactions with the D1-dopamine receptor, wherein certain combinations of SNPs resulted in enhanced D1-mediated cyclic adenosine monophosphate accumulation (although these were not necessarily related to D1-receptor phosphorylation).²⁴

In the coding region of GRK5, there is one nonsynonymous polymorphism at amino acid position 41, where the major allele results in Gln and the minor encodes for Leu.²² This polymorphism is localized to the amino terminus near a calmodulin binding domain. Leu41 is almost exclusively found in individuals of African descent (Table 1). In a transfected cell model (Figure 4a), the Leu41 variant suppressed β_1 -AR signaling to cyclic adenosine monophosphate, indicating that this variant is a gain of function, potentially acting as a "genetic β -blocker." Transgenic mice were generated expressing Gln and Leu41 GRK5 in

myocytes and were studied in the context of a mouse model of heart failure (isoproterenol infusion over 6 days). In Gln41 mice, left ventricular end diastolic dimension increased with isoproterenol infusion, indicative of a dilated cardiomyopathy (Figure 4b). When the β -blocker propranolol was included in the drinking water, cardiomyopathy was completely blocked, as expected. In contrast, only a small increase in left ventricular end diastolic dimension was observed in Leu41 mice, and there was no effect of β -blockade (Figure 4b). This suggested that the gain-of-function GRK5-Leu41 polymorphism partially blocked the effect of isoproterenol infusion, thereby protecting the heart from β_1 -AR-mediated cardiomyopathy. The lack of β -blocker effect is consistent with a “common” mechanism, that is, attenuation of β_1 -AR function.

ADRENERGIC RECEPTOR SIGNALING, GENETIC POLYMORPHISMS, AND CLINICAL DRUG RESPONSES

There are numerous studies in the literature describing associations of *ADRB1*, *ADRB2*, *ADRA2B*, *ADRA2C*, and other ARs or their signaling/regulatory proteins with risk of cardiovascular disease. Although there appears to be some consistency in the reports, genome-wide association studies do not indicate consistent replications of associations for any of these genes. Here, we focus on the pharmacogenomic studies of these genes. We have specifically selected these genes for focus because of the body of literature around them and the availability of replicated findings. As noted above, the vast majority of the cardiovascular pharmacogenomics studies of this signaling cascade have been with β -blockers in the treatment of heart failure or hypertension, and we therefore focus on that literature.

β -BLOCKER PHARMACOGENOMICS IN HEART FAILURE

Given their substantial survival benefits, β -blockers are considered essential therapy in all patients with heart failure who have systolic dysfunction and in whom there is an absence of any contraindication for these drugs.²⁵ Because the primary goal of therapy is prevention of disease progression and death, it is impossible to assess efficacy in an individual patient. There are, however, measurable phenotypes, including β -blocker-induced change in left ventricular performance and anatomic indexes as assessed by echocardiography or other means, that exhibit substantial inter-patient variability and result in outcomes ranging from worsening to substantial improvement. Although such measurements are “snapshots in time,” a substantial body of literature correlates improvements in ventricular indexes with clinical outcomes. Together with the variable outcome responses observed in clinical trials, it is readily apparent that for any given patient, the response to β -blockers in terms of survival prolongation remains unpredictable. Because these agents also reduce BP and heart rate, their use may preclude, or make it difficult to titrate, other drugs, or they may be associated with nontrivial adverse effects. There is therefore potential clinical benefit in identifying patients in whom β -blockers might be (i) of maximal benefit, so that other drugs are “adjusted around” maintenance of the β -blocker (i.e., moderate and extremely good responders), and (ii) of limited value so that alternative therapies could be attempted. It is recognized, however, that a substantial level of evidence would be needed to shift from the paradigm of “ β -blockers for all.”

The β -blocker class includes drugs that are relatively selective for the β_1 -AR, nonselective (block both β_1 -AR and β_2 -AR), and/or possess other ancillary properties. As noted, in the case of β -blockers in heart failure, it seems clear that the most important pharmacological property is β_1 -AR blockade, although it is possible that other properties (e.g., β_2 -AR blockade, vasodilator effects) might be important as well.

A large number of studies have been conducted on adrenergic receptor and signaling/regulatory proteins relative to β -blocker treatment in heart failure. These studies can be broadly categorized into two groups: those focused on β -blocker-induced change in left ventricular ejection fraction (LVEF) and those focused on heart failure–related outcomes, including death, heart transplantation, and hospitalization for heart failure.

β -Blocker pharmacogenomics in heart failure: studies of LVEF

Studies of β -blocker-induced changes in LVEF are summarized in Table 2. The majority of studies have focused on *ADRB1* SNPs; there have been a few on *ADRB2* and one on *ADRA2C*. The findings from these studies are mixed. Three studies have suggested an association, with greater LVEF improvements in *ADRB1* Arg389Arg patients; however, two other studies, including the largest, a randomized controlled trial, showed no such association. The drugs studied include carvedilol, metoprolol, bisoprolol, and bucindolol, each of which differs from the others with respect to certain pharmacological properties. Whether the lack of consistent replication across all the studies is due to differences in the drugs studied, differences in the patient populations (e.g., the percentage with nonischemic heart failure tend to show greater improvements in LVEF), or the absence of a true genetic effect is not known.

Studies of *ADRB2* have generally been negative (or not tested because the study's focus was on a β 1-selective blocker), and the only reported *ADRA2C* study suggested greater LVEF improvement in Del322–325 carriers treated with metoprolol controlled release/extended release.²⁶ It is of interest to note that this association is in contrast with the *ADRA2C* findings for bucindolol (see below), in which Del322–325 carriers had no benefit from bucindolol as compared with placebo. However, these differences may be attributable to the pharmacological differences between metoprolol and bucindolol. With metoprolol, the Del322–325 carriers are likely to experience a greater release of norepinephrine, making β 1-AR blockade with metoprolol beneficial. In contrast, bucindolol has the ancillary property of sympatholysis, which has been shown in other studies to be detrimental in heart failure; this was shown to be more marked with bucindolol treatment in Del322–325 carriers than in noncarriers.²⁷

β -Blocker pharmacogenomics in heart failure: studies of cardiovascular outcomes

More studies, with larger cohorts, have investigated heart failure–related outcomes than have examined LVEF outcomes. These studies, summarized in Table 3, have focused on *ADRB1*, *ADRB2*, *ADRA2C*, and *GRK5*. Although a quick review of Table 3 may suggest that the literature here is also discrepant, a more careful review shows that this is not the case. Consistent with the LVEF studies and the *in vitro*, *ex vivo*, and transgenic mouse studies, several studies that investigated outcomes suggested that patients with *ADRB1* Arg389Arg derive the greatest benefit from β -blocker treatment,^{28–30} whereas other studies did not reveal this association.^{31–33} Data also suggest significant differences in β -blocker-related outcomes, depending on *GRK5* and *ADRA2C* genotype.

What is striking about the positive-vs.-negative studies is the approach to analysis. In all the studies in which outcomes were compared among genotypes (i.e., Arg389Arg vs. Arg389Gly vs. Gly389Gly) in β -blocker-treated patients, no significant associations have been noted (with the exception of an *ADRB2* study). In contrast, all the positive associations with *ADRB1*, *ADRA2C*, and *GRK5* arise from analyses in which β -blocker-treated patients are compared with untreated (or low-dose-treated; <50% target) individuals within a genotype. These findings are consistent with the polymorphism in question having an effect on outcomes (i.e., a risk allele, or main effect) and the β -blocker reducing the risk associated with that allele. There are several lines of evidence to support the hypothesis that in heart

failure, patients with *ADRB1* Arg389Arg,^{28,34} *ADRA2C* Del322–325,³⁴ and *GRK5* Gln41Gln²² genotypes have worse outcomes.

That β -blockers might reduce the risk inherent in carrying these alleles and move outcomes toward those related to lower-risk alleles is not surprising, and there is support by data from several studies. In each case, these findings are also consistent with findings from the various mechanistic studies described above. This effect is perhaps best highlighted by data from the *GRK5* study, which shows that patients with Gln41Gln derived significant survival benefit from β -blockers, that carriers of Leu41 derived no such benefit, and that β -blocker-treated patients with Gln41Gln had survival similar to that of Leu41 carriers who did not receive β -blocker treatment (Figure 4c–e). Importantly, several of the positive association studies provide clear evidence that if β -blocker-treated patients alone were studied for differences in outcomes by genotype, no effect would have been evident.^{14,22,30,31} Statistically, this represents a genotype-by-treatment interaction, which is detectable only if there are contrasting treatments (or no treatment, or placebo) for comparison.

The suggestion that pharmacogenomic effects in heart failure are evident only by comparison against absence of therapy or low doses could have important implications for future targeting of therapy. For example, the replicated data for *GRK5* suggest that Leu41 carriers derive little benefit from β -blocker treatment, but this does not mean they do not experience any adverse effects or that the β -blocker does not limit the use of other therapies because of its BP-lowering and heart rate-lowering effects. This is an example in which Leu41 carriers might be better served by treatment with therapies other than a β -blocker. Substantial additional evidence would be needed to withhold β -blocker therapy from these individuals, but these data highlight the potential clinical advantages implicit in identifying those likely to derive the greatest benefit from a given therapy.

In summary, the literature suggests that, when compared with those treated with a low-dose β -blocker or no β -blocker at all, carriers of *ADRB1* Arg389Arg and *GRK5* Gln41Gln derive the greatest survival benefit from β -blocker treatment. Also, perhaps because of the unique sympatholytic effects of bucindolol, carriers of *ADRA2C* Ins322–325Ins also derive greater benefit. The flip side of the coin is that those with the other genotypes may derive minimal benefit from β -blocker therapy and might be better served with alternative treatment approaches.

β -Blocker pharmacogenomics in hypertension

Numerous drug classes are available for the treatment of hypertension; of these, five are considered appropriate first-line therapy in the United States.³⁵ Specific drug selection is usually empirical, although race and age are sometimes considered. β -Blockers are among the first-line therapy drug classes in the US consensus guidelines for complicated and uncomplicated hypertension, whereas in Europe and elsewhere, they have recently been downgraded from first-line therapy for uncomplicated hypertension. It remains to be seen whether they will be similarly downgraded for uncomplicated hypertension in the upcoming updates to the US consensus guidelines. Like all antihypertensives, β -blockers exhibit substantial variability in efficacy. On average, random selection of drugs from any of the antihypertensive classes leads to adequate BP lowering in about 50% of patients.³⁶ However, clinical crossover design studies have shown that sequential monotherapy leads to response rates as high as 73%.³⁶ These data suggest that hypertension can be controlled in ~75% of patients by means of monotherapy if clinicians could easily identify the best drug for a specific patient and highlight the potential for pharmacogenomic-guided drug therapy selection.

In contrast to β -blockers in heart failure, for which properties beyond β_1 -AR blockade might be beneficial, the bulk of the antihypertensive effect of β -blockers is believed to derive from β_1 -AR inhibition, given that β_1 -AR-selective and nonselective β -blockers have similar antihypertensive efficacies. It is not surprising, therefore, that the majority of pharmacogenomics studies in hypertension center on the nonsynonymous *ADRB1* SNPs, *ADRB1* and *ADRB2*. Several studies, some involving healthy volunteers and others involving patients with hypertension, have tested the association of the *ADRB1* Ser49Gly and Arg389Gly on the heart rate- and BP-lowering effects of β -blockers, and many of these have been previously summarized.³⁷ Consistent with the *in vitro* and *ex vivo* data and the heart failure association data summarized above, several studies have shown that the more active forms of the β_1 -AR (Ser49 and Arg389) are associated with more pronounced BP lowering. Two small but carefully conducted prospective studies involving patients with hypertension had nearly identical findings, showing that Ser49 homozygotes and Arg389 homozygotes experienced greater BP-lowering effects with metoprolol than did the carriers of other variants, with the magnitude of the effect being greater for the codon 389 than for the codon 49 SNP.^{38,39} It is interesting to note that one of these studies was conducted in the United States and the other in China; this suggests a consistency of effect across populations. However, findings from other studies, including two recent, larger prospective studies from Europe did not observe a significant association with *ADRB1* genotype, although in one of these, Ser49Ser homozygotes had numerically greater responses, with each Gly49 allele leading to a lesser BP response.^{40,41} Whether the lack of association in these studies was due to differences in study design, the drugs and/or doses investigated, differences in how the BP response was measured, or lack of a strong genetic effect of *ADRB1* on BP response to β -blockers is unclear. Therefore, the literature at present is not completely clear with respect to associations between *ADRB1* genotype and BP lowering with β -blockers. Like the literature related to heart failure, all the studies with positive associations show the Arg389Arg genotype (and in some studies, Ser49Ser or the haplotype) to be associated with the greatest extent of BP lowering. *ADRB2* was studied in some of the studies, and overall the data do not suggest an important association between *ADRB2* genotype and antihypertensive response. This is perhaps not surprising, given that nearly all the β -blockers tested in hypertension pharmacogenomics studies are β_1 -selective blockers.

The literature on associations with BP-lowering is somewhat mixed, but the majority of the literature suggests no association between the *ADRB1* SNPs and the heart rate response to β -blockers.³⁷ Although somewhat surprising, these findings are consistent with other studies, which predominantly show no association between *ADRB1* genotype and heart response to β -agonist administration or treadmill exercise.

Two studies have tested the association of the *ADRB1* and *ADRB2* SNPs with cardiovascular outcomes in patients with hypertension receiving treatment with β -blockers. In the genetics substudy of INVEST, a randomized, controlled clinical trial of atenolol vs. verapamil in patients with hypertension plus coronary artery disease, nearly 6,000 patients were genotyped for the *ADRB1* and *ADRB2* nonsynonymous SNPs. *ADRB1* Ser49/Arg389 haplotype carrier status was associated with a main effect of an increased risk of the primary outcome (death, nonfatal myocardial infarction, or nonfatal stroke), driven by a greater-than-threefold risk for death.⁴² Evaluation of the death outcome by treatment showed that the risk associated with this allele was attenuated by treatment with atenolol but not with verapamil. Specifically, as shown in Figure 5, there was significant protective effect with atenolol (vs. verapamil) in carriers of Ser49-Arg389 (hazard ratio 0.64; 95% confidence interval 0.41–0.98) but no differences in outcome by treatment in the noncarriers (hazard ratio 1.51; 95% confidence interval 0.27–8.51). These findings are consistent with the association with BP response and the literature related to heart failure, which suggest greater benefit with β -blockers in carriers of Ser49-Arg389 (or Arg389 alone). Also, as highlighted above in the

context of heart failure (and as evident in Figure 5), there were no differences in outcome by genotype in the atenolol-treated individuals; the pharmacogenomic effect was evident only through comparisons within genotype of those treated with β -blocker vs. a contrasting therapy. Specifically, as shown in Figure 5, there were no differences by genotype among atenolol-treated individuals (groups B and C in Figure 5), but Ser49Gly389 carriers treated with verapamil SR (group D) had significantly worse outcomes than the same haplotype group treated with atenolol (group C). Again, this suggests the Ser49-Arg389 is a risk-associated allele for adverse outcomes, with the risk attenuated by β -blocker treatment. This is fully consistent with the literature on heart failure.

This study also found a significant haplotype-by-treatment interaction for *ADRB2*, with increasing copies of the Gly16-Glu27-nt523C being associated with increased risk from treatment with atenolol as compared with verapamil.⁴²

The other data regarding outcomes arise from a case-control study in a large health maintenance organization database.⁴³ The study took a tag SNP approach and also observed significant SNP (or haplotype)-by-treatment interactions for both *ADRB1* and *ADRB2*, although the SNPs were not the common nonsynonymous SNPs reported in other studies. Again, these genetic effects were evident through comparison of β -blocker vs. an alternative treatment.

Overall, the data suggest that *ADRB1* SNPs may influence the antihypertensive response to β -blockers, along with influencing treatment-related outcomes. *ADRB2* SNPs do not appear to be associated with the antihypertensive response but may influence outcomes associated with β -blockers vs. alternative treatments for hypertension. As noted earlier, certain consensus guideline committees outside the United States have recently removed β -blockers from first-line therapy for uncomplicated hypertension because of doubts about the effects on long-term outcomes. The data described suggest that genetic polymorphisms influence efficacy; it is therefore possible that it is in certain genetic groups that β -blockers represent a highly effective antihypertensive therapy, whereas there are minimal benefits to long-term outcomes in those with other genotypes.

Other genes in the adrenergic signaling pathway

Associations with other genes in the adrenergic receptor signaling pathway have been tested, including *GNAS1*, *ADRA2C*, *GNB3*, and *GRK4*; in some cases, there is a single report of a significant association with BP response but no instances of replication of these findings.³⁷ In general, the studies involving these genes are limited in number, and the sample sizes are usually small; it is therefore not clear whether their genetic variants might influence antihypertensive response to β -blockers.

FUTURE DIRECTIONS

A careful review of the literature related to β -blocker pharmacogenomics in hypertension and heart failure leads to several important conclusions. First, intermediate phenotypes (e.g., LVEF improvement in heart failure and BP lowering in hypertension) have been associated in some but not all studies, with greater benefit from β -blockers in individuals with *ADRB1* Arg389Arg (or homozygous Ser49/Arg389 haplotypes). The literature also suggests that *ADRB2* SNPs are not important to these intermediate phenotype responses. Other genes have not been sufficiently studied to draw conclusions about associations with these phenotypes.

Perhaps more important is the high degree of consistency in the literature related to outcomes in both heart failure and hypertension. Specifically, when treatment-related

outcomes are compared within genotype against contrasting treatments, there is evidence of pharmacogenomic effects in patients with *ADRB1* Arg389Arg, *GKR5* Gln41Gln, and *ADRA2C* Ins322–325Ins genotypes, who have the greatest benefit from β -blockers. Seven studies (five in heart failure,^{27–30} two in hypertension)^{42,43} analyzed in this manner show the presence of significant pharmacogenomics effects, whereas there are no studies analyzed for these genes in this manner that do not show a significant pharmacogenomic effect. In contrast, numerous studies suggest that when only β -blocker-treated patients are considered across different genotypes, there is no evidence for a pharmacogenomic effect.

That the literature suggests pharmacogenomic effects might be evident only when contrasting treatments are compared within one genotype could have important implications that extend well beyond β -blockers and cardiovascular disease. First, these data suggest that to rule out a pharmacogenomic effect, particularly for genes involved in the pharmacological pathway (vs. pharmacokinetic effect through drug metabolism or drug transport), it may be essential to design studies such that data on contrasting treatments (or placebo) are available for analysis. This is likely to be most important when a main effect is present but could also occur in the absence of a main effect, as we have seen in other hypertension pharmacogenomics studies. Second, it highlights how carefully the literature must be analyzed when making conclusions about the clinical relevance of pharmacogenomic targets. Finally, this suggests that there are certain genotype groups who may derive minimal benefit from a given therapy. This could lead to therapy guided by genetic information, which is the ultimate goal of pharmacogenomics research.

In considering the next steps, several issues need to be considered. First, in terms of the individual genes for the aforementioned ARs, there are multiple polymorphisms in their promoter, 5' untranslated region, and 3' untranslated region that have been shown to have an impact on receptor expression in recombinant expression systems in which whole genes were transfected with the various polymorphisms present in the combinations found in the population (i.e., haplotypes). In many cases, individual polymorphisms are in low linkage disequilibrium with others in the gene and are therefore poor surrogates for each other and for the haplotype. No study has addressed this issue to date, probably because of the partitioning of the sample sizes into smaller bins and the burden of genotyping. Several analytical approaches have evolved to address the sample-size issue, and it seems prudent to further analyze haplotypes in addition to individual variants. In addition, *in vitro* and physiologic studies support the notion that polymorphisms of several genes within a pathway could act in specific ways to alter phenotype. These could include additivity, synergism, and neutralization. Therefore, knowledge of a variant (or haplotype) in a single gene could have little or no predictive power until it is considered in the context of the effect of another gene on variability. A multigene approach seems to be the next logical step and could be approached using selected genes within a known pathway so as to minimize the issue of multiple comparisons.

It is also clear that basic studies of signal transduction in reductionist systems (such as transfected cells or genetically modified mice), human-derived tissues, and human physiological studies, can lead to insights into potential mechanisms and can deliver new biological insights relevant to drug response. It is well recognized that the results from these types of studies may not correlate with a global clinical outcome, consistent with the notion that we do not know all that we think we do about a given disease or the mechanism of action of a drug. However, the studies will lead to additional insights. Finally, there is a need for more prospective and adequately powered clinical trials. We urge consideration of primary and secondary end points as important outcomes so as to maximize the gain from such trials. We also suggest that add-in polymorphisms be included without the antiquated notion of requiring a sacrosanct *a priori* set of hypotheses prior to enrollment of the first

patient. In essence, we propose that as the tools at the level of discovery, genotyping, high-throughput compound screening, and statistical analysis have undergone a revolution, so should the trial design and approval processes, so that personalized medicine can realize its full potential.

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Figure 1.

Schematic of adrenergic receptor (AR) signaling in the heart. For clarity, only the classic signal transduction pathways are shown, but there are other events from adrenergic signaling that occur through G-protein and non-G-protein interactions (see text). The dual coupling of β₂-AR to G_s and G_i is shown in the myocytes but not in the presynaptic neuron. AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; EP, epinephrine; GRK, G-protein-coupled receptor kinase; IP₃, inositol triphosphate; NE, norepinephrine; PLC, phospholipase.

Figure 2.

Signaling properties of the position 389 β_1 -adrenergic receptor (AR) polymorphism. **(a)** In transfected fibroblasts, the β_1 -Arg389 receptor displays increased agonist-promoted stimulation of adenylyl cyclase as compared with the β_1 -Gly389 receptor. **(b)** In transgenic mice expressing the two human polymorphic β_1 -ARs on cardiomyocytes, baseline and agonist-promoted contractility (first derivative of the positive change in pressure, $+dP/dt$) are greater in β_1 -Arg389 hearts as compared with β_1 -Gly389 hearts. **(c)** β_1 -Arg389 hearts have a greater response (decrease in contractility) to acute infusion of the β -blocker propranolol as compared with β_1 -Gly389 hearts. **(d)** Chronic administration of propranolol for 1 month decreased heart rates as determined by echocardiography in β_1 -Arg389 mice but not in β_1 -Gly389 mice. **(e)** Results from explanted normal hearts stratified by homozygous Arg389 or Gly389 carrier status (at least one Gly allele). Data were acquired in an *ex vivo* model using right ventricular trabeculae. Arg389 hearts showed a greater agonist-promoted contractile response than hearts with the other genotypes. **(f)** Results from similar experiments performed with end-stage failing hearts obtained at the time of cardiac transplantation. The enhanced contractility of Arg389 hearts is apparent from the left shift in the dose-response curve and the greater maximal response as compared with Gly389 carrier hearts, although the magnitude of the differences in maximal response is less than in normal hearts. From refs. 11, 13, and 14 with permission.

Figure 3. Signaling phenotype of the α_{2C} -Del322–325 polymorphism. Human wild type and the Del322–325 α_{2C} -AR were expressed to equivalent levels in Chinese hamster ovary cells. **(a)** Agonist-promoted inhibition of forskolin-stimulated adenylyl cyclase activity in cell membranes was impaired in the deletion receptor. **(b)** α_2 -AR agonist (epinephrine; Epi)-stimulated phosphorylation (P) of extracellular signal-regulated kinases 1/2 is depressed in cells expressing α_{2C} -Del322–325 as compared with wild-type (WT) α_{2C} -AR, whereas thrombin (Thr)-stimulated phosphorylation response and the non-phosphorylated (NP) forms are not different. From ref. 17 with permission.

Figure 4.

G-protein-coupled receptor kinase (GRK) 5-Leu41 properties in transfected cells and transgenic hearts. **(a)** Agonist-promoted cyclic adenosine monophosphate (cAMP) production over time by β_1 -AR is dampened by coexpression of GRK5-Leu41 or -Gln41, but the greatest desensitization is observed with the Leu41 variant. **(b)** Protection from heart failure and lack of β -blocker effect in transgenic mice expressing GRK5-Leu41. Mice expressing equivalent levels of the two GRK variants were treated with isoproterenol (ISO) (or saline control) by implanted mini-pump for 6 days in the absence or presence of the β -blocker propranolol in the drinking water. Gln41 mice displayed a pathologic increase in the cardiac left ventricular end diastolic dimension (LVEDD) in response to ISO (as shown by the red arrow), which was blocked by propranolol (green arrow). In contrast, Leu41 mice had a nonsignificant increase in LVEDD from ISO (red arrow) and no change in dimension with concomitant β -blockade (green arrow). **(c–e)** Kaplan–Meier curves for time from diagnosis of heart failure to death or cardiac transplantation. **(c)** Comparison of GRK5-Q41-only subjects with and without β -blocker use; **(d)** comparison of GRK5-L41-only subjects with and without β -blocker use; **(e)** comparison of GRK5-Q41-only subjects treated with β -blockers and GRK5-L41 carrier subjects without β -blocker use. From ref. 22 with permission.

No. at risk

Non-S49-R389 + AT	626	624	624	624	623	494	324	135	78
Non-S49-R389 + VE	653	652	651	651	651	508	337	131	63
S49-R389 + AT	2,116	2,096	2,096	2,095	2,091	1,669	1,125	454	243
S49-R389 + VE	2,235	2,229	2,228	2,224	2,211	1,737	1,169	478	234

Figure 5.

All-cause mortality and mean on-treatment blood pressure by *ADRB1* Ser49-Arg389 haplotype and atenolol/verapamil sustained-release (SR) therapy. S49-R389–Ser49-Arg389 haplotype. AT, atenolol, DBP, diastolic blood pressure; SBP, systolic blood pressure; VE, verapamil SR. From ref. 42.

Table 1

Coding region variations of adrenergic signaling genes

Gene name	Common name	Nucleotide variability ^a	Amino acid variability ^a	MAF (%) ^b	
				ED	AD
<i>ADRB1</i>	β_1 -AR	145 (A/G)	49 (Ser/Gly)	15	13
		1,165 (C/G)	389 (Arg/Gly)	27	42
		1,166 (G/T)	389 (Arg/Leu)	<0.1	0.9
<i>ADRB2</i>	β_2 -AR	46 (G/A)	16 (Gly/Arg)	40	50
		79 (C/G)	27 (Gln/Glu)	43	27
		491 (C/T)	164 (Thr/Ile)	<4	<4
<i>ADRB3</i>	β_3 -AR	387(T/C)	64 (Trp/Arg)	20	15
<i>ADRA1A</i>	α_{1A} -AR	1,039 (C/T)	347 (Arg/Cys)	57	30
<i>ADRA2A</i>	α_{2A} -AR	53 (C/T)	251 (Asn/Lys)	0.4	40
<i>ADRA2B</i>	α_{2B} -AR	901-909	INS/Del301-303	31	12
<i>ADRA2C</i>	α_{2C} -AR	964-975	INS/Del322-325	3.8	40
<i>GRK4</i>	GRK4	448 (G/T)	65 (Arg/Leu)	35	47
		679 (C/T)	142 (Ala/Val)	40	60
		1,711 (C/T)	486 (Ala/Val)	40	19
<i>GRK5</i>	GRK5	122 (A/T)	41 (Gln/Leu)	1.3	23

AD, African descent; AR, adrenergic receptor; ED, European descent; MAF, minor allele frequency.

^aThe most common allele in the general US population (including 15% African Americans) is the first listed.

^b Allele frequencies as reported in the literature and public databases show some variance, probably due to differences in racial admixture.

β -Blocker pharmacogenomics studies of adrenergic signaling genes in heart failure patients: associations with improvements in left ventricular ejection fraction

Table 2

Study type	β -Blocker	N	Duration	Gene(s) studied	Outcome(s)	Results	P value	Ref.
R	Carvedilol	224	>6 Months	<i>ADRB1</i>	Δ LVEF	Arg389Arg carriers showed greater improvement in LVEF as compared with Gly carriers ($8.7 \pm 1.1\%$ vs. $0.93 \pm 1.7\%$)	0.02	13
P	Metoprolol CR/XL	54	>5 Months	<i>ADRB1</i> , <i>ADRA2C</i>	Δ LVEF	<i>ADRB1</i> Arg389Arg carriers showed greater improvement in LVEF than Gly carriers (from $23 \pm 5\%$ to $29 \pm 10\%$ vs. from $22 \pm 9\%$ to $23 \pm 11\%$). <i>ADRA2C</i> Del322-325 carriers showed greater improvement in LVEF than Ins/Ins (+6 vs. +1 EF units). Considering <i>ADRB1</i> and <i>ADRA2C</i> together, Arg389Arg/Del carriers showed the greatest improvement in EF	0.008 0.025 0.0007	44 26
P	Bisoprolol Carvedilol	199	3 Months	<i>ADRB1</i> <i>ADRB2</i>	Δ LVEF	No associations with <i>ADRB1</i> or <i>ADRB2</i> genotype	NS	45
RCT	Bucindolol	1,040	Median 2 years	<i>ADRB1</i>	Δ LVEF	No associations with <i>ADRB1</i> genotype	NS	14
P/R	Carvedilol	135	>1 Year	<i>ADRB1</i> , <i>ADRB2</i>	Δ LVEF	Arg389Arg carriers showed greater improvement in LVEF ($+18.8 \pm 12.6$) than Arg389Gly carriers ($+9.4 \pm 11.9$) and Gly389Gly carriers ($+6.0 \pm 12.8$)	<0.001	46
R	Carvedilol	80	4 Months	<i>ADRB2</i>	No associations with <i>ADRB2</i> genotype		NS	
R	Carvedilol	80	4 Months	<i>ADRB2</i>	Δ LVEF	Glu27 carriers had a greater percentage of good responders than Gln27Gln carriers (63% vs. 26%)	0.003	47

Δ , Change before and after treatment; *ADRB1*, β_1 -adrenergic receptor gene; *ADRB2*, β_2 -adrenergic receptor gene; *ADRA2C*, α_2C -adrenergic receptor gene; CR/XL, controlled release/extended release; LVEF, left ventricular ejection fraction; N, sample size; NS, not significant; P, prospective study that was designed specifically to test pharmacogenetics hypotheses; R, retrospective study conducted on an existing data set or patient cohort (in some cases, the cohorts were prospectively enrolled and followed, but the genetic/pharmacogenetic questions were posed retrospectively); RCT, randomized, placebo-controlled clinical trial.

β-Blocker pharmacogenomics studies of adrenergic signaling genes in patients with heart failure: associations with clinical outcomes

Table 3

Study type	β-Blocker	N	Duration	Gene(s) studied	Outcome(s)	Results	P value	Ref.
RCT	Bucindolol	1,040	Median 2 years	<i>ADRB1</i>	D	Arg389Arg carriers showed significant benefit from bucindolol (vs. placebo) (HR 0.62; 95% CI 0.40–0.96). No bucindolol benefit over placebo in Gly389 carriers (HR 0.90, 95% CI: 0.62–1.30)	0.03	14
				<i>ADRB1</i>	D+Hos	Arg389Arg carriers showed significant benefit from bucindolol vs. placebo) (HR 0.66; 95% CI: 0.50–0.88)	0.004	
				<i>ADRA2C</i>		Del322–325 carriers had no benefit from bucindolol (vs. placebo) (HR 1.09; 95% CI 0.57–2.08; $P = 0.80$). Ins322–325Ins carriers had significant benefit from bucindolol (HR 0.70; 95% CI: 0.51–0.96; $P = 0.025$)	0.025	27
					<i>Comparisons: Bucindolol vs. placebo, within genotype</i>			
R	Various	375	37–60 Months	<i>ADRB1</i>	D, HT	Gly49 carrier was associated with the longer survival rate than Ser49Ser (HR = 0.31, 95% CI 0.12–0.79)	0.014	30
					When separated by high and low (<50% target) dose; Gly49 carriers benefited from both high and low dose β-blocker, while Ser49Ser required high dose for benefit			
					<i>Comparison: No or low-dose β-blocker vs. high dose within genotype</i>			
R	Various	227	Median 2.8 years	<i>ADRB1 ADRB2</i>	D, HT	<i>ADRB2 Arg16Gln27</i> haplotype homozygotes had increased mortality (HR 1.91, 95% CI 1.09–3.36). The effect was more prominent in those without β-blocker treatment	0.024	32
					No association by <i>ADRB1</i> genotype			
					<i>Comparison: β-blocker treated or untreated; between genotypes</i>			
R	Various	201	3.3 Years	<i>ADRB1</i>	D	Arg389 carriers on low dose (<50% target) or no β-blocker had significantly worse survival than Arg398 carriers on high-dose β-blocker or Gly389Gly	0.0003	28
					Arg389Arg had significantly more nonsustained VT (NSVT) ($P = 0.015$) and derived significant reduction in NSVT ($P = 0.038$), whereas Gly389 carriers had no reduction in NSVT with β-blocker ($P = 0.58$)			
					Arg389 carriers had significantly worse HF survival ($P = 0.025$) but no influence on non-HF related survival ($P = 0.44$)			
					<i>Comparison: β-blocker high dose vs. low dose or no β-blocker; within genotype</i>			
R	Metoprolol Carvedilol	637	2.8 Years	<i>ADRB1 ADRB2 ADRA2C</i>	D, HT	All patients β-blocker treated. No associations noted for <i>ADRB1</i> , <i>ADRB2</i> , <i>ADRA2C</i>	NS	33

Study type	β -Blocker	N	Duration	Gene(s) studied	Outcome(s)	Results	P value	Ref.
<i>Comparison: β-blocker treated; between genotype</i>								
R	Various	810 (HF)		GRK5	D, HT	African Americans: Gln41Gln had significant survival prolongation with β -blocker ($P = 7.96 \times 10^{-8}$); Leu41 carriers had no benefit from β -blocker ($P = 0.53$) due to similar survival in untreated Leu41 carriers and Gln41Gln β -blocker treated ($P = 0.31$). Inadequate power in Caucasians	8e-8	22
<i>Replication cohort of ACS patients: interaction between Leu41 and β-blocker use relative to death outcome ($P = 0.023$)</i>								
<i>Comparison: β-blocker treated vs. untreated, within genotype</i>								
R	Various	2,460	3.8 Years	ADRB1 GRK5	D	No association noted by Arg389Gly genotype in β -blocker treated ($P = 0.80$); untreated Gly389 carriers had borderline difference in survival ($P = 0.0587$)	NS	31
<i>African Americans: GRK5 Gln41 had worse survival ($P = 0.018$) in untreated, but no difference by genotype in β-blocker treated ($P = 0.72$), suggesting treatment benefit in Leu41. Inadequate power in Caucasians</i>								
<i>Comparison: β-blocker treated; between genotypes</i>								
<i>Gly389Gly and Gln41Gln significant benefit from β-blocker treatment in Caucasians ($P = 0.005$) and African Americans ($P = 0.037$)</i>								
<i>Comparison: β-blocker treated vs. untreated, within genotype</i>								

Δ , Change before and after treatment; ADRA2C, α_2C -adrenergic receptor gene; ADRB1, β_1 -adrenergic receptor gene; ADRB2, β_2 -adrenergic receptor gene; CI, confidence interval; D, death; GRK5, G-protein-coupled receptor kinase 5 gene; HR, hazard ratio; HT, heart transplantation; Hos, hospitalization; N, sample size; NS, not significant; P, prospective study designed specifically to test pharmacogenetics hypotheses; R, retrospective study conducted on an existing data set or patient cohort (in some cases, the cohorts were prospectively enrolled and followed, but the genetic/pharmacogenetic questions were posed retrospectively); RCT, randomized, placebo-controlled clinical trial.