Adrenergic Signaling Polymorphisms and Their Impact on Cardiovascular Disease

GERALD W. DORN II

Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri

I. Introduction: Technological and Conceptual Advances That Determined the Evolution of Adrenergic Therapies for Heart Failure

The adrenergic system is one of the body’s major stress-response mechanisms. The classic example of adrenergic stimulation is the “fight-or-flight” response, in which situational factors stimulate massive release of adrenal catecholamines into the bloodstream (Fig. 1). Circulating epinephrine and locally released norepinephrine activate vasoconstricting α1-adrenergic receptors, vasodilatory β2-adrenergic receptors, and myocardial β1- and β2-adrenergic receptors that increase heart rate (called positive chronotropy) and contractility (called pos-
The net result of these catecholamine-induced cardiovascular changes is to increase cardiac output and blood pressure, ensuring adequate perfusion of tissues essential to prevail in a fight, or to ensure success in avoiding lethal injury (running away). Thus adrenergic signaling is a means to metaphorically “turbo charge” normal cardiovascular physiology during periods of acute intense physiological demand. However, we have come to understand that when these same mechanisms are recruited to compensate for chronically diminished cardiac output and blood pressure in heart failure, they contribute to progression of the disease and accelerate clinical deterioration. Extending the automotive metaphor, turbo charging may make a car that is running on only seven cylinders go a little faster for a brief time, but the additional mechanical stress of constantly increasing compression in a broken engine will ultimately lead to failure of other components, and to catastrophic breakdown.

The evolution of our understanding of adrenergic mechanisms in heart disease is a story of technology driving discovery. To fully appreciate how new genomic technologies and discoveries are altering our perception of this important pathway, it is useful to examine the history of its technical and conceptual evolution. Indeed, the second century Greek physician and father of modern medicine, Galen, whose approach to illness was based on correcting disease-producing humoral imbalances (224), might be pleased to find that current treatments for heart failure recapitulate this approach, correcting imbalances in neurohormones with β-adrenergic receptor antagonists (“β-blockers”) and angiotensin-converting enzyme inhibitors. Although the notion that illness is caused by an imbalance between blood, green and black bile, and phlegm did not survive the Renaissance, the state-of-the-art for treating “dropsical” heart failure in the 17th century continued to be blood-letting as described by G. Baglivi in De Praxi Medica (ca. 1669) (137), and phlebotomy remained a therapeutic mainstay for heart failure throughout the 18th and 19th centuries as modern pathophysiological concepts of cardiac remodeling and pulmonary congestion suggested relieving cardiac overloading in this manner(127): “The overworked ventricle first hypertrophies and then dilates. As it dilates the blood gets dammed up behind it and an increased venous pressure is transmitted ultimately to the capillaries where the edema is formed.” Even after the more mechanistically insightful description of disease progression from compensated hypertrophy to “broken compensation” (now known by the term decompenated heart failure) by Sir William Osler, the recommended treatments for the condition continued to be blood-letting by venesection and volume depletion by bowel evacuation through administration of Epsom salts (228). Only after the development of mercurial diuretics in the 1940s (319) and loop diuretics in the 1960s (318) was phlebotomy finally supplanted as the primary means of intravascular volume reduction in heart failure, although it is still taught as an urgent measure for acute, severe pulmonary edema in the emergency setting (75).

Phlebotomy is an example of a therapy that was effective despite being advocated based on a flawed understanding of the disease process. Unfortunately, the history of β-adrenergic blocking agents (β-blockers) in heart failure inverts that paradigm. Current medical practice is based on irrefutable data accumulated from tens of thousands of study subjects showing that β-blockers improve cardiac function and prolong life expectancy in heart failure (22). This therapeutic efficacy is counterintuitive because catecholamines increase cardiac function, whereas β-blockers antagonize these effects. Indeed, based in part on this rational bias, and on a misinterpretation of experimental data generated concomitantly with the development of β-blockers in the early 1960s, they were initially contraindicated in heart failure. It had been appreciated for some time that adrenal catecholamines raise heart rate, stimulate cardiac contractility, and increase blood pressure. It was also observed that the clin-

---

**Fig. 1.** Role of the adrenergic system in the “fight-or-flight” response.
ical picture of heart failure includes rapid heart rate and peripheral vasoconstriction, i.e., signs of increased adrenergic stimulation. With the development of techniques for quantitative measurement of tissue and circulating catecholamines in the 1960s, it was further shown that heart failure is associated with markedly diminished myocardial norepinephrine content (19, 100). Integrating the clinical observation of adrenergic hyperactivation in heart failure and biochemical findings of myocardial catecholamine depletion led to the conclusion that catecholaminergic activation was a compensatory response in heart failure (increasing cardiac output and blood pressure) and that the observed deficiency of myocardial catecholamines in heart failure contributed to functional cardiac insufficiency. It was therefore reasoned that restoring catecholamine levels would improve cardiac function in heart failure. During approximately the same time period, reports surfaced that administration of the new β-blocker propranolol in large doses acutely exacerbated heart failure (80, 301), reinforcing the notion that adrenergic stimulation is essential to maintaining clinical compensation of heart failure. Consequently, the use of β-blockers was absolutely contraindicated in heart failure for the following decade, and trials were undertaken to evaluate the therapeutic efficacy of agents that increased overall adrenergic signaling, such as type 1 phosphodiesterase inhibitors and parental catecholamines, in heart failure. The unfortunate result of pharmacological stimulation of the adrenergic system in heart failure was increased arrhythmic sudden death and appearance of an entirely new disease, iatrogenic catecholamine cardiomyopathy (111, 171).

The adverse consequences of adrenergic stimulation in clinical trials, together with an evolving understanding of mechanisms for adrenergic desensitization and cardiac β-adrenergic receptor downregulation in heart failure (24), suggested that increased adrenergic signaling in heart failure contributed to disease progression (Fig. 2). The observed myocardial catecholamine deficiency was the consequence of unrelenting catecholamine release from sympathetic neurons in the heart and did not reflect an insufficiency of catecholamine stimulation as previously thought. This diagnostically opposing perspective on the impact of adrenergic signaling in heart failure was reinforced by the pioneering 1975 observation by Waagstein et al. (322) that judicious use of β-receptor blocking agents, administered at low doses under close clinical supervision, enhanced cardiac function in heart failure; subsequent work (118) showed that β-blockers restored adrenergic responsiveness in heart failure by upregulating β-receptors. This, in turn, led to large-scale clinical trials demonstrating improved cardiac function and prolonged survival of heart failure patients treated with β-blockers (49, 123, 231, 313), and ultimately to the modern approach of heart failure treatment by comprehensive pharmacological inhibition of hyperactive neurohormone signaling pathways (230).

Paralleling the evolution of our understanding of heart failure and its associated clinical advances was a scientific revolution that redefined concepts of adrenergic receptors in molecular terms. Cloning of the first mammalian β-adrenergic receptor by Lefkowitz and colleagues (63), and subsequent cloning-by-homology of other adrenergic receptors, redefined adrenergic receptor subtypes that had previously been categorized strictly by pharmacological profile (260). Cardiac-specific recombinant expression of specific adrenergic receptors in genetically manipulated mice revealed striking differences between major β-adrenergic subtypes on cardiac function and long-term adaptation: overexpression of β2-adrenergic receptors at lower levels enhanced cardiac function without long- or short-term deleterious consequences (213), and at high levels led to dilated, fibrotic cardiomyopathy (180), whereas β1-adrenergic receptor overexpression at even low levels produced a lethal dilated cardiomyopathy (79). Taken together, these data demonstrated that differences in signaling profiles between adrenergic subtypes, and in signaling intensity within a given receptor subtype, have the potential to contribute to cardiac maladaptation and failure. The extension of this idea to naturally occurring adrenergic receptor variants having atypical pharmacological profiles as the result of common nucleotide sequence variations is one of the factors contributing to the further evolution of our understanding of heart failure and is the basis for the idea that interindividual variability in disease course or response to β-blocker therapy is caused, at least in part, by genetic polymorphisms. Here, we review available clinical and experimental studies of genetically variant adrenergic receptors and their functional partners.
II. GENETIC VARIATION AND ITS EFFECTS ON CARDIOVASCULAR AND RELATED DISEASES

A. Challenges of Studying Genetics of Complex Diseases

The Online Mendelian Inheritance in Man website is a comprehensive resource for information on Mendelian disorders, listing all known mutations and their associated phenotypes (Online Mendelian Inheritance in Man 2009: http://www.ncbi.nlm.nih.gov/omim/Johns Hopkins University). In many instances, the original findings for a monogenic disorder were derived from genetic testing and linkage disequilibrium studies of large families in which genetic loci and/or specific sequence variants could be statistically linked to rare disease phenotypes. In some cases, these findings have not only led to diagnostic tests that can anticipate development of the disease itself, but have presaged a more complete understanding of previously unsuspected aspects of disease pathophysiology. In contrast, delineating the genetic factors that meaningfully contribute to or modify more common sporadic diseases such as hypertension, diabetes, or heart failure has not advanced as rapidly and, indeed, may be much more challenging to accomplish. Because these “complex” syndromes develop as a consequence of interactions between multiple genetic and nongenetic factors, the role of any individual genetic factor is relatively small compared with those for monogenic diseases. While Framingham data suggest a strong familial pattern for common heart failure (166), and it is estimated that the aggregate contribution of genetics to common heart failure is a high as \( \sim 30\% \), penetrance of genetic phenotypes is incomplete, and the diseases themselves tend to be highly variable. Thus specific contributory genetic factors have been difficult to identify.

What might one anticipate of genetic risk factors or disease modifiers involving adrenergic signaling pathways? As is the case with inherited cardiomyopathies (124, 144), multiple rare risk alleles within the same mechanistic pathway likely produce similar phenotypic effects. With the use of hypertrophic cardiomyopathy as an example, there are over 800 separate mutations in a dozen or more genes that are reported as causing hypertrophic cardiomyopathy. One of the two most common genetic causes of hypertrophic cardiomyopathy mutations is the MYH7 gene encoding the \( \beta \)-myosin heavy chain protein, the major large contractile protein in the heart (149). Over 250 individual mutations in this one gene can cause phenotypic hypertrophic cardiomyopathy. Thus hundreds of different individual genetic events all produce the same disease, probably because all disease-causing MYH7 mutations have a similar biochemical/functional phenotype. By analogy, genetic events in any component of the adrenergic signaling pathway that have the same overall effect on adrenergic signaling might be expected to have the same consequence on cardiac health and disease. This suggests that an overall assessment of the influence of adrenergic signaling polymorphisms on cardiovascular disease must incorporate the net effect of multiple polymorphisms across the entire signaling pathway, which has not yet been achieved. Further complicating this issue, human genetic diversity is greater than originally anticipated (0.5% of total genome differs between individuals and at least 44% of annotated genes exhibit DNA sequence variation, Refs. 175, 331), and interindividual genetic variation clearly contributes to uneven susceptibility to, progression of, and therapeutic response in heart failure. There are over 9 million single nucleotide polymorphisms (SNPs) in the human genome, with each individual carrying between 3 and 4 million SNPs. Nonsynonymous polymorphisms, i.e., those that change the encoded amino acid, have the potential to alter protein function by changing its structure. Properly powered genome-wide association analysis (GWAS) gene-association studies for hypertension, heart failure, or other sporadic polygenic diseases therefore require thousands or tens of thousands of carefully phenotyped individuals. This demands tremendous effort and is accomplishable only at significant expense.

As an example of the complexities involved in identifying genetic causes of complex syndromes, consider a prototypical polygenic complex trait that is readily and unambiguously assayed, height. Height has a dozen or more major genetic factors and is also affected by environmental influences such as nutritional status. Several major efforts to identify “height genes” in thousands of individuals using the technique of GWAS have used microarray-based platforms that simultaneously map up to one million common SNPs located at defined intervals across the genome (45, 81, 169, 183). Yet, the results of these studies have not been concordant, likely because of the large number of genetic and nongenetic factors that contribute to height. In these GWAS studies, “unbiased” association analysis of widely distributed genomic DNA markers had the potential to identify putative genetic associations with disease. However, GWAS studies are limited by relatively poor genetic resolution (arrays with the highest SNP density cover the genome with one marker per \( \sim 1,000 \) bp) and implicate relatively large genetic loci rather than specific genes or gene variants; they cannot detect the influence of rare or private gene variations. Thus while GWAS studies have identified specific genetic loci in cancer, diabetes, hypertension, coronary atherosclerosis, and other diseases (43, 173, 208, 330, 346), assignment of the basis for SNP effects and understanding the mechanistic basis for their disease associations has not been achieved for most of these findings.
With respect to heart failure, for which assessment and characterization is fraught with ambiguity and day-to-day variability, the only GWAS reported to date found no genotype-disease association that achieved genome-wide significance (163).

An alternate approach to unbiased GWAS studies is to choose a candidate gene for detailed genotyping or resequencing based on knowledge of its pathophysiological role. In particular, polymorphism discovery has revealed that G protein-coupled receptors, including those for α- and β-adrenergic receptors, are highly polymorphic. Many candidate gene studies have evaluated the association of one or more polymorphisms of adrenergic receptors in heart failure and asthma, based on compelling data suggesting involvement of adrenergic pathways in these diseases (reviewed in Ref. 68). Although there have been positive findings from these studies, the general approach of genotyping functional elements in individual candidate genes limited by inherent investigator bias. Consequently, associations resulting from candidate gene studies have often failed to be replicated in other populations (1). As described in detail in section IV of this review, that has been the case with most polymorphism association studies of adrenergic signaling pathways in cardiovascular disease.

Pharmacogenomic interactions (the effects of gene variants on drug effects, e.g., β1-adrenergic polymorphism effects on β-blocker response) may represent a special case more amenable to targeted genetic analysis because the questions being addressed tend to be focused narrowly upon the action of a drug on its known molecular target(s) (the field of pharmacodynamics) or on its distribution, metabolism, and bioavailability (pharmacokinetics). As opposed to the almost impossibly broad question of identifying genetic modifiers of complex diseases limited by inherent investigator bias. Consequently, associations resulting from candidate gene studies have often failed to be replicated in other populations (1). As described in detail in section IV of this review, that has been the case with most polymorphism association studies of adrenergic signaling pathways in cardiovascular disease.

Pharmacogenomic interactions (the effects of gene variants on drug effects, e.g., β1-adrenergic polymorphism effects on β-blocker response) may represent a special case more amenable to targeted genetic analysis because the questions being addressed tend to be focused narrowly upon the action of a drug on its known molecular target(s) (the field of pharmacodynamics) or on its distribution, metabolism, and bioavailability (pharmacokinetics). As opposed to the almost impossibly broad question of identifying genetic modifiers of complex diseases limited by inherent investigator bias. Consequently, associations resulting from candidate gene studies have often failed to be replicated in other populations (1). As described in detail in section IV of this review, that has been the case with most polymorphism association studies of adrenergic signaling pathways in cardiovascular disease.

B. Terminology and Definitions for Polymorphism Studies

This review focuses almost entirely on nonsynonymous, or amino acid changing, polymorphisms that have the potential to alter the function of adrenergic receptors and their associated signaling factors. Because protein function is determined by structure, which in turn is determined by amino acid sequence, I describe nonsynonymous variants by referring to the change in encoded amino acid. For example, Arg389Gly in the β1-adrenergic receptor indicates the reference allele first, the amino acid position in the middle, and the changed or “polymorphic” allele second. There is, however, no accepted standard terminology used to label or describe polymorphisms in different contexts. From an historical perspective, receptors have been described as “wild-type” (typically the first cloned, and therefore assumed to also be the most common) or “variant,” i.e., differing from wild-type (and widely assumed to be less common). However, in some instances, the original or reference receptor clone has turned out to be less common, and so describing one or the other as wild-type can result in confusion. In population studies, it is common to refer to genes as having “major” and “minor” alleles, representing the more and less common forms, respectively. However, adrenergic receptor allele frequencies vary between populations of differing genetic ancestry, so what is a major allele in
one normal population group may be the minor allele in another (205). For purposes of this review, nonsynonymous polymorphisms are designated on the basis of the two differentially encoded amino acids and their position, i.e., $\beta_1$-adrenergic receptor Arg389Gly. For clarity, rather than using the proper gene name when describing genotype (i.e., $ADRA1B$ for the $\beta_1$-adrenergic receptor gene) in descriptions of clinical studies, I have continued to use the protein descriptor and amino acid position to describe genotype, i.e., $\beta_1$-adrenergic receptor Arg/Arg389 to indicate homozygous Arg at amino acid position 389, or Arg/Gly389 for heterozygous at the same position. Finally, when indicating results of comparative experimental studies in which signaling factors are expressed in cultured cells or genetic mouse models, I simply indicate to which receptor the data refer (i.e., Arg389 or Gly389). In some instances, such as the rare and highly dysfunctional Thr to Ile polymorphism at position 164 in the human $\beta_2$-adrenergic receptor, I may remind the reader of which form is wild-type, meaning functionally normal (in this case Thr164), and which is variant, meaning functionally abnormal (e.g., Ile164). Finally, the term polymorphism is intended to describe relatively common gene variations that do not themselves cause disease. Originally, the term was used to describe genetic events with allele frequencies of greater than 0.05 (5%), although with increased awareness of the vast number of individual variations, the term is now applied to gene variants with allele frequencies of 0.01 or greater (1%). Even this level is arbitrary and open to debate. I prefer not to engage in discussions that may largely be semantic, and will probably soon be outdated. For the current purposes, “polymorphism” is used to describe individual gene variations that, because their functional consequences are modest, are not considered to cause monogenic disease. This contrasts with mutations, which can/do cause disease. Mutations are therefore typically rare because they are subject to negative evolutionary selection, whereas this is not a major event with polymorphisms. These definitions tacitly recognize that many gene variants are both uncommon and benign, and which I would describe as “rare” (<1% prevalence) or “private” (seen in only a single instance) polymorphisms.

III. ADRENERGIC POLYMORPHISMS

A. Fundamentals of Receptor Signaling

The basic signal transduction steps initiated by agonist-receptor binding are shared for all G protein-coupled receptors. For adrenergic receptors, there are four molecules critically involved in signaling initiation by ligand: the receptor itself, the heterotrimeric G protein to which it couples for activation of downstream signaling factors, G protein receptor kinases that regulate receptor-G protein interactions, and regulators of G protein signaling (RGS) proteins that regulate G protein activity (Fig. 3). Additionally, cytochrome P-450 enzymes can metabolize some $\beta$-blockers and therefore impact adrenergic signaling in response to $\beta$-blocker therapy (Fig. 3). Because genetic polymorphisms of adrenergic receptors and their associated factors exert their putative disease-modulating effects by altering signaling function, the basic roles of these factors and their functional interactions are briefly reviewed.

Adrenergic receptors are catalytically inactive in the absence of bound agonist, i.e., they have little intrinsic signaling activity. Instead, they act as highly sensitive and specific sensors for extracellular neurohormones and therefore function as transducers of local or system-wide hormonal stimuli into specific molecular intracellular signals. Translation by receptors of an extracellular stimulus to a specific intracellular signal is critically dependent on physical and functional receptor coupling to heterotrimeric G proteins, which determine the nature of the intracellular response between different types of receptors. Binding of an agonist to its receptor induces a conformational change that facilitates interaction of the receptor intracellular domains with one or more of ~15 heterotrimeric G proteins (Table 1). Coupling to agonist-occupied receptor stimulates dissociation of the trimeric G protein into two subunits, each of which has the capacity to modulate a signaling pathway. Depending on the specific predetermined receptor G-$\alpha$ subunit protein interaction, either adenyl cyclase is stimulated (by $G_{\alpha_o}$ as by $\beta_1$-adrenergic receptors) or inhibited (by $G_{\alpha_i}$ as by $\beta_2$-adrenergic receptors), or phospholipase C is activated (by $G_{\alpha_q}$ as by $\alpha_1$-adrenergic receptors). The ability of one dissociated G protein-$\alpha$-subunit molecule to activate multiple copies of its downstream signaling effector amplifies and propagates the initiating signal. These major components of adrenergic signaling, i.e., agonist, receptor, and G protein, follow the same general paradigm as does signaling for virtually all of the hundreds of seven transmembrane-spanning receptors for which an agonist has been identified. Indeed, the adrenergic receptor system became the prototype for studies that defined G protein signaling in general. The interested reader is referred to Dr. Robert Lefkowitz’ extraordinary personal account of the road that he and his co-workers followed from relative primitive “grind and bind” studies of tissue membrane preparations to the molecular cloning and characterization of adrenergic receptor subtypes and their interacting molecules (167).

The fundamental characteristics of adrenergic signaling were initially defined in classical physiological studies that determined the dose-dependent physiological effects
of catecholamines and their antagonists in various organ preparations. Based on variable rank-order potency of tissue responses to different adrenergic agonists (see Table 1), Ahlquist (4) pioneered the concept of α- and β-adrenergic receptor subtypes at a phenomenological level. Biochemical advances during the 1960s and 1970s described two distinct catecholamine second messenger pathways, the cAMP/protein kinase A (PKA) pathway mediated through adenylyl cyclase, and the inositol 1,4,5-trisphosphate (IP3)/protein kinase C (PKC) pathway mediated through phospholipase C. Shortly thereafter, Gilman and co-workers (104) delineated the critical role of heterotrimeric G proteins in transducing the extracellular signal of catecholaminergic agents and other hormonal stimuli to an intracellular transduction response. It is interesting that these developments occurred in an environment where the nature, and even existence, of hormone receptors continued to be debated. Indeed, Ahlquist is noted for characterizing the α- and β-adrenergic receptors he initially proposed as “abstract concepts,” and there were those who argued that adenylyl cyclase was the actual β-adrenergic “receptor” (258).

**FIG. 3.** Proximal components of the β-adrenergic signaling pathway. Epinephrine (adrenaline) interacts with membrane-bound β-adrenergic receptors to activate a heterotrimeric G protein that transduces signal to distal effectors. β-Blockers propanolol and metoprolol competitively inhibit agonist-receptor binding. Metoprolol, but not propanolol, is metabolized and eliminated by the cytochrome P-450 enzyme CYP2D6. Agonist-occupied receptors are uncoupled from G proteins through phosphorylation by G protein receptor kinases (GRK). G protein activity is depressed by regulators of G protein signaling (RGS). Each of these factors has functional polymorphisms that can impact overall adrenergic signaling.
Establishing the existence and defining the nature of β-adrenergic receptors required their purification from biological membranes and reconstitution of their biological activity. Only in this manner could it be proven that a candidate protein both bound its predicted agonist and transduced the proper cell signal. The key technologies that made this possible were as follows: 1) development of radioligand binding assays that could accurately measure ligand binding affinity and density of low abundance receptors, 2) generation of affinity columns to purify receptor protein from solubilized membrane preparations, and 3) reconstitution assays where a given purified β-receptor protein could be proven to stimulate adenylyl cyclase in an agonist-dependent manner (37, 64, 219). Subsequent amino acid sequencing of purified β2-adrenergic receptor peptide fragments provided sufficient information to design the degenerate oligonucleotide probes employed to clone full-length hamster and human β2-receptor cDNAs (48, 63, 154). This, in turn, led to cloning the other members of the adrenergic receptor family by low-stringency homology screening (64). Adrenergic receptor structure was inferred from amino acid sequences predicted by their respective nucleotide sequences and revealed that they were all closely related members of a superfamily of seven transmembrane spanning receptors. Structure-function studies relying heavily on mutational analysis proved that the extracellular and transmembrane domains were more important for ligand binding, whereas intracellular domains and the carboxy terminus were important for G protein coupling and in receptor downregulation (Fig. 4) (229) and set the stage for analyses of naturally occurring receptor polymorphisms.

### B. Cardiovascular Responses Regulated by Adrenergic Pathways

As reviewed above, adrenergic receptor subtypes exhibit specificity for agonists and couple to different cell signaling pathways. Not surprisingly, they also exhibit tissue-specific patterns of expression that contribute to their physiological and pathological effects (Fig. 5): α1-adrenergic receptors respond somewhat more to norepinephrine than to epinephrine, couple via Gq to phospholipase C signaling, and are expressed at high levels in vascular smooth muscle and at low levels in cardiac myocytes. Accordingly, α1-adrenergic receptors potentiate vascular constriction and therefore increase blood pressure. As first described in 1978 by Yanagisawa (77), subsequently confirmed by a number of other groups (2, 32, 286), and ultimately reproduced by cardiac-specific transgenic overexpression (182), myocardial α1-adrenergic receptors have a measurable, but modest, positive inotropic (procontractile) effect. Initially, α1-adrenergic receptor-mediated cardiac inotropy was, like the prototypical vasoconstrictor response, attributed to increased IP3-stimulated calcium release (240). However, it is now recognized that IP3 has very little effect on cardiac myo%

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Agonist Rank Order</th>
<th>Coupling</th>
<th>Effect</th>
<th>Agonist</th>
<th>Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-AR</td>
<td>Norepi &gt; Epi &gt;&gt; Iso</td>
<td>Gq/PLC/IP3/PKC</td>
<td>Vasodilation</td>
<td>Phenylephrine</td>
<td>Phenolamine</td>
</tr>
<tr>
<td>α2-AR</td>
<td>Epi &gt; Norepi &gt;&gt; Iso</td>
<td>Gi/AC/decrease cAMP</td>
<td>Sympathoinhibit</td>
<td>Clonidine</td>
<td>Yohimbine</td>
</tr>
<tr>
<td>β1-AR</td>
<td>Iso &gt; Epi = Norepi</td>
<td>Gi/AC/increase cAMP</td>
<td>Vasoconstrict</td>
<td>Dobutamine</td>
<td>Metoprolol</td>
</tr>
<tr>
<td>β2-AR</td>
<td>Iso &gt; Epi &gt;&gt; Norepi</td>
<td>Gi/AC/increase cAMP</td>
<td>+ Inotropy + Chronotropy</td>
<td>Terbutaline</td>
<td>Propranolol</td>
</tr>
<tr>
<td>β3-AR</td>
<td>Iso = Norepi &gt; Epi</td>
<td>Gi/AC/decrease cAMP</td>
<td>Vasodilation</td>
<td>Lipolysis</td>
<td>Solabegron</td>
</tr>
</tbody>
</table>

Agonists are as follows: Epi, epinephrine; Norepi, norepinephrine; Iso, isoproterenol/isoprenaline. Coupling factors are as follows: Gq, Gi, Go, or Gs.

---

**FIG. 4. Structure-function relationships of adrenergic receptors.** A representative adrenergic receptor is shown with extracellular amino terminus (NH2), seven transmembrane domains (blue cylinders), and intracellular carboxy terminus (COOH). Amino-terminal glycosylation site is shown (“martini glass” icon) that can affect receptor localization and internalization. Approximate catecholamine binding domain is shown by red oval. Intracellular G protein coupling domains that can be phosphorylated by GRKs are shown by green rectangle. Polymorphisms in these regions typically affect the indicated functions.
cyte calcium signaling that is coupled to the contraction response (8, 195, 335, 347). Instead, the weak positive inotropism induced by \(\beta_1\)-adrenergic receptor stimulation is the result of enhanced myofilament sensitivity (312), almost certainly mediated by increased myosin light-chain phosphorylation observed in \(\beta_1\)-adrenergic receptor-stimulated rodent and human heart tissue (6, 108).

Notwithstanding these interesting findings, \(\beta_1\)-adrenergic receptors are probably more important in stimulating cardiac hypertrophy (214, 302), which at the macroscopic level is a reactive increase in myocardial mass that attempts to compensate for increased work load. At the cellular level, the gain in mass is accomplished by increasing cardiomyocyte size, rather than cell number. The stimulus for cardiac hypertrophy is any imbalance between demand and functioning muscle available to perform the work. Thus hypertension or aortic outflow obstruction increases the pressure against which the left ventricle must eject its blood volume, representing increased demand (pressure overload). With aortic or mitral valvular insufficiency, the forward ejection fraction is reduced, and the ventricle must eject the same blood volume over and over again, representing increased demand (volume overload). In the case of myocardial infarction or myocarditis, the damage to cardiac muscle decreases availability of functioning contractile units. All of these conditions increase release of catecholamines. Genetic manipulation studies in mice have proven that hormonal stimulation of \(G_q\)-coupled signaling pathways, including the \(\alpha\)-adrenergic pathway, play critical roles in stimulating cardiac hypertrophy (5, 55).

\(\alpha_2\)-Adrenergic receptors are expressed in the sympathetic nervous system, are coupled to \(G_i\), and are critical effectors of synaptic norepinephrine reuptake. Although they function in a variety of ways in the central nervous system, within the cardiovascular system they are most important as negative modulators of myocardial contractility stimulated by direct neuronal stimulation.

As noted above, myocardial \(\alpha_1\)-adrenergic receptors can modestly increase cardiac contractility (182), but it is myocardial \(\beta\)-adrenergic receptors, which outnumber myocardial \(\alpha_1\)-adrenergic receptors by an order of magnitude, that play the major roles in regulating minute-by-minute cardiac contractile function. Myocardial \(\beta_1\) and \(\beta_2\)-adrenergic receptors are both coupled via \(G_{\alpha}\) to stimulation of adenylyl cyclase, and through it to activation of PKA that phosphorylates contractile and calcium regulatory proteins to enhance contractility. \(\beta_2\)-Adrenergic receptors have, in addition, the potential to couple to the
adenyl cyclase inhibitory G protein, Ga. The physiological relevance of dual β2-adrenergic receptor coupling to Ga and Gt in the heart is a matter of some debate, as are the conditions under which it can or does occur. Lefkowitz first described β2-adrenergic receptor coupling to Ga and Gt in transfected cultured human fibroblasts in the late 1990s and further demonstrated the phosphorylation of the receptor by PKA (downstream of Gt) could trigger the switch from β2-adrenergic receptor-Ga coupling to β2-adrenergic receptor-Gt coupling (56). These fundamental observations, and extension of the findings to include a similar G protein switching function for G protein receptor kinase (GRK) 2, have been replicated many times in other fibroblast tissue culture models (189, 342), cultured endothelial cells and rat arterial preparations (50), β2-adrenergic receptor overexpressing isolated mouse cardiac myocytes (337) and transgenic mice (114), genetically normal isolated rat cardiac myocytes (241), and the canine heart (116). Controversy exists, however, about the effects of β2-adrenergic receptor G protein switching on the heart. For myocardial contraction, activation of Gt is proposed to negate any positive inotropic effect of (Gs-coupled) β2-adrenergic receptor stimulation (337). On the other hand, β2-adrenergic receptor stimulation may be less cardiotoxic because of its ability to signal in part through Gt (90, 116, 345).

Whether β2-receptor coupling to two G proteins has meaning in human hearts can be argued based on two studies with opposing results. Using photoaffinity labeling of active Gt, Kilts et al. (151) showed that β2-adrenergic receptor stimulation biochemically activated Gt in human atrial membranes. On the other hand, in a more recent study that used traditional physiological assessments, Molenaar et al. (216) were unable to identify functional consequences of β2-adrenergic receptor-Gt signaling in human atrial tissue. In fact, these two data sets are not incompatible, as β2-adrenergic receptor signaling to Gt may impact myocardial contraction and relaxation minimally and have greater effects on programmed cell death, channel function, etc. (52). In any case, β2-adrenergic receptor coupling studies have not been performed on human ventricular tissue or cardiac myocytes, so the essential data to begin the process of ascertaining pathophysiological relevance are lacking. Nevertheless, defining the differences in β-receptor subtype signaling is important because β1- and β2-adrenergic receptors are differentially regulated in human heart failure (26). Bristow was the first to report that β-adrenergic receptor density is decreased in heart failure and that the remaining receptors are largely uncoupled from adenylyl cyclase (24), as a consequence of homologous downregulation and desensitization (317). Differences conferred by adrenergic subtypes are explored in more detail in the next section.

C. Adrenergic Receptor Subtypes and Their Individual Characteristics

There are nine adrenergic receptors: three α1, three α2, and three β. All of the genes presumably arose from a common seven transmembrane-spanning, G protein-coupled ancestral gene. The coding regions of the three β-adrenergic receptor genes and three α2-adrenergic receptor genes are each encoded in single exons, while all three α1-adrenergic receptor genes have single large intron separating regions that encode the body of the receptor from those that encode the seventh transmembrane domain and carboxy terminus (Fig. 6). The similar genetic structures of immediate family members, but distinctions between more general subtypes, suggest that adrenergic receptor diversity has evolved through a branching series of gene-duplication events. Thus it is not surprising that each of the nine adrenergic receptors binds the endogenous catecholamines epinephrine and norepinephrine and that their distinct end-organ effects are attributable to differences in agonist binding affinity and specifics of G protein coupling (see Table 1).

I. α1-Adrenergic receptors

There are three α1-adrenergic receptor subtypes: α1A, α1B, and α1D. The out-of-sequence nomenclature arose from initial confusion matching newly cloned receptor cDNAs with previously described pharmacological recep-
tor subtypes, and with preconceptions about patterns of tissue expression. These ambiguities have since been resolved (122). The major biological effects of α₁-adrenergic receptor stimulation are vasoconstriction and vascular smooth muscle growth via the actions of vascular smooth muscle receptors, and myocardial hypertrophy via the actions of cardiomyocyte receptors (see Fig. 5).

Each of the α₁-adrenergic receptors is coupled to the Gq/G11 heterotrimeric G protein and activates phospholipases C and A2 and protein kinases C and D (67, 83, 235). A major effecter of α₁-adrenergic receptor signaling is intracellular free calcium, which is released from internal calcium stores by the actions of IP₃ and enters the cell by capacitative influx through membrane calcium channels. The α₁A- and α₁B-adrenergic receptor subtypes, which are the most common in myocardial tissue, are more efficiently coupled to agonist-mediated calcium release than is the α₁D-adrenergic receptor, possibly because a larger proportion of the latter subtype is intracellular and therefore uncoupled from signal transducers (38). Each of the α₁-adrenergic receptors has the potential also to activate p42 and p44 ERK kinases, Jun amino-terminal kinase (JNK), or p38 mitogen-activated protein kinase (together, the MAPKs), which are responsible for mediating their trophic effects in vascular smooth muscle and in cardiac myocytes (132, 133). However, the extent of activation of each MAPK appears highly dependent on receptor subtype and tissue type.

Given the variability of tissue- and cell type-specific expression for α₁-adrenergic receptors, it was initially difficult using purely physiological and pharmacological approaches to determine their subtype-specific effects on integrated cardiovascular function. Absence of completely subtype-specific pharmacological agonists further complicated these types of studies. On the basis of in vitro functional studies and patterns of receptor expression, it would be expected that major consequences would be on systemic vascular tone and blood pressure and on cardiac growth and reactive hypertrophy (131, 237, 327). Indeed, tissue-specific receptor overexpression using transgenic techniques supports differences in the abilities of α₁-adrenergic receptor subtypes to confer disease phenotypes. For example, cardiac-specific overexpression of α₁D-adrenergic receptors induces pathological hypertrophy (214, 324), whereas overexpression of α₁A-adrenergic receptors increases cardiac inotropy (contraction) without stimulating hypertrophy (182), although this (like other forms of unremitting contractile stimulation) can ultimately lead to adverse myocardial remodeling (40). Transgenic overexpression studies are potentially confounded by promiscuous signaling of receptors expressed at nonphysiological levels, by unrestrained signaling of constitutively active receptors that were sometimes used, and by ectopic expression of receptors not normally expressed in a given cell type. For these reasons, the development of knockout mice lacking each of the α₁-adrenergic receptor subtypes, individually and in combination, provided in vivo systems better suited for evaluating the true relevance of specific α₁-adrenergic subtypes to various physiological and pathological processes.

Ablation of α₁A-adrenergic receptors resulted in mice with ~10% lower resting blood pressures and a ~15% decrease in the pressor response to phenylephrine, suggesting a major role for this receptor subtype in regulating resting and reactive blood pressure (264), although it is notable that a subsequent study from this same group reported normal blood pressures in α₁A/α₁B double-knockout mice (225). Ablation of α₁B-adrenergic receptors reduced myocardial total α₁-adrenergic receptor density to ~25% of normal values, without provoking a cardiac phenotype. α₁D-Adrenergic receptor knockout mice also had normal resting blood pressures, although the phenylephrine pressor response was decreased by 45% compared with controls, suggesting that this subtype is also important in mediating acute vasoconstrictor responses (35). The α₁D-adrenergic receptor knockout phenotype resembled a more complete α₁A-adrenergic receptor knockout phenotype, with a ~8% decrease in basal blood pressure and an α-adrenergic agonist pressor response that was decreased by ~25% (129). In the same study, the major effects of combined α₁B- and α₁D-receptor ablation seemed to be mediated largely by the α₁D subtype, because the double knockout showed only a ~13% decrease in basal mean arterial pressure, and exhibited a similar degree of diminished pressor response as the α₁A-adrenergic receptor knockout. Thus, in the systemic circulation of mice, the accumulated data support the largest role for α₁A-adrenergic receptors in acutely regulating blood pressure. As discussed in more detail below, this conclusion becomes important when attempting to correctly interpret correlative studies of α₁-adrenergic receptor polymorphisms in human hypertension.

The same α₁-adrenergic receptor knockout mouse models have been used to explore roles of α₁-adrenergic receptors on vascular smooth muscle growth, employing experimental models of neointimal proliferation after intraluminal vascular injury. These models attempt to recapitulate features of coronary restenosis that sometimes occurs as a late complication of balloon coronary angioplasty. In a study comparing the extent of femoral artery restenosis in α₁A, α₁B, and α₁A/α₁B double-knockout mice, reductions in intimal thickness were seen in the α₁A/α₁B double adrenergic receptor knockouts, suggesting a role for both of these subtypes in reactive vascular smooth muscle proliferation (128).

Finally, the various α₁-adrenergic receptor knockout mice have been used to answer questions regarding the role of this signaling pathway in cardiac hypertrophy, which is transduced largely via Gq-coupled signaling pathways (217). It is worth reviewing a bit of the history of this
area and the terminology used to describe cardiac growth, since an incomplete understanding of the former can lead to experimental misinterpretations, and the latter can be confusing even for cognoscenti. The idea that α₁-adrenergic signaling promotes pathological hypertrophy is deeply ingrained in the field of molecular and cellular cardiology because phenylephrine-stimulated hypertrophy of cultured neonatal rat cardiac myocytes, as originally described by Paul Simpson (284) and subsequently adopted for world-wide usage, is the "gold-standard" in vitro assay for cardiomyocyte hypertrophy. Although cultured neonatal rat cardiac myocytes have many advantages, they are not adult myocytes, and events that are observed in this system may not apply to either the postnatal or adult in vivo heart. Indeed, this appears to be the case for α₁A-adrenergic receptors. These receptors clearly mediate a type of pathological hypertrophy in cultured neonatal cardiac myocytes (153); however, the major cardiac phenotype of α₁A-adrenergic receptor knockout mice is significantly reduced cardiac growth in young mice, which the authors termed "physiological hypertrophy" (225). This raises the issue of proper terminology and how conclusions can sometimes be misinterpreted. "Hypertrophy" literally means excessive growth, which is not the same as the normal growth of an organ (the proper term is actually "eutrophy," but it is rarely used; Ref. 70). "Physiological hypertrophy" does occur; the classic example is breast enlargement during lactation. In the heart, the analogous situation would be cardiac enlargement in trained athletes (69). Thus there are three different forms of cardiac growth that are, and should be treated as, separate responses to different stimuli: 1) developmental cardiac growth, which is normal in embryonic and juvenile mammals, 2) physiological hypertrophy that occurs in response to increased physiological work, and 3) pathological hypertrophy that typically is a response to chronic and unremitting pressure or volume overload, or to myocardial injury.

So what are the effects of α₁-adrenergic receptors on cardiac growth? Absence of α₁A-receptors diminishes normal postnatal cardiac growth, resulting in modestly hypoplastic hearts (225). Absence of both the α₁A- and α₁B-adrenergic receptors increased cardiac maladaptation to pressure overload, which is one of the most potent naturally occurring stimuli for pathological hypertrophy (226). This interesting finding bears careful examination and evaluation, because of the multiple interacting factors at play. Concentric hypertrophy itself is an adaptive response to increased pressure, because it favorably affects cardiac geometry, decreases wall stress and afterload, and therefore increases the efficiency of contraction (62). In α₁A-adrenergic receptor knockout mice, the hearts are hypoplastic to begin with because of the aforementioned suppression of normal postnatal developmental growth. The α₁A- and α₁B-adrenergic receptor double-knockout mice appear not to develop the concentric hypertrophy that is a hallmark of the early adaptive response to pressure overloading. Wall thickness (an increase of which is the sine qua non for compensatory concentric hypertrophy) was not reported in this paper but must have actually decreased compared with control pressure overloaded mice, as estimated by back-calculating from the left ventricular diastolic dimensions and heart weights. Furthermore, the characteristic expression of fetal gene markers for hypertrophy, β-myosin heavy chain, and α-skeletal actin (17, 327) did not occur after pressure overloading in the α₁-receptor double-knockout mice, whereas the marker for heart failure, atrial natriuretic factor (ANF), was robustly expressed. Thus, although there was an increase in cardiac mass (i.e., hypertrophy) in the α₁-receptor double-knockout mice after pressure overloading, the benefits of this hypertrophy were not seen. These results strongly suggest that aspects of reactive hypertrophy induced by α₁-adrenergic stimulation can benefit the heart by compensating for increased hemodynamic loading, i.e., that the characterization of α-adrenergic receptor-mediated hypertrophy, and perhaps of early reactive hypertrophy in general, as "pathological" is not accurate. These results and this conclusion have interesting implications for functional polymorphisms of α₁-adrenergic receptors in human hypertensive and valvular heart disease.

2. α₂-Adrenergic receptors

There are three α₂-adrenergic receptor subtypes: α₂A, α₂B, and α₂C, each coupled to the inhibitory G protein G₁. α₂-Adrenergic receptors are expressed primarily in the nervous system and platelets. Presynaptic α₂A and α₂C-adrenergic receptors inhibit norepinephrine release from sympathetic nerves (119). Thus, depending on subtype, the major biological effects of α₂-adrenergic receptors can be on platelet aggregation, regulation of sympathetic outflow from the central nervous system, reuptake of norepinephrine from within peripheral sympathetic nerve synapses, insulin secretion and lipolysis, or to a limited extent, vasoconstriction (101, 125).

Each of the three α₂-adrenergic receptors has been ablated in mice. The most detailed cardiovascular studies have examined the consequences of receptor knockouts on blood pressure. Genetic deletion of α₂A-adrenergic receptors prevented the characterization hypotension induced by pharmacological α₂-adrenergic receptor stimulation with clonidine (196), showing that the central hypotensive effects of α₂A-adrenergic receptor stimulation are mediated by this subtype (93, 155). A concomitant study in mice lacking either α₂B or α₂C-adrenergic receptors supported this conclusion and further suggested that vascular α₂B-adrenergic receptors can produce vasoconstriction (184) and exert hyperten-
sive effects, whereas there was no detectable cardiovascular phenotype from $\alpha_{2C}$-receptor ablation. Subsequent studies evaluating the hypertensive response to salt loading in various $\alpha_2$-adrenergic receptor knockout mice found that absence of $\alpha_{2A}$ or $\alpha_{2C}$-adrenergic receptors did not impact the development of salt-dependent hypertension, whereas absence of $\alpha_{2B}$-adrenergic receptors prevented hypertension. This finding provides additional support for a role of the $\alpha_{2B}$-receptor subtype in maintaining blood pressure in opposition to the central hypotensive effects of $\alpha_{2A}$-adrenergic receptors (198, 199). It is worth noting that whether the vasopressor effects of $\alpha_{2B}$-adrenergic receptors reflect peripheral vasoconstriction, or are a central effect mediated by brain receptors, has been vigorously debated (102).

The inhibitory effects of presynaptic $\alpha_{2A}$- and $\alpha_{2C}$-adrenergic receptors on sympathetic stimulation suggest that these subtypes could play a protective role in heart failure. Indeed, absence of $\alpha_{2A}/\alpha_{2C}$-adrenergic receptors in double-knockout mice produces a form of “catecholamine cardiomyopathy,” demonstrating that basal sympathetic tone normally constrained by $\alpha_2$-adrenergic inhibition of neuronal catecholamine release is sufficient to induce cardiac toxicity (119). Mice lacking just $\alpha_{2C}$-adrenergic receptors developed a similar cardiomyopathy after surgical pressure overloading (21). These experimental results suggest that loss-of-function polymorphisms of $\alpha_{2A}$- and $\alpha_{2C}$-adrenergic receptors could modify the risk of developing, or the progression of, human heart failure. Some clinical data support this idea (see below).

3. $\beta$-Adrenergic receptors

There are three $\beta$-adrenergic receptor subtypes: $\beta_1$, $\beta_2$, and $\beta_3$-receptors. The $\beta_1$- and $\beta_2$-receptors play the major roles in modulating cardiac output and blood pressure. $\beta_3$-Adrenergic receptors are primarily expressed in adipocytes (76), although they are detectable in myocardium and seem to exert a significant negative inotropic effect in opposition to the positively inotropic $\beta_1$- and $\beta_2$-receptors. For this reason, $\beta_3$-receptors have sometimes been proposed to act as protective “endogenous $\beta$-blockers” in heart failure (308). In contrast, transgenic overexpression of $\beta_3$-adrenergic receptors at fairly high levels in cardiac myocytes (330 fmol/mg protein) had a positive inotropic effect in mice (157), suggesting that they do not act in opposition to $\beta_1$- and $\beta_2$-adrenergic receptors under all conditions. However, these transgenic studies must be viewed critically because high levels of receptor overexpression have the potential to produce promiscuous receptor coupling to atypical signaling effectors, generating nonphysiological phenotypes. In any case, because most genetic work to date in cardiovascular disease has focused on the $\beta_1$- and $\beta_2$-receptor subtypes, and the handful of studies performed on $\beta_3$-adrenergic receptor polymorphisms have failed to show an association between the common $\beta_3$-adrenergic receptor Trp64Arg polymorphism and heart disease (91, 218, 245, 278, 306, 341), this review focuses on $\beta_1$- and $\beta_2$-adrenergic receptors.

The major myocardial/cardiomyocyte isoform is the $\beta_1$-adrenergic receptor, which accounts for ~80% of all myocardial $\beta$-adrenergic receptors in normal myocardium (25). $\beta_1$-Adrenergic receptors are positively coupled to adenylyl cyclase via the stimulatory G protein, $G_s$, and therefore mediate those effects that are stimulated by $G_s$/PKA, i.e., an increase in heart rate (chronotropy), an increase in myocardial contraction (inotropy), and an increase in electrical automaticity. The critical role of $\beta_1$-adrenergic receptors in regulating cardiac contraction was best demonstrated through ablation of its gene and investigations of cardiac physiology in $\beta$-receptor knockout mice. Although lethal developmental abnormalities in germ-line $\beta_1$-adrenergic receptor knockout mice initially prevented detailed studies of cardiac function (262), breeding the knockout allele onto a different genetic background produced viable mice having a normal basal cardiovascular phenotype (i.e., normal resting heart rate, cardiac function, and blood pressure), but lacking the normal positively chronotropic (heart rate) and inotropic (contractility) responsiveness to catecholamines (263). Virtual elimination of the “fight-or-flight” response after $\beta_1$-adrenergic receptor ablation suggests that, at least in normal mice, acute regulation of cardiac performance is mediated almost entirely by this subtype; $\beta_2$-receptors normally have more importance as regulators of vascular tone and blood pressure (261).

As noted previously, long-term exposure to, or endogenous production of, catecholamines can cause cardiac dysfunction and contribute to development or clinical deterioration of heart failure. There are compelling data to support the notion that the $\beta_1$-adrenergic receptor subtype is largely responsible for this catecholamine cardiotoxicity (52) and that chronic $\beta_1$-adrenergic receptor activity accounts for at least some of the deleterious effects of chronic sympathetic stimulation in heart failure as described in Milton Packer’s “neurohormonal hypothesis” (230). Cardiac pathology stimulated specifically through $\beta_1$-adrenergic receptors was best demonstrated by transgenic overexpression studies in which a relatively modest level of receptor overexpression (as little as 5 times the normal number) was sufficient to induce an aggressive dilated, fibrotic cardiomyopathy, even in the absence of exogenous catecholamines (79). The observation that $\beta_1$-adrenergic receptors can be cardiotoxic in the absence of catecholamine excess has important implications for genetic polymorphisms that increase intrinsic signaling activity of this receptor (see below), and has provided additional mechanistic insight into the striking
clinical efficacy of \(\beta\)-blockade in human heart failure (49, 123, 232, 313).

\(\beta_2\)-Adrenergic receptors are less common than \(\beta_1\)-adrenergic receptors in normal myocardium, but are the dominant subtype in vascular and bronchial smooth muscle where they decrease muscle tone and mediate vasodilation (important in hypertension) and bronchodilation (important in asthma). For this reason, \(\beta_2\)-adrenergic receptor knockout mice exhibited normal basal cardiovascular function, but develop abnormalities of vascular resistance that are provoked by exercise-induced catecholamine release (47). The absence of a clear effect of \(\beta_2\)-adrenergic receptor ablation on cardiac contraction was initially surprising because these receptors are detected in myocardial homogenates (although perhaps predominantly in the non-myocyte fraction), and they enhance cardiac contraction when forcibly expressed in cardiac myocytes. Indeed, it has been proposed that \(\beta_2\)-adrenergic receptor gene therapy might be used to treat heart failure based on enhanced cardiac contractile function without the adverse consequences commonly associated with positively inotropic therapies seen in transgenic mice overexpressing \(\beta_2\)-adrenergic receptors specifically in cardiac myocytes (213). However, subsequent studies in our laboratory revealed that \(\beta_2\)-adrenergic receptors are not as cardiotoxic as \(\beta_1\)-receptors, but at high expression levels (i.e., \(\sim 300\)-fold normal myocardial \(\beta\)-receptor levels) produce a dilated fibrotic cardiomyopathy similar to that provoked by expression of \(\beta_1\)-adrenergic receptors at much lower levels (71, 180). These studies in genetically manipulated mouse models proved that \(\beta_1\)-adrenergic receptor signaling has greater potential than \(\beta_2\)-adrenergic receptor signaling to cause or contribute to heart failure. Consistent with this notion, functionally significant human DNA sequence variations of \(\beta_2\)-adrenergic receptors appear to have greater impact on myocardial contraction and heart failure, whereas \(\beta_2\)-adrenergic receptor polymorphisms seem to impact hypertension or asthma.

IV. HUMAN ADRENERGIC RECEPTOR POLYMORPHISMS AND CARDIOVASCULAR DISEASE

In the following section, a comprehensive list of the common nonsynonymous polymorphisms (i.e., those that change amino acid coding) and other functionally significant adrenergic receptor gene variants is provided, by individual subtype. For each receptor subtype, the functional ramifications of polymorphisms are described, based largely on results of recombinant expression in cultured cells. For those few polymorphic receptors in which genetic mouse models have been developed to assess consequences on integrated cardiovascular function, these results are also reviewed. Finally, the results of human genotyping studies are described, and the overall implications of cell, mouse, and human studies are discussed. Table 2 summarizes genetic data for disease-associated polymorphisms reviewed below.

A. \(\alpha_1\)-Adrenergic Receptor Polymorphisms

Of the three subtypes of \(\alpha_1\)-adrenergic receptor, the \(\alpha_{1A}\)-subtype is most abundant in vascular smooth muscle. It has therefore been implicated in regulating arterial resistance and blood pressure (264, 266). Table 3 lists reported nonsynonymous polymorphisms of human \(\alpha_1\)-adrenergic receptors and their associations with disease. Figure 7 shows the location of the important \(\alpha_{1A}\)-adrenergic receptor polymorphism relative to the functional receptor domains illustrated in Figure 4.

The \(\alpha_1\)-adrenergic receptor polymorphism most often studied in human hypertension is \(\alpha_{1A}\)-Arg347Cys (previously reported as Arg492Cys; Refs. 136, 292). This substitution occurs within the carboxy terminus of the receptor and introduces a potential palmitoylation site that can theoretically alter subcellular receptor localization. However, comparative studies of the Arg and Cys receptor expressed in Chinese hamster ovary (CHO) cells revealed no differences in agonist or antagonist binding affinity, in calcium signaling stimulated by norepinephrine, or in receptor desensitization after prolonged agonist exposure (279). Thus there is as yet no evidence supporting an effect of this common polymorphism on in vitro \(\alpha_{1A}\)-adrenergic receptor function. Nevertheless, this polymorphism has been examined in a number of populations with results suggesting a possible in vivo effect. It is important to note that the frequency of the Cys347 polymorphism varies greatly between populations of different geographic ancestry (338), so the nature of the study population may be a variable in human investigations. For this reason, clinical studies are introduced as needed with

![Figure 7. Location of clinically significant \(\alpha_{1A}\)-adrenergic receptor polymorphisms.](http://physrev.physiology.org/)}
TABLE 2. Genetic data for adrenergic polymorphisms

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Refseq ID</th>
<th>Gene Chromosome Position* (Strand)</th>
<th>mRNA Length, bp</th>
<th>mRNA Size, amino acids</th>
<th>dbSNP SNP ID</th>
<th>SNP Chromosome Position*</th>
<th>Intronic Position</th>
<th>Major/Minor Allele</th>
<th>Codon</th>
<th>African/ American MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRA1A</td>
<td>NM_000680</td>
<td>chr6:2667222-26722822 (-)</td>
<td>2,281</td>
<td>466</td>
<td>rs1048101</td>
<td>chr8: 26628028</td>
<td>+1039</td>
<td>C/T</td>
<td>R347C</td>
<td>0.568 0.208</td>
</tr>
<tr>
<td>ADRA2A</td>
<td>NM_000681</td>
<td>chr10:1128370-112840600 (+)</td>
<td>3,889</td>
<td>465</td>
<td>rs1000035</td>
<td>chr10: 11283552</td>
<td>+738</td>
<td>C/G</td>
<td>N266K</td>
<td>0.090 0.030</td>
</tr>
<tr>
<td>ADRA2A</td>
<td>NM_000681</td>
<td>chr10:1128370-112840600 (+)</td>
<td>3,889</td>
<td>465</td>
<td>rs1000035</td>
<td>chr10: 11283552</td>
<td>+738</td>
<td>C/G</td>
<td>N266K</td>
<td>0.090 0.030</td>
</tr>
<tr>
<td>ADRA2B</td>
<td>NM_000682</td>
<td>chr9:2678262-26782888 (-)</td>
<td>2,288</td>
<td>450</td>
<td>rs10000772</td>
<td>chr9: 26782957</td>
<td>+901</td>
<td>CAAAGGAGGAGCG-</td>
<td>del310–303</td>
<td>0.570 0.210</td>
</tr>
<tr>
<td>ADRA2B</td>
<td>NM_000683</td>
<td>chr7:376296-37727051 (+)</td>
<td>1,958</td>
<td>462</td>
<td>rs10176701</td>
<td>chr7: 37602097</td>
<td>+964</td>
<td>GGCGGGGGGCGCGC-</td>
<td>del325–325</td>
<td>0.606 0.420</td>
</tr>
<tr>
<td>ADRA2C</td>
<td>NM_000684</td>
<td>chr1:1158060-11580665 (+)</td>
<td>2,862</td>
<td>477</td>
<td>rs10180252</td>
<td>chr1: 11580603</td>
<td>+145</td>
<td>A/G</td>
<td>S49G</td>
<td>0.270 0.420</td>
</tr>
<tr>
<td>ADRA2B</td>
<td>NM_000685</td>
<td>chr1:1158060-11580665 (+)</td>
<td>2,862</td>
<td>477</td>
<td>rs10180252</td>
<td>chr1: 11580603</td>
<td>+145</td>
<td>A/G</td>
<td>S49G</td>
<td>0.270 0.420</td>
</tr>
<tr>
<td>ADRA2B</td>
<td>NM_000686</td>
<td>chr1:1158060-11580665 (+)</td>
<td>2,862</td>
<td>477</td>
<td>rs10180252</td>
<td>chr1: 11580603</td>
<td>+145</td>
<td>A/G</td>
<td>S49G</td>
<td>0.270 0.420</td>
</tr>
<tr>
<td>ADRA2C</td>
<td>NM_000687</td>
<td>chr5:1840621-184063196 (+)</td>
<td>3,033</td>
<td>413</td>
<td>rs1014573</td>
<td>chr5: 18406410</td>
<td>+66</td>
<td>G/A</td>
<td>G51R</td>
<td>0.388 0.480</td>
</tr>
<tr>
<td>ADRA2C</td>
<td>NM_000688</td>
<td>chr5:1840621-184063196 (+)</td>
<td>3,033</td>
<td>413</td>
<td>rs1014573</td>
<td>chr5: 18406410</td>
<td>+66</td>
<td>G/A</td>
<td>G51R</td>
<td>0.388 0.480</td>
</tr>
<tr>
<td>ADRA2C</td>
<td>NM_000689</td>
<td>chr5:1840621-184063196 (+)</td>
<td>3,033</td>
<td>413</td>
<td>rs1014573</td>
<td>chr5: 18406410</td>
<td>+66</td>
<td>G/A</td>
<td>G51R</td>
<td>0.388 0.480</td>
</tr>
<tr>
<td>GRK4</td>
<td>NM_002075</td>
<td>chr9:8033260-8033316192 (-)</td>
<td>2,188</td>
<td>359</td>
<td>rs772466452</td>
<td>chr9: 80347069</td>
<td>-904</td>
<td>GCC/TT</td>
<td>S275H</td>
<td>0.450 0.340</td>
</tr>
<tr>
<td>GRK4</td>
<td>NM_002076</td>
<td>chr10:12946375-12946556 (+)</td>
<td>1,923</td>
<td>340</td>
<td>rs5443</td>
<td>chr12: 604875</td>
<td>+825</td>
<td>C/T</td>
<td>T164L</td>
<td>0.000 0.044</td>
</tr>
<tr>
<td>GRK4</td>
<td>NM_002077</td>
<td>chr18:27816-182781406 (+)</td>
<td>1,375</td>
<td>211</td>
<td>rs9053226</td>
<td>chr18: 18278003</td>
<td>-18</td>
<td>-/TC</td>
<td>H131I</td>
<td>0.490 0.220</td>
</tr>
<tr>
<td>GRK4</td>
<td>NM_002078</td>
<td>chr19:27816-182781406 (+)</td>
<td>1,375</td>
<td>211</td>
<td>rs9053226</td>
<td>chr19: 18278003</td>
<td>-18</td>
<td>-/TC</td>
<td>H131I</td>
<td>0.490 0.220</td>
</tr>
<tr>
<td>GRK4</td>
<td>NM_002079</td>
<td>chr4:392543-3925447 (-)</td>
<td>2,321</td>
<td>578</td>
<td>rs2986005</td>
<td>chr4: 3926451</td>
<td>+94</td>
<td>C/T</td>
<td>R66L</td>
<td>0.348 0.469</td>
</tr>
<tr>
<td>GRK4</td>
<td>NM_002080</td>
<td>chr4:392543-3925447 (-)</td>
<td>2,321</td>
<td>578</td>
<td>rs2986005</td>
<td>chr4: 3926451</td>
<td>+94</td>
<td>C/T</td>
<td>R66L</td>
<td>0.348 0.469</td>
</tr>
<tr>
<td>GRK5</td>
<td>NM_005308</td>
<td>chr10:120967197-12101530 (-)</td>
<td>2,575</td>
<td>509</td>
<td>rs10768070</td>
<td>chr10:12108069</td>
<td>+122</td>
<td>A/T</td>
<td>Q4IL</td>
<td>0.013 0.230</td>
</tr>
</tbody>
</table>

MAF, minor allele frequency. *Chromosome positions were obtained from assembly GRCh37.
biological readouts support a likely effect of this polymorphism on cardiovascular responses to sympathetic stimulation. Snapir et al. (292) performed a detailed examination of the effects of several common adrenergic receptor polymorphisms on hemodynamic responses to intravenous epinephrine in 16 young healthy male subjects. Epinephrine (adrenaline) stimulates both $\alpha_1$- and $\beta$-adrenergic receptors. In this small patient group, the homozygous $\alpha_1$-adrenergic receptor Cys347 genotype was associated with slightly higher systolic blood pressure and an attenuated blood pressure response to intravenous epinephrine. Individuals homozygous for $\alpha_1$-adrenergic receptor Cys347 in this study had longer electrocardiographic P-R intervals, suggesting modest atrioventricular conduction delay. A similar type of result was found by Iacoviello et al. (136) who genotyped for adrenergic receptor polymorphisms and examined their distribution in 109 individuals with familial or sporadic hypertension. Whereas there was no association with familial hypertension for any of the receptor polymorphisms, individuals carrying the $\alpha_1$-adrenergic receptor Cys347 polymorphism had significantly higher heart rates, measured as electrocardiographic R-R intervals, and lower heart rate variability. The jury is still out, but the accumulated data support a marginal effect of the $\alpha_1$-adrenergic receptor position 347 polymorphism on cardiovascular responses to sympathetic stimuli.

A number of nonsynonymous polymorphisms have been described for $\alpha_1$- and $\alpha_2$-adrenergic receptors (Tables 4 and 5, respectively), but no functional phenotypes or cardiovascular disease associations have been reported.

### B. $\alpha_2$-Adrenergic Receptor Polymorphisms

There are three subtypes of $\alpha_2$-adrenergic receptors, designated $\alpha_2A$, $\alpha_2B$, and $\alpha_2C$. As reviewed above, the mouse gene knockout studies suggested that $\alpha_2A$ and $\alpha_2C$-adrenergic receptors may be more important as modulators of sympathetic tone, whereas $\alpha_2B$-receptors are more likely to direct vasoconstriction. $\alpha_2A$-Adrenergic receptors are presynaptic autoinhibitory receptors for sympathetic neurons and mediate synaptic norepinephrine reuptake. Table 6 lists reported nonsynonymous polymorphisms of human $\alpha_2A$-adrenergic receptors and any assoc-
associations with disease. An Asn251Lys polymorphism in the receptor’s third intracellular loop has the potential to affect receptor-effector coupling or desensitization, as this region is critical for G protein interactions and GRK-mediated phosphorylation (Fig. 8). Small and Liggett and co-workers (288) expressed recombinant Asn251 and Lys251 2A-adrenergic receptors in CHO and COS-7 cells to evaluate the functional consequences of the variant. Ligand binding affinity and basal receptor signaling were the same. However, the Lys251 variant exhibited 40% greater agonist-mediated signaling, measured as binding of [35S]GTPγS to active receptors, and of increased epinephrine-stimulated inositol phosphate accumulation (149). Thus this is a gain-of-function polymorphism.

Other 2A-adrenergic receptor polymorphisms are notable for their effects not on receptor function, but on the level of receptor expression. The Liggett group comprehensively analyzed polymorphisms surrounding the small, intronless 2A-adrenergic receptor gene, described 17 major haplotypes, and evaluated their functional significance by expression in a human neuroblastoma cell line (288). Different haplotypes were associated with lower, equivalent, or higher expression than the reference group.

Clinical genotype-phenotype association studies of 1A-adrenergic receptors mostly genotyped by restriction fragment length polymorphism (RFLP), and therefore did not account for combinations of linked nucleotide changes, or haplotypes, as did Liggett. Nevertheless, studies have described associations between the noncoding rs553668 SNP and hypertension or other autonomic responses (sweating, platelet aggregation) (89, 95, 191, 212, 303). However, the largest and most comprehensive examinations of 2-adrenergic receptor polymorphisms and blood pressure response, performed by the Insel (82) and Victor laboratories (176), failed to detect any effect of 2A-, 2B-, or 2C-adrenergic receptor polymorphisms on extremes of blood pressure or on the pressor response to yohimbine. Thus existing data are not sufficient to support a major effect of 2-receptor polymorphisms in essential hypertension.

2A-Adrenergic receptors also transduce epinephrine-stimulated suppression of pancreatic beta cell insulin secretion. For this reason, the 2A-adrenergic receptor knockout mice exhibit enhanced insulin secretion (84), and 2-cell specific 2A-adrenergic receptor overexpression mimics diabetes (60). Recently, a convincing role for 2A-adrenergic receptor polymorphisms in human type 2 diabetes was elucidated in a fascinating study that took advantage of experimental models to bridge the gap to human type 2 diabetes (265). Rosengren et al. (265) identified a tight genetic locus for diabetes using congenic Goto-Kakizaki diabetic rat strains carrying the Niddm1i insulin resistance locus. The candidate gene segment con-

### Table 4. Nonsynonymous polymorphisms of human α1B-adrenergic receptors (ADRA1B)

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>SNP Type</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs192448</td>
<td>Coding NS</td>
<td>152</td>
<td>T</td>
<td>G</td>
<td>51</td>
<td>V</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1759418</td>
<td>Coding NS</td>
<td>292</td>
<td>G</td>
<td>A</td>
<td>98</td>
<td>V</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1759420</td>
<td>Coding NS</td>
<td>428</td>
<td>G</td>
<td>A</td>
<td>143</td>
<td>R</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1759427</td>
<td>Coding NS</td>
<td>1168</td>
<td>C</td>
<td>T</td>
<td>300</td>
<td>P</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3432383</td>
<td>Coding InDel</td>
<td>1480</td>
<td>G</td>
<td>—</td>
<td>494</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Indel, insertion-deletion polymorphism. Other abbreviations are as in Table 3.

### Table 5. Nonsynonymous polymorphisms of human α1D-adrenergic receptors (ADRA1D)

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>SNP Type</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs55926349</td>
<td>Coding NS</td>
<td>1315</td>
<td>C</td>
<td>T</td>
<td>439</td>
<td>R</td>
<td>W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs61759832</td>
<td>Coding NS</td>
<td>1603</td>
<td>C</td>
<td>T</td>
<td>535</td>
<td>Q</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3.
tained five genes, including \(\alpha_2A\)-adrenergic receptors. Interestingly, expression of \(\alpha_2A\)-adrenergic receptors was increased in the diabetic rats, and receptor blockade with yohimbine rescued the diabetic phenotype. Based on these findings, over 900 subjects phenotyped for insulin secretion were genotyped for 19 known \(\alpha_2A\)-adrenergic receptor polymorphisms, and the results were validated in a second cohort of almost 5,000 individuals. The rs553668 noncoding SNP was associated with impaired insulin secretion. Case-control analysis showed that this SNP increased risk of type 2 diabetes 1.3- to 1.4-fold. Given the association of this SNP with hypertension, its genotyping will no doubt be included in any future clinical tests for cardiovascular risk gene variants. The gene-association studies with this noncoding polymorphism also provide an example of how focusing on nonsynonymous amino acid changing polymorphisms can miss genuine genetic disease modifiers. Notwithstanding that the preponderance of studies have focused on nonsynonymous SNPs, it will be important in future studies to examine polymorphisms in adrenergic receptor promoter regions that regulate expression, in micro-RNAs that regulate adrenergic receptor translation, and in the case of multi-exon genes, for splice site polymorphisms that can modulate alternate splicing events.

C. \(\alpha_2B\)-Adrenergic Receptor Polymorphisms

Vascular \(\alpha_2B\)-adrenergic receptors have the potential to increase blood pressure by mediating vasoconstriction. Table 7 lists nonsynonymous polymorphisms of human \(\alpha_2B\)-adrenergic receptors and any associations with disease. There is only one relatively common \(\alpha_2B\)-adrenergic receptor polymorphism, a nine nucleotide deletion (del) that eliminates three glutamic acids at positions 301–303 in the receptor’s third intracellular loop (Fig. 9). This area of the receptor is critical for receptor-G protein coupling and can be phosphorylated by GRK to induce homologous desensitization (see Fig. 6). Comparative studies of “wild-type” (also called “ins” for insertion, the opposite of “del” for deletion) and \(\alpha_2B\)-adrenergic receptor del301–303 recombinantly expressed in CHO and COS cells showed no difference in ligand binding affinity, and a minimal difference in coupling to G\(_i\) (287). However, when coexpressed with GRK2, the del301–303 variant was phosphorylated only half as efficiently and failed to undergo homologous desensitization. These results indicate that the \(\alpha_2B\)-adrenergic receptor del301–303 variation might increase long-term receptor signaling by preventing normal agonist-mediated desensitization.

Because of their roles in vascular smooth muscle, increased \(\alpha_2B\)-adrenergic receptor signaling as a consequence of diminished GRK-mediated receptor desensitization has the potential to enhance vasoconstriction, and therefore to exacerbate hypertension. Michael Stein’s group (221, 222) looked carefully for in vivo effects of the \(\alpha_2B\)-adrenergic receptor del301–303 polymorphism by using pharmacological provocation and measuring hand vein constriction. In two small studies of this general design (221, 222), there were no discernable effects of \(\alpha_2B\)-adrenergic receptor del301–303 genotype on venocon-

---

**TABLE 7. Nonsynonymous polymorphisms of human \(\alpha_2B\)-adrenergic receptors (ADRA2B)**

<table>
<thead>
<tr>
<th>rs</th>
<th>SNP Type</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1836146</td>
<td>Coding NS</td>
<td>109</td>
<td>G</td>
<td>A</td>
<td>37</td>
<td>G</td>
<td>S</td>
<td>Increased coupling</td>
<td>289</td>
</tr>
<tr>
<td>rs1800034</td>
<td>Coding NS</td>
<td>119</td>
<td>C</td>
<td>G</td>
<td>40</td>
<td>A</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs8135467</td>
<td>Coding NS</td>
<td>232</td>
<td>A</td>
<td>G</td>
<td>108</td>
<td>N</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800035</td>
<td>Coding NS</td>
<td>798</td>
<td>C</td>
<td>G</td>
<td>251</td>
<td>N</td>
<td>K</td>
<td>Increased coupling</td>
<td>289</td>
</tr>
<tr>
<td>rs34007165</td>
<td>Coding Indel</td>
<td>810</td>
<td>CGAGCGCAGCCGCGCCGC</td>
<td>—</td>
<td>270–276</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1836146</td>
<td>Coding NS</td>
<td>1064</td>
<td>C</td>
<td>T</td>
<td>355</td>
<td>T</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800036</td>
<td>Coding NS</td>
<td>1150</td>
<td>G</td>
<td>T</td>
<td>384</td>
<td>E</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs55368213</td>
<td>Coding NS</td>
<td>1246</td>
<td>T</td>
<td>A</td>
<td>416</td>
<td>C</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs55368213</td>
<td>3’-UTR</td>
<td>1780</td>
<td>G</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>Hypertension, diabetes</td>
<td>89, 95, 191, 212, 265, 303</td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3.
TABLE 7. Nonsynonymous polymorphisms of human α2B-adrenergic receptors (ADRA2B)

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>SNP Type</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs28365031</td>
<td>Coding NS</td>
<td>901 GAAGAGGAG</td>
<td>—</td>
<td></td>
<td>301–304</td>
<td>G</td>
<td>A</td>
<td>Altered coronary vasoconstriction, hypertension, MI, sudden death</td>
<td>164, 291–293, 320</td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3. MI, myocardial infarction.

striction, although the second study described an association with one haplotype consisting of two noncoding polymorphisms in the same gene. It is possible that non-coding polymorphisms in the α2B-adrenergic receptor gene can have functional consequences, for example, on receptor expression (36), but there are no definitive data demonstrating that in this instance. It is also possible that the dorsal hand vein is not the optimal vessel for examining α2B-adrenergic receptor-mediated vasoconstriction and that a greater difference may be discerned in more reactive arterial tissue. For example, Snapir et al. (292) examined coronary vasoconstriction (measured as coronary flow velocity) in 16 young individuals without heart disease and found that individuals heterozygous for the del301–303 α2B-adrenergic receptor polymorphism had a smaller increase in coronary flow after intravenous administration of epinephrine. Thus there is at least one study supporting the notion that the loss-of-function del301–303 α2B-adrenergic receptor is associated with loss of α-adrenergic-stimulated vasoconstriction in humans in vivo.

An interesting study from Sweden pursued a positive genetic association between a locus on chromosome 2 that included the α2B-adrenergic receptor gene and hypertension in Scandinavians (321). The prevalence of α2B-adrenergic receptor del301–303 was assessed in over 900 individuals with primary hypertension, compared with ~800 normotensive controls (320). The homozygous deletion genotype was associated with early-onset hypertension (onset at <50 years of age). Excluding early onset disease, homozygous deletion was weakly associated with hypertension in nondiabetic subjects.

Two studies have explored associations between multiple adrenergic receptor polymorphisms and hypertension. Iacoviello et al. (136) did not detect an association between del301–303 and family history of hypertension, and Snapir et al. (291) also did not see an association of this polymorphism with hypertension in a prospective population-based study of cardiac risk factors. Thus the aggregate data to date do not suggest an important effect of this polymorphism on systemic hypertension.

As noted above, available data (much of it generated by the same group) are more suggestive that the α2B-adrenergic receptor del301–303 polymorphism can alter coronary vasoreactivity, the risk of coronary artery disease, and associated clinical sequelae. Based on the observation that vascular α2B-adrenergic receptors could mediate coronary vasoconstriction after pharmacological provocation (292), a number of studies have examined the relationship between α2B-adrenergic receptor del301–303 and acute myocardial infarction or sudden cardiac death, both of which involve coronary vasospasm. Snapir et al. (291) first reported that α2B-adrenergic receptor del301–303 is associated with acute myocardial infarction in a prospective analysis of 900 middle-aged Finnish men. Subjects homozygous for del301–303 had over twice the risk of an acute coronary event than individuals who carried at least one wild-type α2B-adrenergic receptor allele [relative risk (RR) = 2.2]. In a follow-up study, the same group assessed α2B-adrenergic receptor del301–303 genotype in 683 cases of out-of-hospital sudden death (293). Individuals homozygous for del301–303 were at increased risk for sudden cardiac death (RR = 2.0) and fatal myocardial infarction (RR = 2.1). The risk was greater in individuals who had experienced their lethal event before age 55 (RR = 4.5–5.0).

Recently, Laukkanen et al. (164) reported the results of a prospective study of middle-aged Finnish men that strongly supports the conclusion that α2B-adrenergic receptor del301–303 confers increased risk for sudden cardiac death. Over 1,600 middle-aged Finnish men were followed for an average of 17 years, during which there were 76 sudden cardiac deaths. All were genotyped for α2B-adrenergic receptor del301–303 and classified as homozygous del (21%), homozygous ins (i.e., “wild-type”) (29%), or heterozygous del/ins (50%). Risk-adjusted Cox modeling showed that carriers of the del polymorphism (i.e., those subjects with one or two del301–303 alleles)
had increased risk of sudden cardiac death (RR = 1.97). These data are provocative but represent a very select population. The relevance of these findings to more diverse populations is uncertain but is certainly testable in existing large cohorts.

Given the intense interest in possible relationships between the analogous deletion polymorphism of \( \alpha_{2c} \)-adrenergic receptors and heart failure (see below), it is interesting that there have been no large clinical studies that have specifically examined a similar association with the \( \alpha_{2b} \)-adrenergic receptor deletion polymorphism.

**D. \( \alpha_{2c} \)-Adrenergic Receptor Polymorphisms**

Presynaptic \( \alpha_{2c} \)-adrenergic receptors inhibit norepinephrine release from myocardial (and other) presynaptic sympathetic nerves (119). Thus activation of \( \alpha_{2c} \)-adrenergic receptors initiates a negative-feedback mechanism that interrupts sympathetic tone in conditions, including hypertension and heart failure, where catecholaminergic stimulation is increased.

Table 8 lists nonsynonymous polymorphisms of human \( \alpha_{2c} \)-adrenergic receptors and their associations with human disease. The most studied \( \alpha_{2c} \)-adrenergic receptor polymorphism is an in-frame 12 nucleotide deletion that eliminates four amino acids (Gly-Ala-Gly-Pro 322–325) within the third intracellular loop, which is critical for G protein coupling and GRK-mediated desensitization (see Fig. 10). Functional analyses of recombinant wild-type and del322–325 receptor expressed in CHO cells revealed that the del322–325 receptor has decreased coupling to \( G_i \), manifested as loss of the high-affinity binding characteristic of coupled receptors and impaired agonist-mediated inhibition of adenylyl cyclase (289).

A number of studies have looked for associations between the \( \alpha_{2c} \)-adrenergic receptor del322–325 polymorphism and hypertension, but the results of large trials have shown no association (82, 160, 176). On the basis of these results, it seems reasonable to exclude this polymorphism as a major risk modifier for hypertension.

The data are more suggestive for a meaningful pathophysiologic effect of \( \alpha_{2c} \)-adrenergic receptor del322–325 in heart failure. The first, and most provocative, study was by Small et al. (290) who reported that the combination of homozygosity for \( \alpha_{2c} \)-receptor del322–325 and \( \beta_1 \)-adrenergic receptor Arg389, seen almost exclusively in individuals of African decent, increased the risk of developing heart failure 10-fold. The scientific rationale for this clinical finding is very attractive, as the net effect of the combination of these two polymorphisms would be to dramatically increase myocardial adrenergic signaling via gain of function by the Arg389 \( \beta_1 \)-adrenergic receptor and loss of normal synaptic inhibition by the \( \alpha_{2c} \) del 322–325 adrenergic receptor. If true, this also represents one of the most powerful genetic risk factors for heart failure ever described (increasing risk 10-fold) and would certainly merit genetic testing for increased surveillance and to implement preventative measures. However, there are reasons to be cautious about these results before accepting them and applying them more generally to medical practice. The study was relatively small by today’s standards, consisting of only 159 heart failure patients and 189 controls. Furthermore, the positive results were seen only in the subgroup of 78 African American heart failure subjects, of whom 23 were homozygous for Arg389 at the \( \beta_1 \)-receptor and 41 were homozygous for \( \alpha_{2c} \)-adrenergic receptor del322–325. Case-control analysis showed no dif-

**TABLE 8. Polymorphisms of human \( \alpha_{2c} \)-adrenergic receptors (ADRA2C)**

<table>
<thead>
<tr>
<th>SNP</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs73076814</td>
<td>Coding NS</td>
<td>760</td>
<td>G</td>
<td>C</td>
<td>254</td>
<td>D</td>
<td>H</td>
<td>289, 290</td>
</tr>
<tr>
<td>rs11767072</td>
<td>Coding Indel</td>
<td>964</td>
<td>GGGCGGGGCG</td>
<td>—</td>
<td>322–325</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800037</td>
<td>Coding NS</td>
<td>1113</td>
<td>G</td>
<td>C</td>
<td>371</td>
<td>K</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>rs1133450</td>
<td>Coding NS</td>
<td>1202</td>
<td>G</td>
<td>T</td>
<td>401</td>
<td>S</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>rs1133452</td>
<td>Coding NS</td>
<td>1337</td>
<td>G</td>
<td>C</td>
<td>446</td>
<td>R</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>rs11269124</td>
<td>3′-UTR Indel</td>
<td>1457</td>
<td>GGGAGCTTTCGCCAGACCC</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3.
ference in heart failure risk (i.e., no disproportionate representation by genotype in cases versus controls) for either adrenergic receptor genotype in Caucasians, and no difference in risk conferred by the Arg389 β1-adrenergic receptor polymorphism in African Americans. The data indicated a fivefold increase in heart failure risk for African Americans homozygous for the α2C-deletion polymorphism, and a further doubling of this risk in subjects homozygous for both the α2C-del and the β1-adrenergic receptor Arg389. However, there were only 17 African Americans (2 controls and 15 heart failure) with this combination of genotypes, which is a very small number for case-control comparison and raises the possibility of type 1 error, i.e., a false-positive association.

Because of these limitations, confirmation of an association between the α2C-adrenergic receptor del322–325 polymorphism and increased heart failure risk in a much larger cohort is essential before this genotype-phenotype correlation can be fully accepted. Unfortunately, such a study has not been completed. The largest attempt at validation included 1,121 African American and 740 Caucasian subjects from the Dallas Heart Study, a cross-sectional study that looked at associations between markers or precursors of heart failure and the α2C-receptor deletion and β1-receptor position 389 polymorphisms (34). Although this was not a case-control study of heart failure, it was adequately powered to detect associations between the polymorphisms and heart failure-associated measures of ventricular contractile performance, ventricular dimension, and circulating levels of the heart failure marker brain natriuretic peptide (BNP). However, no significant associations were detected. A small case-control study from South Africa also found no difference in prevalence of the α2C-del polymorphism in 37 African heart failure cases, compared with 34 controls (73). A small case-control study from Japan (n = 91 idiopathic cardiomyopathy patients and 119 controls) looked for associations with the α2C-receptor deletion polymorphism and the β1-receptor position 49 and 389 polymorphisms (223). Although none of their results achieved statistical significance, there was a trend for the α2C-receptor deletion polymorphism to protect against heart failure, i.e., the association was in the opposite direction of the original Small/Liggett report. Finally, a small study found that the prevalence of the α2C-del polymorphism was similar in heart failure patients and controls, but when they compared heart failure patients with and without the α2C-del polymorphism, those who carried the allele had more severe heart failure, measured as symptomatic status and by objective invasive hemodynamic measures (21). While these results are intriguing, at this time the accumulated data are not sufficient to indicate that the α2C-receptor deletion polymorphism constitutes a significant risk factor for development of heart failure.

An alternate hypothesis is that the α2C-receptor del322–325 polymorphism can affect heart failure outcome after onset of the disease. Indeed, a recent larger study by the Liggett group proposed an effect of the α2C-del polymorphism on heart failure survival, but the association was complicated by multiple epistatic interactions between the α2C- and β1-adrenergic receptor genes that have both the potential to influence heart failure outcome and to confound interpretation of the results (146). Other studies have also looked for differences in heart failure outcome as a function of α2C-adrenergic receptor del genotype. A German longitudinal survey of outcomes in 345 severe heart failure patients followed for an average of almost 5 years found that the α2C-receptor del polymorphism was a powerful positive predictor of outcome, i.e., that it was protective against death or transplant (255). This study benefits from a relatively long follow-up period and a high proportion of subjects who reached one of the study end points of death, implantation of a left ventricular mechanical assist device (LVAD) as a bridge to cardiac transplantation, or cardiac transplantation itself. In contrast, an analysis of adrenergic receptor polymorphisms in the 526 patient MERIT-HF cohort found no association between α2C-adrenergic receptor del322–325 genotype, alone or in combination with β1-adrenergic receptor Arg389 genotype, on adverse events in heart failure (273). Likewise, Sehnert et al. (274) reported no association between α2C-receptor del322–325 and heart failure outcome in 637 patients followed for an average of 3 years.

A limitation of the MERIT-HF genetic substudy (273) is that the results were not stratified by β-blocker treatment status. Likewise, in the Sehnert study, all patients were treated either with carvedilol or metoprolol (274). Thus there is the potential for the α2C-adrenergic receptor del polymorphism to have undetected effects that are influenced or obfuscated by β-blocker therapy. This has been examined. A positive association between the left ventricular functional response to β-blocker therapy in 54 heart failure patients and carriers of the α2C deletion who were also homozygous Arg for the β1-adrenergic receptor 389 polymorphism was described with the β1-adrenergic receptor specific blocker metoprolol (190). A recent analysis of the BEST genetic substudy (14) correlated absence of a mortality benefit with increased sympathetic effects of the experimental β-blocker bucindolol in α2C-adrenergic receptor del322–325 carriers (27). This is an interesting observation because bucindolol has sympatholytic effects that are not shared by most β-blockers in clinical use. Subjects carrying one or two α2C-adrenergic receptor del322–325 alleles showed enhanced sympatholysis, measured as venous norepinephrine levels, in response to bucindolol, indicating an additive pharmacogenetic effect. However, only subjects homozygous for “wild-type” α2C-adrenergic receptor ins322–325 showed evidence for
any mortality benefit from bucindolol. Notwithstanding these provocative findings, a pharmacogenomic interaction between the \( \alpha_{2C} \)-adrenergic receptor ins/del322–325 polymorphism and \( \beta \)-blocker therapy has not been seen in all studies. Thus, before the \( \alpha_{2C} \)-deletion polymorphism can be accepted as a risk modifier or determinant of therapeutic response in human heart failure, either alone or in synergism with \( \beta_1 \)-adrenergic receptor Arg389 (290), gene variant-disease and gene variant-therapeutic response associations need to be confirmed and validated in large, prospective studies.

E. \( \beta_1 \)-Adrenergic Receptor Polymorphisms

1. Experimental studies

Through their effects to increase heart rate and cardiac contractility, \( \beta_1 \)-adrenergic receptors are the most important receptors acutely modulating minute-by-minute cardiac output. Table 9 lists nonsynonymous polymorphisms of human \( \beta_1 \)-adrenergic receptors and any reported associations with human disease. The two most studied \( \beta_1 \)-adrenergic receptor polymorphisms are the Ser49Gly variant at the extracellular amino terminus of the receptor, and the Arg389Gly variant located within a predicted fourth intracellular loop formed by a stretch of \( \sim \)12 amino acids from the distal seventh transmembrane-spanning domain to the membrane-anchoring palmitoylated cysteines (Fig. 11). Like the third intracellular loop, this region is important for receptor-\( G_s \) coupling and for GRK-dependent desensitization.

![Location of clinically significant \( \beta_1 \)-adrenergic receptor polymorphisms.](http://physrev.physiology.org/)

2. Experimental data

The Liggett group evaluated the function of \( \beta_1 \)-adrenergic receptor Ser49 and Gly49 variants in comparative studies after recombinant expression in CHW and HEK-293 cells and found no difference in Gly49 \( \beta_1 \)-adrenergic receptor-expressing HEK-293 cells. In contrast, Levin et al. (174) found that basal and agonist-stimulated adenylyl cyclase activities were increased in Gly49 \( \beta_1 \)-adrenergic receptor-expressing HEK-293 cells. Both studies agreed that agonist-stimulated downregulation of Gly49 \( \beta_1 \)-adrenergic receptors was enhanced. Since the position of Gly49...

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>SNP Type</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs34844626</td>
<td>Coding NS</td>
<td>77</td>
<td>C</td>
<td>T</td>
<td>26</td>
<td>A</td>
<td>V</td>
<td>Downregulation</td>
<td>174, 250</td>
</tr>
<tr>
<td>rs35720003</td>
<td>Coding NS</td>
<td>85</td>
<td>G</td>
<td>A</td>
<td>29</td>
<td>A</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs35290916</td>
<td>Coding NS</td>
<td>92</td>
<td>G</td>
<td>A</td>
<td>31</td>
<td>R</td>
<td>Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs72566521</td>
<td>Coding NS</td>
<td>118</td>
<td>G</td>
<td>A</td>
<td>40</td>
<td>A</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1801252</td>
<td>Coding NS</td>
<td>145</td>
<td>A</td>
<td>G</td>
<td>49</td>
<td>S</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1760024</td>
<td>Coding NS</td>
<td>434</td>
<td>G</td>
<td>T</td>
<td>145</td>
<td>S</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs238741</td>
<td>Coding NS</td>
<td>952</td>
<td>C</td>
<td>A</td>
<td>318</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs822307</td>
<td>Coding NS</td>
<td>971</td>
<td>A</td>
<td>G</td>
<td>324</td>
<td>K</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs180897</td>
<td>Coding NS</td>
<td>1027</td>
<td>G</td>
<td>A</td>
<td>343</td>
<td>A</td>
<td>T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs189429</td>
<td>Coding NS</td>
<td>1056</td>
<td>G</td>
<td>T</td>
<td>352</td>
<td>E</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1801253</td>
<td>Coding NS</td>
<td>1165</td>
<td>C</td>
<td>G</td>
<td>389</td>
<td>R</td>
<td>G</td>
<td>Gain of function, heart failure risk and outcome</td>
<td>54, 143, 202, 211, 251, 259, 271, 290, 323</td>
</tr>
<tr>
<td>rs17875425</td>
<td>Coding NS</td>
<td>1166</td>
<td>G</td>
<td>T</td>
<td>389</td>
<td>R</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs90050253</td>
<td>Coding NS</td>
<td>1196</td>
<td>G</td>
<td>A</td>
<td>399</td>
<td>R</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs171170</td>
<td>Coding NS</td>
<td>1199</td>
<td>G</td>
<td>T</td>
<td>400</td>
<td>R</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs57038389</td>
<td>Coding NS</td>
<td>1213</td>
<td>C</td>
<td>T</td>
<td>405</td>
<td>H</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs61760026</td>
<td>Coding NS</td>
<td>1223</td>
<td>G</td>
<td>C</td>
<td>408</td>
<td>R</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs238740</td>
<td>Coding NS</td>
<td>1380</td>
<td>C</td>
<td>A</td>
<td>460</td>
<td>D</td>
<td>E</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3.
is ~10 amino acids from a known β1-adrenergic receptor glycosylation site, it is possible that altered glycosylation is a mechanistic explanation for the otherwise paradoxical finding that an amino acid substitution in the extracellular receptor domain can affect receptor downregulation (which is determined by phosphorylation of intracellular residues). Indeed, the pattern of migration of Ser49 β1-adrenergic receptors on polyacrylamide gels differs from that of the Gly49 receptor (consistent with altered glycosylation), and the high-molecular-weight species of Ser49 β1-adrenergic receptors is sensitive to inhibitors of N-glycosylation (250). Although these are interesting observations, a precise molecular mechanism for apparent altered glycosylation, or for accelerated downregulation, of the Gly49 β1-adrenergic receptor has not been proposed.

The function of recombinantly expressed β1-adrenergic Arg389 and Gly389 receptors has also been evaluated in cultured Chinese Hamster fibroblasts (CHW cells) (202). Radioligand binding studies showed that the high-affinity agonist binding site, which is characteristic of an active, G protein-coupled receptor, was apparent only for Arg389 receptors and difficult to detect for Gly389 receptors. Arg389 radioligand binding curves likewise showed a typical rightward shift upon addition of the nonhydrolyzable GTP analog GppNHp (showing a transition from the G protein-coupled to uncoupled state), while Gly389 binding curves were unaffected by the guanine nucleotide. Both of these results suggest that, compared with Arg389 receptors, the Gly389 β1-adrenergic receptor resists adopting the high-affinity G protein-coupled conformation necessary to transduce a cell signal. In other words, the Arg389 receptor signals through its G protein (Gαs) more readily than Gly389 receptors. Furthermore, in the presence of GppNHp (i.e., in the uncoupled-from-signaling receptor conformation), the low-affinity (G protein-uncoupled) binding site was the same for both receptors, demonstrating that β1-adrenergic receptor affinity for ligands is not affected by the position 389 polymorphism. Consistent with the conformational changes noted above, basal and isoproterenol-stimulated adenyl cyclase activities were greater for the Arg389 receptor than Gly389 receptor. Together, these studies show that the Arg389 β1-adrenergic receptor has greater coupling to adenyl cyclase than the Gly389 receptor, with no change in ligand binding. These conclusions were reinforced in a more recent study (143), showing that the Arg389 β1-adrenergic receptor exhibits enhanced coupling to Gαs without any change in affinity for the β-blockers metoprolol, carvedilol, bisoprolol, or propranolol. The observation that Arg389 β1-adrenergic receptors have a conformation more favorable for agonist-promoted coupling to Gαs than the Gly389 receptors further suggested that agonist-promoted, GRK-mediated desensitization might also be enhanced. Indeed, desensitization was ~50% greater for Arg389 β1-adrenergic receptors, compared with Gly389 β1-adrenergic receptor (251).

To assess the relevance of the position 389 β1-adrenergic receptor polymorphism on integrated cardiovascular function, the Liggett and Dorn laboratories created and analyzed transgenic mice with cardiac myocyte-specific (α-myosin heavy chain promoter driven) expression of the Arg389 or Gly389 human β1-adrenergic receptors (211). Since we had previously shown that high levels of β-adrenergic receptors expressed with this transgenic system can result in spontaneous cardiomyopathy, and that the development of cardiomyopathic features was not only receptor “dose” dependent but also time dependent (180), for these studies we selected two lines with modest and equivalent receptor expression levels, and then performed comparative studies of young and old Arg389 or Gly389 β1-adrenergic receptor transgenic mice. Cardiac function was examined using noninvasive echocardiographic measures for serial studies, and microminiaturized invasive catheterization-based techniques for terminal studies. Histological examinations looked for evidence of cardiac myocyte drop-out in the form of replacement fibrosis. Consistent with findings from the cell-based studies (that the Arg389 β1-adrenergic receptor couples more efficiently to Gαs; see above), basal and peak dobutamine-stimulated cardiac isovolumic contraction (measured as the first derivative of pressure by time, +dP/dt) in younger mice was greater for Arg389 than Gly389 receptor transgenic mice. Interestingly, by 6 mo of age, Arg389 mice no longer exhibited a contractile response to dobutamine, although basal contractile function remained elevated compared with Gly389 mice. We interpreted these findings as revealing receptor desensitization specific to the Arg389 receptor at this age and identified three contributory mechanisms: decreased functional Gαs coupling of Arg389 receptors, decreased myocardial Gαs protein levels, and diminished expression of cardiac adenyl cyclase. Each of these biochemical findings paralleled a loss of contractile performance, increased myocardial fibrosis, and signs of overt heart failure by 9 mo of age.

These data were consistent with the broader notion that increased cardiomyocyte β-receptor signaling by any means, including chronic agonist stimulation (215), increased receptor expression (71, 180), or enhanced intrinsic receptor signaling (211) can ultimately result in cardiac toxicity and contribute to heart failure. These data also provided an additional rationale for β-blocker therapy in heart failure. Accordingly, we evaluated whether the β1-adrenergic receptor Arg389 polymorphism might not only affect β-receptor signaling and predispose to experimental heart failure, but would also help determine β-blocker responsiveness. Experiments performed in isolated, perfused mouse hearts revealed that acute infusion of the nonselective β-blocker propranolol induced a greater negative inotropic (decreased contractility) re-
sponse for Arg389 β1-adrenergic receptor transgenic hearts, while Gly389 receptor hearts were relatively less sensitive to the β-blocker and exhibited a negative inotropic response only at high concentrations. Likewise, in vivo examination of the effects of chronic oral propranolol administration showed a negative chronotropic effect (decreased heart rate) only in Arg389 mice.

Liggett’s group recently extended these studies by comparing transcriptional (mRNA) profiles in mice expressing the human β1 Arg389 or Gly389 adrenergic receptor, and a mouse that has receptor-independent activation of adenylyl cyclase signaling, the adenylyl cyclase type V transgenic mouse (305). Over 1,000 mRNAs were uniquely regulated in Arg389 transgenic hearts, whereas only 245 mRNAs were uniquely regulated in Gly389 hearts. Gene-ontology analysis identified mRNA clusters that were uniquely modulated by Arg389, the most significant of which involved regulation of extracellular matrix-associated genes known to be activated directly or indirectly by transforming growth factor (TGF)-β. Since these studies were performed on young mice before they developed their respective myocardial pathologies (211, 309), these results are more likely to reflect direct effects of altered β-adrenergic signaling by the Arg389 β1-adrenergic receptor on gene expression and suggest a mechanism for pathological effects of this receptor in addition to simple induction of cardiomyocyte death.

A novel experimental platform supports the findings of functional differences between Arg389 and Gly389 β1-adrenergic receptors and has provided additional insight into the mechanisms involved and the different pharmacokinetic interactions of β-blockers used in heart failure. In the first report to employ intramolecular fluorescence resonance energy transfer (FRET) to explore differences in drug effect on polymorphic β1-adrenergic receptors, Rochais et al. (259) examined the molecular configuration of the Arg389 and Gly389 receptors after binding of an agonist (norepinephrine) or the three β-blockers used in heart failure. Different conformations of agonist- and β-blocker-occupied receptor were assessed as the read-out for inverse agonist activity, i.e., a receptor conformation that turns off all signaling. The researchers observed that the conformation of metoprolol- and bisoprolol-occupied receptor did not differ between Arg389 and Gly389, whereas the Arg389 receptor was much more sensitive to inverse agonism by carvedilol than the Gly389 receptor. These studies further demonstrate that polymorphisms at β1-adrenergic receptor amino acid 389 can direct different responses to individual β-blockers.

Taken together, biochemical, functional, and structural studies that compared position 389 β1-adrenergic receptor polymorphism effects using cell expression or genetic mouse models all indicated that (compared with the Gly389 receptor) the Arg389 β1-adrenergic receptor exhibits a gain-of-signaling function that can initially improve cardiac contractility, but ultimately predisposes to cardiomyopathic decompensation. The accumulated data further indicate that the abnormally active Arg389 receptor is more sensitive to pharmacological β-blockade and that the distinct pharmacological properties of different β-blockers can affect polymorphic receptor-drug interactions. From the opposite perspective, it might be construed that the Gly389 β1-adrenergic receptor functions almost as if it were already “β-blocked.” Thus β1-adrenergic receptor hyperactivity conferred by Arg at position 389 may represent an opportunity to use β-blockers with greater effect in diseases responsive to antiadrenergic therapy.

3. Human studies in hypertension

Of the three β-adrenergic receptor subtypes, β1-adrenergic receptors are more common in myocardium and represent a minor isoform in vascular smooth muscle where β2-adrenergic receptors predominate. However, blood pressure is not determined only by changes in vascular tone, but also by the cardiac output, which itself is determined by heart rate and myocardial contraction. Thus a number of groups have examined the consequences on hypertension of the β1-adrenergic receptor position 49 polymorphism that is thought to alter receptor downregulation and the position 389 polymorphism that alters receptor signaling.

An early study from Sweden analyzed 102 discordant sibling pairs and described higher heart rates for individuals homozygous for Arg389. The same report describes a case-control analysis of 292 nondiabetic hypertensives and 265 normotensive controls that showed increased risk for hypertension in homozygous Arg389 subjects (9); there was no association between the Ser49Gly polymorphism and hypertension. Similar associations between parameters of increased cardiac function and Arg389 were concomitantly reported by the Johnson group (134), and subsequently by the CAREGENE study from Belgium (59). In only two studies does the position 49 β1-adrenergic receptor polymorphism show any apparent association with cardiac function. One study evaluated heart rate and blood pressure at baseline and after mental challenge as a function of several α- and β-adrenergic receptor polymorphisms (206). The other was a study of >1,000 Asian subjects genotyped for the β1-adrenergic receptor position 49 and 389 polymorphisms (248). In both instances, Ser49 was associated with a higher resting heart rate.

The result described above by Bengstsson and colleagues, in which the Arg389 genotype correlated with blood pressure in a Swedish cohort (9), was confirmed for male subjects only in a subsequent large (n = 1,880) Japanese study (281). However, other studies have failed
to identify allele differences between hypertensive and normotensive subjects (141, 147, 188). In their report on the effects of adrenergic receptor polymorphisms on hemodynamic changes induced by intravenous epinephrine in healthy males, Snapir et al. (292) found no effects of the β₁-adrenergic receptor Arg389Gly polymorphism. Thus the data have not consistently found an association between either the position 49 or 389 β₁-adrenergic receptor polymorphisms and blood pressure. On the basis of these results, and in the context of greater importance of β₂-adrenergic receptors in vasoconstrictor responses, it is reasonable to conclude at this time that β₁-adrenergic receptor polymorphisms do not play major roles as risk factors in human hypertension.

The possibility that β₁-adrenergic receptor position 49 or 389 polymorphisms might still exert pharmacogenomic interactions that could modify individual responses to β-blocker therapy in hypertension has also been studied. As described in detail in the next section, pharmacogenomics of β₁-adrenergic receptor polymorphisms and β-blockers have been an area of intense interest in heart failure, in which β-blockers not only improve symptoms of the disease, but also prolong life. While hypertension is typically not associated with symptoms, and affects life span only because of its major complications, β-blockers continue to be among the first-line therapeutic choices, and the individual response to antihypertensive therapy is easy to measure in objective terms (systolic and diastolic blood pressure and heart rate). Accordingly, a number of studies have looked at possible effects of β-receptor polymorphisms and hypertensive responses to those β-blockers used to treat clinical hypertension. In a retrospective study, O’Shaughnessy et al. (227) found no difference between baseline blood pressure or response to β-blocker treatment for the Arg389 polymorphism. A similar lack of association was reported from Sweden in essential hypertensive patients treated with atenolol (147), and from Italy in hypertensive subjects treated with bisoprolol (88). In contrast, in a prospective study of 40 subjects, Johnson et al. (141) found that the homozygous Ser49 and Arg389 β₁-adrenergic receptor haplotype had a superior antihypertensive response to metoprolol. A group from China likewise found that the homozygous Ser49 and Arg389 haplotype conferred enhanced metoprolol response in a small study (n = 61). These data suggest that position 49 and 389 β₁-adrenergic receptor polymorphisms, which are in linkage disequilibrium, can have a modest affect on individual cardiovascular responses to β-blocker therapy. It is worth noting that these data do not distinguish between alterations in cardiac contractility, heart rate, or vascular tone as the major determinants of the putative gene-drug interactions. For this reason, it is conceivable that β₁-adrenergic receptor polymorphisms can have a measurable pharmacogenomic effect on blood pressure response to β-blocker therapy, without themselves independently altering the risk of developing hypertension.

4. Human studies of heart failure

Myocardial dominance of β₁-adrenergic receptors points to a possible role in cardiac function, especially for the prevalent Arg389Gly polymorphism that has powerful effects on receptor-Gs coupling and, therefore, on receptor signaling to downstream effectors and end-organ response. It is notable that the region containing this polymorphism, the receptor’s fourth intracellular loop, is highly conserved between species. Indeed, Arg is found at the position analogous to human amino acid 389 in every species from which sequence data are available; the only known exception is the human Gly389 variation. As reviewed above, compared with Gly389, the Arg389 β₁-adrenergic receptor demonstrates enhanced agonist stimulation of adenylyl cyclase, with no change in receptor-ligand binding. A number of studies support a meaningful impact of the β₁-adrenergic receptor 389 polymorphism to modify the risk or progression of, and response to β-blocker therapy in, clinical heart failure.

Bruck et al. (31) directly examined the effects of the β₁-adrenergic receptor 389 polymorphism on cardiac function and plasma renin activity in response to intravenous infusion of the β₁-adrenergic selective agonist dobutamine in 18 male subjects (10 homozygous Arg389 and 8 homozygous Gly389, all homozygous for Ser at amino acid 49) (31). These carefully designed and controlled investigations demonstrated that the response to dobutamine for every end point was greater in Arg389 than Gly389 subjects. Importantly, they also found that the β-blocker bisoprolol was a more effective antagonist of dobutamine-stimulated cardiac and renin responses in the Arg389 subjects. The same group subsequently examined whether the position 389 β₁-adrenergic receptor polymorphism likewise affected the cardiovascular response to exercise, in which the sympathetic nervous system is only one of multiple factors that enhance cardiac function. A similar group of subjects as in the previous study underwent bicycle ergometry after parasympathetic blockade with atropine. However, no differences were detected between homozygous Arg389 and Gly389 subjects on any cardiac end point (170). The results of these two studies demonstrate that enhanced signaling by Arg389 β₁-adrenergic receptors has measurable consequences on human cardiovascular function in response to pharmacological stimulation or inhibition of β-receptors, but also that any such effects are less evident under physiological conditions where multiple factors can interact combinatorially to increase cardiac function. Therefore, it is not surprising that two studies from Scotland have described different associations between β₁-adrenergic receptor polymorphisms and cardiac hypertrophy. Stanton et al. (300) stud-
ied 249 renal failure patients (who typically have elevated blood pressures and develop reactive cardiac hypertrophy from combined pressure and volume overload) and described a significant association between homozygous Gly389 genotype and increased echocardiographic left ventricular mass. In contrast, an analysis of 110 twin pairs suggested a nonsignificant association between homozygosity for Arg389 and increased left ventricular mass (304). Thus the relationship, if any, between the β1-adrenergic receptor position 389 polymorphism and cardiac hypertrophy in humans requires additional study.

In the area of heart failure, Wagoner et al. (323) performed one of the early studies evaluating β1-adrenergic receptor 389 polymorphism effects on exercise in heart failure patients. This study measured peak oxygen consumption during graded exercise testing as an index of clinical heart failure status in 263 patients with moderate to severe (clinical class III/IV) heart failure (323). Peak exercise oxygen consumption (VO2) is a commonly used test to assess clinical status and quantify integrated cardiovascular function in heart failure; maximal oxygen consumption of greater than 14 ml/min/kg is associated with a favorable prognosis (200). Homozygous Arg389 subjects had significantly greater VO2 than homozygous Gly389 subjects (17.7 ± 0.4 vs. 14.5 ± 0.6 ml·kg⁻¹·min⁻¹, P = 0.006), with Arg/Gly 389 heterozygotes having an intermediate result. This study also examined the β1-adrenergic receptor 49 polymorphism and observed an association with VO2 at this position as well. This finding is important because it raises the possibility that linkage disequilibrium between amino acids 49 and 389 in this small, intronless gene contributes to apparent two-genotype associations. Indeed, when the data were analyzed according to the two-locus haplotypes rather than independently for the amino acid 49 and 389 loci, the highest VO2 was in the homozygous Arg389, Ghu49 carrier group, and the lowest VO2 was in the homozygous Gly389, Ser49 carrier group (P = 0.001). An even greater difference in peak exercise VO2 between Arg389 homozygous subjects and individuals carrying at least one Gly389 allele was observed in a subsequent report (271) that, however, did not see any association with the position 49 polymorphism. Thus the apparent position 49 β1-adrenergic receptor polymorphism effect on peak exercise oxygen consumption reported by Wagoner et al. (323) is probably being driven by linkage with the 389 β1-adrenergic receptor polymorphism. Together, these two studies seem to provide strong evidence supporting enhanced cardiac function conferred by the Arg389 β1-adrenergic receptor and demonstrate that these effects can have a meaningful impact in the context of human heart failure.

If increased signaling by Arg389 β1-adrenergic receptors is largely ligand dependent (as suggested by the cell-based and transgenic mouse experiments reviewed above), then pharmacological β-blockade may have increased efficacy in patients having one or two Arg389 β1-adrenergic receptor alleles. This hypothesis was specifically tested and supported by our transgenic mouse studies (see above) (211). It has also been evaluated in several clinical investigations. In a small retrospective study of 224 heart failure patients that accompanied our transgenic mouse data, we examined the effects of the 389 polymorphism on therapeutic response to the β-blocker carvedilol by measuring echocardiographic left ventricular ejection fraction (LVEF) after at least 6 mo of treatment (211). Carvedilol dose was the same in both genotype groups, but Arg389 homozygotes showed a greater improvement in LVEF than Gly389 homozygotes (8.7 ± 1.1 vs. 0.93 ± 1.7%, P < 0.02). Arg/Gly389 heterozygotes were similar to the Arg homozygotes, suggesting a dominant effect of the gain-of-receptor signaling function allele on left ventricular contractile performance. A group from China examined the response to the β1-selective antagonist metoprolol in eight young men homozygous for Arg389, compared with eight men homozygous for Gly389 (187), and likewise found that the heart rate response to metoprolol at rest and with exercise was greater for Arg389 subjects. Johnson’s group (311) prospectively analyzed the effects of β1-adrenergic receptor 389 and 49 genotypes on metoprolol up-titration in 61 β-blocker naive heart failure patients. Although cardiac decompensation did not differ between either the 389 or 49 locus genotypes, subjects carrying the Gly389 polymorphism required greater increases in their heart failure medications than Arg389 patients, suggesting a higher sensitivity to low-dose β-blockade in Arg389 subjects. (An association was also seen with homozygous Ser49 subjects, although as noted above, this may be driven by linkage with the Arg389 genotype.) A follow-up paper that reported the baseline and follow-up echocardiographic data from this same cohort showed that the Arg389 homozygous subjects underwent beneficial remodeling after 3 mo of the maximum metoprolol therapy, defined as reduction in left ventricular systolic and diastolic dimensions (310).

Greater left ventricular reverse remodeling (in simplistic terms, normalization of heart size) in heart failure patients with the Arg389 β1-adrenergic receptor polymorphism after nonselective β-blockade with carvedilol was recently confirmed by Chen et al. (42). This study from Australia compared pre- and posttreatment (at least 1 yr) echocardiograms of 135 mild to moderate severity (clinical class II–III) heart failure patients and found that ejection fraction improved by 18.8% in Arg389 homozygotes, by 9.4% in heterozygotes, but only by 6% in Gly 389 homozygotes (P < 0.001). Finally, the Johnson group (190) also evaluated effects of the β1-adrenergic receptor position 389 polymorphism in combination with the α2c-receptor del polymorphism on echocardiographic cardiac function ~6 mo after starting metoprolol in 54 β-blocker...
naive heart failure patients. (The combination of Arg389 β1-adrenergic receptor and α2C-receptor del polymorphism had previously been identified as increasing the risk for heart failure, Ref. 290). Patients homozygous for β1-adrenergic receptor Arg389 showed greater improvement than Gly389 carriers. Homozygous Arg389 patients who also carried the α2C-receptor del polymorphism had an even greater improvement in left ventricular ejection fraction, whereas the reciprocal genotype (homozygous Gly389 and no α2C-receptor del allele) showed no significant improvement from metoprolol. The consensus of all these studies is that subjects with the more active and potentially harmful combination of α2C- and β1-adrenergic receptor polymorphisms appeared to derive the greatest benefit from β-blocker therapy.

The transgenic mouse experiments and these human clinical studies point to a pharmacogenomic interaction between the 389 β1-receptor variant and β-adrenergic agonists/antagonists. This observation led to a detailed and intriguing study performed by Liggett et al. (179) that characterized interactions between β1-adrenergic receptor position 389 genotype and clinical response to the experimental β-blocker bucindolol in heart failure subjects. This study is especially valuable because it evaluated the functional consequences of β1-adrenergic receptor Arg or Gly389 on in vitro myocardial contraction assessed in studies of human failing and nonfailing right ventricular trabeculae, as well as on heart failure survival using data from the randomized Beta-Blocker Evaluation of Survival Trial (BEST) cohort (179). For the organ bath studies, “nonfailing” trabeculae were obtained from unused transplant donor hearts and from end-stage “failing” hearts (the explants from cardiac transplant recipients). Radioligand binding experiments showed similar β-adrenergic receptor expression in membranes from Arg389 and Gly389 hearts. However, trabeculae from nonfailing and failing homozygous Arg389 hearts displayed greater maximal contractile force than those from Gly389 carriers (i.e., one or two Gly389 alleles), although the magnitude of differences between genotypes was only half as great in the failing tissue. Liggett et al. (179) have proposed that this difference between normal and failing hearts is due to enhanced desensitization of Arg389 β1-adrenergic receptors under the conditions of catecholamine excess that typify severe heart failure. Of most interest, forskolin-contracted trabeculae from failing Arg389 or Gly389 hearts showed equivalent negative inotropic responses to carvedilol (i.e., carvedilol acted as a neutral β-adrenergic receptor antagonist for both genotypes), whereas bucindolol decreased contractile force only in Arg389 hearts (i.e., bucindolol acted as an inverse β-adrenergic agonist for Arg389 β1-adrenergic receptors only). Although the experimental design used forskolin to precontract the muscle fibers, which completely bypasses β-receptor signaling to directly stimulate downstream adenyl cyclase, the results support the idea that different intrinsic pharmacological properties of β-blockers can determine pharmacogenomic interactions.

The consequences of the pharmacogenomic interaction between bucindolol and the β1-adrenergic receptor position 389 polymorphism on heart failure survival were assessed in the same study. Genotyping was performed on 1,040 DNAs archived from BEST (179), a prospective, double-blinded, placebo-controlled trial of the β-blocker bucindolol in moderate-to-severe (clinical class III–IV) heart failure (14). Study end points included all-cause mortality, heart failure hospitalizations, and the combined end point of mortality and hospitalization. The study design compared outcomes between placebo and bucindolol treatment groups, stratified by 389 position β1-adrenergic receptor genotype. Because the randomized study included a placebo arm, effects of the polymorphism on heart failure progression were assessed both in the absence of β-blocker and with bucindolol treatment. It is important to note that this design makes for four study groups (treated or not, stratified by Arg or Gly polymorphism), but the Kaplan-Meier survival and Cox proportional-hazards regression analyses were assessed with significance defined as a P value of <0.05, without the usual Bonferroni-type adjustment for multiple-group comparisons. The placebo data showed that Arg389 or Gly389 β1-adrenergic receptor genotype did not change survival in β-blocker-untreated subjects with moderate to severe heart failure. The only group with survival that differed (at P < 0.05) from others consisted of patients homozygous for Arg389 β1-adrenergic receptor who were treated with bucindolol; Gly389 carriers had identical survival whether they received placebo or bucindolol. These results indicate either absence of efficacy of bucindolol for these heart failure outcomes, or resistance to bucindolol for patients carrying one or two Gly389 β1-adrenergic receptor alleles. Among homozygous Arg389 patients, bucindolol was superior to placebo, with a hazard ratio (HR) = 0.62, 95% confidence interval (C.I.) = 0.40–0.96, P = 0.03. This same comparison in Gly carriers revealed no difference in survival (HR = 0.90, 95% C.I. = 0.62–1.30, P = 0.57), indicating improved longevity with bucindolol for the homozygous Arg389 genotype only (although bucindolol does not appear to have the same efficacy for this end point as the β-blockers approved for heart failure, carvedilol, and metoprolol, so this conclusion must be viewed in the proper context; Ref. 14). An effect of the 389 polymorphism on the secondary end point of heart failure hospitalization in the bucindolol-treated group was also suggested: Arg389 patients receiving bucindolol had diminished risk (hazard ratio; HR) for hospitalization (0.64, 95% C.I. = 0.46–0.88, P = 0.006 compared with same genotype receiving placebo), whereas Gly389 carriers showed no benefit of bucindolol compared with placebo (HR = 0.86, 95% C.I. = 0.64–1.15, P = 0.30). For the
combined outcome of mortality or time to first heart failure hospitalization, a significant benefit was seen with bucindolol treatment in homozygous Arg patients compared with placebo (HR = 0.66, 95% C.I. = 0.50–0.88, P = 0.004), but not for Gly389 carriers (HR = 0.87, 95% C.I. = 0.67–1.11, P = 0.250). As in many other studies, stratifying the data by the position 49 β1-adrenergic receptor polymorphism provided no additional predictive value. In summary, this study concluded that Arg389 homozygotes are bucindolol responders, whereas Gly 389 carriers are not.

It is difficult to know how important these findings may ultimately be. The study is strengthened because it included a placebo-controlled analysis of heart failure survival. Future studies of this design are unlikely as it would be unethical to remove heart failure subjects from β-blocker therapy that clearly is life-prolonging. A limitation, however, is that the BEST genetic substudy utilized the β-blocker bucindolol, which has unique pharmacological properties not shared by β-blockers approved and commonly used to treat heart failure. Most importantly, bucindolol has sympatholytic effects that are as great as the imidazoline I1-receptor agonist moxonidine, which has been associated with adverse outcomes in heart failure (23, 51). On the other hand, bucindolol has an inverse agonist property that was observed only in Arg389 heart tissue, whereas metoprolol and carvedilol acted as neutral antagonists for both β1-adrenergic receptor position 389 polymorphisms. Finally, the original BEST study was started in the late 1990s, and intercurrent improvements in heart failure therapy unrelated to β-blockers are not reflected in results of retrospective genotyping, so it is possible that the placebo group does not reflect outcomes achieved with current state-of-the-art therapeutic regimens.

Taken together, the results with bucindolol point to a pharmacogenomic interaction between the β1-receptor 389 polymorphism and an experimental β-blocker that has unique sympatholytic and inverse agonist properties (23). The question is whether this gene-drug interaction extends to β-blockers in clinical use to treat heart failure in the United States and elsewhere, metoprolol and carvedilol. Data from other clinical studies may be useful in addressing this question. In an older substudy of MERIT-HF, β-adrenergic receptor polymorphisms were genotyped and used to compare outcomes (332). In this study, almost half of the study subjects had mild heart failure, and the mean follow-up period was only 12 mo, so the study end point was combined hospitalization and death. The analysis compared outcome by genotype in the combined cohort of placebo- and metoprolol-treated patients, and the major conclusion was that Gly389 β1-adrenergic receptor subjects did not have improved overall outcomes. However, since the metoprolol and placebo groups were combined for this genetic substudy, it is not possible to determine whether or not there may have been a pharmacogenetic interaction. de Groot et al. (57) studied 199 β-blocker naive heart failure patients from France before and after treatment with β-blockers, but found no association between β1-adrenergic receptor 389 polymorphism and heart rate or left ventricular ejection fraction. Shin et al. (280) found no correlation between β1-adrenergic receptor 389 genotype and survival in a longitudinal observational study of outcomes in 227 heart failure patients followed for up to 4 yr. A recent retrospective 2-center catheterization-laboratory registry study of 637 heart failure patients treated with carvedilol or metoprolol also found no effect of several adrenergic receptor polymorphisms, including the β1-receptor 389 variant, on survival.

Our group also examined mortality as a function of β1-adrenergic receptor position 389 genotype and β-blocker treatment in a longitudinal study of 2,460 heart failure patients from Cincinnati and Philadelphia (54). Although this was not a placebo-controlled study, since only 82% of patients were being treated with β-blockers (largely carvedilol and metoprolol), we were able to assess outcomes as a function of genotype and nonrandomized β-blocker treatment status. β-Blocker untreated Caucasian patients homozygous for Arg389 had significantly longer survival times (P = 0.044) than β-blocker untreated Gly389 carriers. No survival difference was seen in African Americans, although lower numbers in this subgroup gave the study only marginal power to detect a difference. Compared with untreated patients, subjects who were treated with β-blockers exhibited prolonged survival that was not different between Arg389 homozygotes and Gly389 carriers in either ethnic group. These results support a benefit for the Arg/Arg389 β1-adrenergic receptor genotype in heart failure, which are consistent with findings of smaller studies that evaluated contractile function and/or Vo2 (31, 271, 323). However, the data also suggest that conventional β-blocker therapy is sufficient to overcome any signaling differences between the Arg389 and Gly389 receptors, at least for the end point of mortality in heart failure. Indeed, no clinical heart failure study to date has confirmed a pharmacogenetic interaction between any currently approved β-blocker and the Arg389 β1-adrenergic receptor polymorphism, as was suggested by the BEST substudy. But it must be noted that these other studies were not randomized to placebo, which introduces an unavoidable selection bias between β-blocker-treated and untreated subjects. (Why were the non-β-blocker-treated subjects not treated with this life-saving therapy? Were they intolerant of β-blockers? Was their heart failure so unstable as to preclude β-blocker therapy?) Furthermore, in our study and the others, there was no formal method of monitoring β-blocker treatment compliance; treated or untreated groups were assigned based on subject and physician history, and not actual pill
counts. Finally, in a nonrandomized trial, even the definition of “treated” and “untreated” can be arbitrary. If a subject received β-blockers for 3 mo, but they were discontinued for unacceptable side effects, and the study subject was then followed for 5 yr, is this individual appropriately designated as “treated” or “not treated”? In short, because there have been no prospective, interventional trials, there have been no perfect studies of β₁-adrenergic receptor polymorphism effects on β-blocker response in heart failure. Unlike hypertension, where one can readily measure blood pressure, in heart failure the preferred end point (death from heart failure) is difficult to establish and is achieved only after many years. The only randomized, placebo-controlled mortality study in heart failure was performed with an experimental β-blocker that has atypical pharmacological properties. Finally, recent surveys of heart failure outcome with clinically approved β-blockers are limited because they are not (and should not be) randomized to a placebo treatment group.

In aggregate, available human data support a modest effect of the gain-of-function Arg389 β₁-adrenergic receptor polymorphism on cardiac function. This polymorphism may impact either heart failure risk or progression, but it appears that β-blockers currently in use are sufficient to overcome the subtle differences that polymorphic receptor function may have on heart failure survival. However, it is probable that β-blockers with different pharmacological profiles (inverse agonist versus neutral antagonist versus partial agonist; intrinsic sympatholytic activity; receptor subtype specificity) will be developed for increasingly specific and individualized applications, such as heart failure with or without hypertension, or heart failure with preserved cardiac systolic function. Thus the studies of Arg389 β₁-adrenergic receptor polymorphisms in heart failure are important because they have defined a paradigm whereby genetic testing can be used to personalize therapy with specific pharmacological agents that have the optimal pharmacological profile for a given individual’s genotype/phenotype.

Although the 389 polymorphism has received the most attention of the β₁-adrenergic receptor variants, the Gly49Ser polymorphism at the receptor’s extracellular amino terminus has also been studied, either alone or in the same clinical trials in which the position 389 polymorphism was assessed. As noted above, the exact mechanisms by which this variant modifies receptor function are unclear, but there appears to be a consensus that the Gly49 β₁-adrenergic receptor exhibits greater agonist-promoted desensitization than the Ser49 receptor (174, 250). There are only a few clinical studies that looked for pathophysiological consequences of the β₁-adrenergic receptor 49 polymorphism. Individuals homozygous for Ser49 were described as having increased myocardial ischemia (113) and β-blocker resistance (197), whereas individuals carrying at least one Gly49 allele were described as having increased aerobic power on exercising testing (59) and of being protected from heart failure (91). Lanfear et al. (161) found no effect on mortality for either of the β₁-adrenergic receptor SNPs, independent of β-blocker treatment status, in patients admitted with acute coronary ischemic events. In the absence of confirmatory studies that validate these exploratory findings in large, multiple, and independent cohorts, results suggesting cardiovascular disease risk or outcomes associations with the β₁-adrenergic receptor position 49 polymorphism are best considered as preliminary.

F. β₂-Adrenergic Receptor Polymorphisms

2. Experimental studies

Due to the important role of β₂-adrenergic receptors in promoting vasodilation, it was anticipated that gain-of-function polymorphisms of this receptor would be protective against hypertension, while loss-of-function β₂-adrenergic receptor variants might act as risk factors for the disease. Table 10 lists reported nonsynonymous polymorphisms of β₂-adrenergic receptors and their putative associations with disease. Figure 12 shows the location of these polymorphisms relative to functional β₂-adrenergic receptor domains.

Green et al. (107) evaluated the functional impact of the extracellular amino terminus β₂-adrenergic receptor Arg16Gly and Gln27Glu polymorphisms by expression in Chinese Hamster CHW fibroblasts (107). Neither ligand binding nor receptor coupling to Gₛ was affected by these polymorphisms. However, reciprocal effects on receptor desensitization were described: the Gly16 receptor had increased agonist-promoted desensitization (compared with Arg16), and the Gln27 receptor was severely deficient in desensitization.

The Liggett group also performed detailed in vitro and in vivo functional studies of the less common Ile164 β₂-adrenergic receptor polymorphism located within the putative ligand binding pocket of the receptor (see Fig. 12). In a hallmark study that was the first to actually compare function between a “wild-type” and naturally occurring genetically variant receptor, “wild-type” Thr164 and “variant” Ile164 human β₂-receptors were recombinantly expressed in cultured CHW cells, revealing that the Ile164 receptor exhibits impaired epinephrine, norepinephrine, and isoproterenol binding, with diminished G protein coupling (106). This study established the paradigm that most have followed for subsequent in vitro functional evaluation of human adrenergic receptor variants. A follow-up study by the same group demonstrated the value of expressing human variant receptors in mouse models to evaluate possible consequences on integrated organ system function (315). The Thr164 and Ile164 hu-
man $\beta_2$-adrenergic receptors were transgenically expressed in mouse cardiac myocytes using the now standard $\alpha$-myosin heavy chain ($\text{MYH6}$) promoter, and biochemical and functional responses to $\beta$-receptor stimulation were measured. Consistent with the previous studies in CHW cells, hearts expressing the Ile164 receptor exhibited decreased basal and isoproterenol-stimulated adenylyl cyclase activity and diminished cardiac contraction, both reflecting decreased receptor-mediated activation of $G_s$. Thus the Ile164 polymorphism confers a loss-of-receptor signaling functional phenotype.

Finally, there are polymorphisms of the $\beta_2$-adrenergic receptor that alter its expression by changing either the 5'-untranslated promoter region or amino acid coding within a 19-amino acid leader peptide (the “leader cistron” or “LC”) that regulates translation of $\beta_2$-adrenergic receptor mRNA (207). The LC leader peptide is encoded by a short open reading frame located just over 100 nucleotides upstream of the $\beta_2$-adrenergic receptor translation initiation codon and is polymorphic at position 19 for either Cys (the major allele, 0.63 frequency) or Arg. Comparative expression in COS-7 fibroblasts of recombinant vectors containing both the leader cistron and the receptor open reading frame, with either Arg or Cys at LC position 19, showed an approximately twofold greater receptor expression with LC Cys-19. These findings were recapitulated in the same study using cultured human airway smooth muscle cells from genotyped patients (207). On the other hand, the LC-19 polymorphism did not affect $\beta_2$-receptor expression or signaling in a study of lymphocytes from asthmatic subjects (185). A factor that may explain different findings between experimental studies that recombinantly express receptor cDNAs that by only one nucleotide in the LC region and clinical studies in diverse human subjects is the significant linkage disequilibrium that exists between polymorphisms in the small, single-exon $\beta_2$-adrenergic receptor genes. This is especially relevant to linkage between the physically proximate $\beta_2$-adrenergic receptor LC-19 polymorphism and the position 16 and 27 polymorphisms in the same gene. The high degree of linkage disequilibrium between these sites suggests that multiple functionally significant gene variants are simultaneously impacting $\beta_2$-receptor signaling in unanticipated ways in individuals carrying different haplotypes. Consequently, extensive haplotyping may be necessary to distinguish between complex haplotypes.

### TABLE 10. Polymorphisms of human $\beta_2$-adrenergic receptors ($\text{ADRAB2}$)

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>SNP Type</th>
<th>mRNA Position</th>
<th>Ref Allele</th>
<th>Var Allele</th>
<th>Codon</th>
<th>Ref AA</th>
<th>Var AA</th>
<th>Phenotype</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1042711</td>
<td>5'-UTR</td>
<td>-47</td>
<td>T</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td>Obesity, diabetes</td>
<td>339</td>
</tr>
<tr>
<td>rs1801704</td>
<td>5'-UTR</td>
<td>-20</td>
<td>T</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td>Obesity, diabetes</td>
<td>339</td>
</tr>
<tr>
<td>rs39734603</td>
<td>Coding NS</td>
<td>44</td>
<td>A</td>
<td>G</td>
<td>15</td>
<td>N</td>
<td>S</td>
<td>Desensitization</td>
<td>107</td>
</tr>
<tr>
<td>rs1042713</td>
<td>Coding NS</td>
<td>46</td>
<td>G</td>
<td>A</td>
<td>16</td>
<td>G</td>
<td>R</td>
<td>Desensitization</td>
<td>107</td>
</tr>
<tr>
<td>rs1042714</td>
<td>Coding NS</td>
<td>79</td>
<td>G</td>
<td>C</td>
<td>27</td>
<td>E</td>
<td>Q</td>
<td>Desensitization</td>
<td>107</td>
</tr>
<tr>
<td>rs5336948</td>
<td>Coding NS</td>
<td>172</td>
<td>C</td>
<td>—</td>
<td>58</td>
<td></td>
<td></td>
<td>Loss of function, heart failure, coronary disease</td>
<td>7, 106, 181, 238, 315, 344</td>
</tr>
<tr>
<td>rs5680672</td>
<td>Coding Indel</td>
<td>312</td>
<td>A</td>
<td>—</td>
<td>104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1800888</td>
<td>Coding NS</td>
<td>491</td>
<td>C</td>
<td>T</td>
<td>164</td>
<td>T</td>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3729043</td>
<td>Coding NS</td>
<td>659</td>
<td>C</td>
<td>G</td>
<td>220</td>
<td>S</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs41320345</td>
<td>Coding NS</td>
<td>718</td>
<td>T</td>
<td>C</td>
<td>240</td>
<td>F</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs41358746</td>
<td>Coding NS</td>
<td>741</td>
<td>G</td>
<td>T</td>
<td>247</td>
<td>Q</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs56100672</td>
<td>Coding NS</td>
<td>769</td>
<td>G</td>
<td>A</td>
<td>257</td>
<td>G</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs61769539</td>
<td>Coding NS</td>
<td>821</td>
<td>C</td>
<td>T</td>
<td>274</td>
<td>T</td>
<td>M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3.
disease phenotypes (61), as opposed to simple biochemical read-outs in cell-based systems. Indeed, by using phase analysis to impute β2-adrenergic receptor haplotypes, Drysdale et al. (72) described 13 coding and non-coding β2-adrenergic receptor polymorphisms organized into just 12 haplotypes (out of 2^{12}, or 8,192 theoretically possible combinations) and suggested that only four major haplotypes determined in vivo effectiveness of bronchodilator therapy in human asthma. The importance of considering the consequences of multiple individual genetic events within adrenergic signaling pathways, and of organizing individual genotypes into common genetic clusters, or haplotypes, is a recurring theme in this field of research.

2. Human studies in hypertension

The overall functional implications of β2-adrenergic receptor position 16 and 27 polymorphisms on receptor desensitization established through cell culture studies can be summarized as follows: the Gly16 receptor that improves desensitization has the potential to chronically diminish β2-adrenergic signaling, whereas decreased desensitization of the Glu27 receptor has the potential to dramatically increase β2-adrenergic receptor signaling. On the basis of these inferences, the hypothesis that β2-adrenergic receptor polymorphisms are associated with altered hypertension risk has been repeatedly tested. Again, it must be kept in mind that the β2-adrenergic receptor polymorphisms at amino acids 16 and 27 exhibit significant linkage disequilibrium and are known to occur as only 3 of the 4 possible haplotypes: Arg16/Gln27, Gly16/Glu27, and Gly16/Gln27 (207). This must be considered when interpreting the many clinical studies that report association with altered hypertension risk has been repeatedly tested. In aggregate, the data supporting an association for the position 16 polymorphism with hypertension also tended to find an association for position 27 and that those finding no association at one position likewise found none at the other, since there is a high degree of linkage disequilibrium between these two loci.

Data are scarce for the β2-adrenergic receptor Thr164Ile polymorphism. Iaccarino et al. (135) found no association with hypertension in 775 cases, whereas the large Danish study (276) detected an association with increased blood pressure limited to female Ile164 carriers (i.e., having at least one allele). These results have not been confirmed. In aggregate, the data supporting an association between β2-adrenergic receptor polymorphisms and hypertension are inconclusive and suggest that any effects of β2-adrenergic receptor polymorphisms on blood pressure are modest (112).

3. Human studies of heart failure

Because most data support a relatively minor role for the β2-adrenergic receptor in minute-by-minute cardiac function (compared with the β1-adrenergic receptor), studies of β2-adrenergic receptor polymorphism effects on cardiomyocytes in cell-based systems, and on cardiac function in genetic mouse models or ex vivo human heart tissue, are scarce. On the basis of its known biochemical coupling, β2-receptor stimulation should promote cardiac contraction (inotropy) and relaxation (lusitropy) by activating Gs, but could also diminish these effects by concomitantly activating Gi. In a very careful and comprehensive study, Molenaar et al. (216) attempted to determine whether stimulating Gs, or Gi, signaling was the dominant effect of β2-adrenergic receptor stimulation in isolated atrial muscle strips from nonfailing or failing hearts, using multiple functional and biochemical end points. Importantly, these studies also controlled for β2-adrenergic receptor Arg16Gly and Glu27Glu genotype. In both failing and nonfailing hearts, Gs signaling was potently stimulated by β2-adrenergic receptors, and there was no mea-
surable effect of the position 16 or 27 polymorphisms on contraction or relaxation.

A number of in vivo studies have interrogated the impact of the $\beta_2$-adrenergic receptor position 16 and 27 polymorphisms on cardiac function. Hoit et al. (126) found no differences between heart rate or echocardiographic ventricular function after infusion of the $\beta_2$-adrenergic receptor-specific agonist terbutaline in normal individuals homozygous for either Gly16 or Arg 16. Likewise, Bruck et al. (30) found that position 16 or 27 genotype did not affect the extent of cardiac heart rate or contractile response to chronic terbutaline treatment. On the other hand, Eisenach et al. (74) interpreted results from hand-grip stress as showing that Gly16 homozygotes generated a higher echocardiographic cardiac output than subjects homozygous for Arg16. Finally, Tang et al. (307) compared $\beta_2$-adrenergic receptor position 16 genotypes with results of echocardiography performed in hypertensive or normotensive subjects and described enhanced ventricular contractile performance in normotensive, but not hypertensive, Gly16 homozygotes. It is evident that a significant deficiency in this field is the almost complete absence of studies of the same design and with the same end points that could be compared in a meaningful way. Given this weakness, the data indicate that these $\beta_2$-adrenergic receptor position 27 polymorphisms have little impact on cardiac function, but a meaningful effect of the Gly16 $\beta_2$-receptor to enhance cardiac function cannot be ruled out based on currently available data.

Two studies have asked whether $\beta_2$-adrenergic receptor polymorphisms are associated with altered risk of having or developing heart failure. An Italian study of 236 heart failure cases and 230 controls found no association between $\beta_2$-receptor Arg16Gly or Gln27Glu polymorphisms and heart disease (53). Our group also recently reported results of a multigene resequencing study in a large ischemic and idiopathic heart failure cohort and their controls that included resequencing of the $\beta_2$-adrenergic receptor gene. Again, no $\beta_2$-adrenergic receptor polymorphisms were independently associated with having heart failure (205).

As previously noted, it is certainly possible that an adrenergic receptor polymorphism might not be a risk factor for developing cardiovascular disease, but could still alter its progression once the disease is contracted. Liggett et al. (181) described a powerful negative association between the $\beta_2$-adrenergic receptor Ile164 polymorphism and heart failure outcome in 259 well-characterized patients with class II-IV (mild, moderate, and severe) heart disease. In this report, Ile164 carriers with heart failure (there were no homozygotes, and homozygous Ile164 may indeed be incompatible with life) had a 1-yr survival of only 42%, compared with 76% for those without a variant allele (181). In transfected cells, the Ile164 receptor had diminished adenylyl cyclase signaling, and transgenic mice expressing the Ile164$\beta_2$-adrenergic receptor in addition to endogenous cardiac myocyte $\beta$-receptors exhibited decreased cardiac contractility (106, 315). Impaired signaling of this rare $\beta_2$-adrenergic receptor polymorphism was confirmed by Brodde et al. (29) in a detailed comparative study of acute terbutaline responses in 6 Ile164 carriers compared with 12 individuals with the "wild-type" $\beta_2$-adrenergic receptor (i.e., subjects homozygous for Arg16, Gln27, and Thr164). The Ile164 carriers exhibited blunted inotropic and chronotropic responses to terbutaline. In a follow-up study of similar design that assessed cardiac function after 2 wk of oral terbutaline treatment (a time sufficient for receptor desensitization to have occurred), decreased desensitization was observed. Interestingly, given blunted initial terbutaline responses in Ile164 carriers, and then decreased long-term desensitization, the long-term inotropic and chronotropic effects of chronic $\beta_2$-adrenergic receptor stimulation were ultimately the same between Ile164 carriers and Thr164 homozygotes. This may explain variable results of clinical outcome studies. The rarity of this polymorphism has made properly powering outcome studies difficult. However, the original striking observation that $\beta_2$-adrenergic receptor Ile164 can adversely impact heart failure outcome was recently confirmed by Barbato et al. (7) in terbutaline challenge studies on a small group of heart failure subjects who are Ile164 carriers (n = 18), compared with 37 matched controls. The results not only demonstrated that Ile164 carriers had blunted cardiac contractile response to terbutaline, but that they also had an increased incidence of adverse outcomes over a 2-yr follow-up period. On the other hand, adverse outcomes were not observed in a larger study of 443 heart failure patients from New Zealand (186).

Finally, two studies of coronary artery disease support some type of as yet undetermined effect of the position 164 $\beta_2$-adrenergic receptor polymorphism. Ridker’s group analyzed the three major $\beta_2$-adrenergic receptor genotypes in ~2,500 subjects (from a study of almost 15,000 healthy American men) and found that Ile16 carriers who were homozygous for both Gly16 and Gln27 had a lower risk of developing a myocardial infarction (odds ratio = 0.178), whereas individuals homozygous at Thr164 and carrying Arg16 or Gln27 were at increased risk (odds ratio = 1.235) (344). Piscione et al. (238) found a significant adverse effect of the Ile164 polymorphism on disease progression in an important study of 330 patients who underwent percutaneous coronary intervention. The Ile164 polymorphism was disproportionately represented in the coronary artery disease cases (12.1%), compared with a control population (3%, consistent with other control data). Within the coronary artery disease cohort, Ile164 allele carriers had significantly earlier age of onset of coronary atherosclerosis, a higher incidence of multivessel coronary artery disease, and more serious com-
applications. Overall, Ile164 increased the risk of cardiac death in this coronary disease cohort by 3.7-fold. Importantly, the general finding of an adverse cardiac outcome associated with carrying a β₂-adrenergic receptor Ile164 allele was confirmed in the same study in a different at-risk population (150 peripheral atherosclerosis subjects). To date, these data constitute the strongest evidence for a deleterious effect of the Ile164 β₂-adrenergic receptor polymorphism in human heart disease.

Because the allele frequency for the β₂-adrenergic receptor Ile164 polymorphism is <5%, all published studies that have examined the response to β-blockers in heart failure have been underpowered to detect any potential pharmacogenomic effects, although an adverse impact of β-blockers on heart failure survival in Ile164 carriers has been suggested in one study (186).

The results of studies relating β-adrenergic receptor polymorphisms and β-blocker responsiveness in heart failure have not achieved consensus. In an Italian study, Forleo et al. (91) assessed development of heart failure over an average of 33 mo in 171 consecutive idiopathic cardiomyopathy patients genotyped at β₂-adrenergic receptor positions 16, 27, and 164, plus the 2 major β₁-adrenergic receptor polymorphisms and the β₃-adrenergic receptor position 64 polymorphism (91). After adjusting for covariates, the β₂-adrenergic receptor variant alleles, except for the rare Ile164 polymorphism, were all associated with lower risk of developing heart failure. A more recent case-control study from the same group analyzed the prevalence of β₂-adrenergic and β₁-adrenergic receptor polymorphisms in 189 idiopathic dilated cardiomyopathy patients from southern Italy, compared with 378 age- and gender-matched controls (92). Here, they found that subjects homozygous for β₂-adrenergic receptor Gly16 or that carried a β₁-adrenergic receptor Gly49 allele were at increased risk for developing dilated cardiomyopathy. de Groote et al. (58) examined 444 heart failure subjects, all of whom were treated with bisoprolol or carvedilol, and explored associations between β₂- and β₁-adrenergic receptor polymorphisms and survival over a period averaging 3 yr, but found no independent association. There was, however, a two-locus haplotype of the β₂-adrenergic receptor (Gly16Gly, Gln27Gln) associated with decreased survival by univariate analysis only. In a study that genotyped 227 heart failure subjects for 8 polymorphisms, including the β₁-adrenergic receptor position 49 and 389 polymorphisms and the β₂-adrenergic receptor position 16 and 27 polymorphisms, Shin et al. (280) reported that the homozygous β₂-adrenergic receptor Arg16/Gln27 haplotype was associated with poor survival. In contrast, a recent case-control study from Poland (97 cases, 105 controls) found no association of the common β₂- or β₁-adrenergic receptor polymorphisms with idiopathic dilated cardiomyopathy (233), although this small study lacks sufficient power to exclude many genuine associations.

Several groups have carefully looked for potential interactions between β₂-adrenergic receptor polymorphisms and β-blocker treatment effects. In their study of 199 consecutive β-blocker naive heart failure cases before and after initiation of β-blocker therapy, de Groote et al.
looked for associations between \(\beta\)-blocker response (measured as increased left ventricular ejection performance) and common \(\beta_2\) and \(\beta_1\)-adrenergic receptor polymorphisms. While they observed a definite benefit of \(\beta\)-blockade in this patient group (increase in echocardiographic ejection fraction from 30 ± 10 to 40 ± 13%), they saw no association between individual variability in \(\beta\)-blocker treatment effect and \(\beta\)-receptor genotype.

Lanfear et al. (161) examined the effects of \(\beta_1\)-adrenergic receptor position 49 and 389 polymorphisms and of \(\beta_2\)-adrenergic receptor position 16 and 27 polymorphisms on 3-yr survival in 735 individuals admitted with acute coronary ischemic events. The results were stratified by \(\beta\)-blocker treatment status (161). There were no differences in mortality by any genotype for patients not discharged on \(\beta\)-blockers. However, a protective effect was reported for the \(\beta_2\)-receptor Gly16 and Glu27 genotypes in patients discharged on \(\beta\)-blockers. Since this represents a single haplotype (207), these individuals may represent a group with unique responses to \(\beta\)-blocker protection (and the reciprocal haplotype a group relatively less protected) in the postischemic heart. However, these interesting results have not yet been confirmed, so there continues to be no consensus about \(\beta_2\)-adrenergic receptor polymorphisms and heart disease. The reasons for discrepancies in clinical studies of \(\beta_2\)-adrenergic receptor gene variants may be due to small effects of this receptor on cardiac disease, to small effects of these polymorphisms on differential drug response, or to absence of large-scale studies with consistent (one drug) study designs.

G. \(\beta_3\)-Adrenergic Receptor Polymorphisms

Polymorphisms of the \(\beta_3\)-adrenergic receptor are associated with diabetes phenotypes and, as noted above, have not received a great deal of attention in clinical cardiac studies. Table 11 lists the nonsynonymous polymorphisms of \(\beta_3\)-adrenergic receptors and their clinical associations. Figure 13 shows the position of the relevant \(\beta_3\)-adrenergic receptor polymorphism.

V. POLYMORPHISMS OF ADRENERGIC RECEPTOR ACCESSORY PROTEINS AND CARDIOVASCULAR DISEASE

A. \(G_a\) Polymorphisms

Cardiovascular stimulation by \(\beta_1\)- and \(\beta_2\)-adrenergic receptors is transduced by \(G_a\) (stimulatory G protein)-mediated activation of adenylyl cyclase. In the inactive state, \(G_a\) exists as a GDP-bound heterotrimer (one \(\alpha\)-, \(\beta\)-, and \(\gamma\)-subunit). Activation of \(\beta\)-adrenergic receptors causes \(G_a\) to bind GTP and dissociate from the \(G\beta\gamma\) subunit, permitting each to transduce signals (Fig. 1; reviewed in Ref. 78). Polymorphisms of \(G_a\) are rare (193), and no nonsynonymous variants have yet been described that contribute to cardiovascular disease. However, a common synonymous polymorphism (ATT>ATC, Ile131) in exon five has been associated with hypertension and blood pressure response to \(\beta\)-blockade (138). It is notable that rare activating \(GNAS\) (\(G_a\) gene) mutants resulting in constitutive cAMP production, such as Arg201Leu, cause hormonal oversecretion associated with spontaneous tumors of pituitary, thyroid, and adrenal glands as well as café-au-lait skin lesions in McCune-Albright’s syndrome (329). In contrast, inactivating \(G_a\) mutations cause Albright’s hereditary osteodystrophy and resistance to parathormone and pituitary-derived thyroid stimulating hormone (TSH) and growth hormone releasing hormone (GHRH) (297). Amino acid conservation in \(G_a\) and the striking pathological effects of nonsynonymous mutations in \(GNAS\) suggest that there is little tolerance for functional variability in this critical signaling factor.

A noncoding dimodulate polymorphism of the \(\alpha\)-subunit of the \(G_\text{q}\) signaling protein that transduces signals by \(\alpha_\text{q}\)-adrenergic, and other, receptors has been associated with increased heart failure mortality among African Americans (178) and with cardiac hypertrophy in a European study (98). These observations are of interest (even though the polymorphism is noncoding, i.e., it is not within an amino acid coding exon and therefore does not affect amino acid sequence) because the polymorphism interrupts binding of suppressive transcription factors to a critical region in the \(G_\text{q}\) promoter (98, 178). The effect of the polymorphism is therefore to increase \(G_\text{q}\) mRNA expression. This is especially of interest because a large body of work using transgenic mice has demonstrated that even a modest increase in cardiomyocyte \(G_\text{q}\) expression can be a powerful stimulus for both cardiac hypertrophy and development of heart failure (3, 55, 115, 130, 246, 268, 272). Although larger studies need to be performed, the convergence of biochemical and genetic mouse data with a human \(G_\text{q}\) disease phenotype is intriguing.

Heterotrimeric G proteins are made of \(\alpha\)-subunits and \(\beta\gamma\)-subunits (\(G\beta\gamma\)) that dissociate upon binding to ligand-occupied receptors. Multiple \(G\beta\gamma\) combinations can occur, and polymorphisms of \(G\beta\) have been described. In particular, the C825T variant of \(G\beta_3\) causes alternate splicing that produces a truncated protein thought to increase G protein signaling (283). This polymorphism is reported to reduce the risk of myocardial infarction and increase the effectiveness of cholesterol-lowering statin therapy (236) and has been associated with features of the metabolic syndrome, including hypertension, obesity, dyslipidemia, and insulin resistance (150, 282). Not every study has been positive, however (18,
209), and these data should be considered as preliminary until more rigorously tested in larger studies.

B. RGS Polymorphisms

RGS proteins terminate Gα-mediated signaling by accelerating hydrolysis of GTP to GDP, which promotes reassociation of Gα, β, and γ subunits into inactive heterotrimers (see Fig. 1). A recent study that explored candidate genes within GWAS linkage regions on chromosome 1 demonstrated an association between RGS5 and essential hypertension (39). These are interesting data because RGS5 is highly expressed in vascular smooth muscle, and RGS5 gene knockout mice have a phenotype of persistently low blood pressure, dilated arterial vessels, and enhanced vasodilatory responses (44). In contrast, RGS2 knockout mice have a reciprocal phenotype of hypertension with impaired vascular relaxation (121, 165). Phenotypic opposites likely result from preferential targeting of different G proteins by the two RGS proteins, G1 and G5 by RGS5 versus G1 by RGS2. A case control multigene association study recently identified RGS2, RGS19, and RGS20 as hypertension susceptibility genes (156). These findings are particularly interesting because several noncoding polymorphisms of RGS2 have been associated with essential hypertension or metabolic syndrome (97, 256, 275). While these data are preliminary, they suggest that polymorphic variation of RGS proteins that regulate critical cardiovascular signaling pathways can alter the risk of, or predisposition to, hypertension.

C. GRK Polymorphisms

Under conditions of extreme exertion or physiological stress, heart rate and cardiac contractility (i.e., cardiac output) are rapidly increased by β-adrenergic receptor signaling in response to systemically circulating or locally released sympathetic catecholamines. After the exertion is over or stress is relieved, catecholamine levels decrease and cardiac output normalizes. Thus the normal mechanism for terminating the adrenergic response is relief from stimulatory catecholamines. Under some conditions however, especially the chronically depressed cardiac output that is the hallmark of systolic heart failure, these same catecholinergic/β-adrenergic signaling pathways are persistently activated. Experimental studies and rare clinical syndromes (iatrogenic catecholine toxicity, the cardiomyopathy of pheochromocytoma) have demonstrated that unremitting adrenergic stimulation is itself a cause of cardiomyocyte toxicity and can create a downward functional spiral of worsening heart failure that stimulates more catecholamines, which further injure the heart, etc. Nature has provided a mechanism for desensitization that can “unplug” receptors from their signaling effectors under conditions of persistent agonist stimulation and can therefore potentially protect myocardium from the pathological consequences of uninterrupted βAR signaling: GRK-mediated phosphorylation of myocardial β-adrenergic receptors (87, 239) (see Fig. 3).

Agonist-occupied adrenergic receptors adopt a conformation that not only permits coupling to G protein signaling effectors, but simultaneously permits them to be phosphorylated by GRKs. GRK-phosphorylated receptors then attract a family of proteins, β-arrestins, that disrupt receptor-G protein interactions, thus uncoupling the receptors from their signaling effectors, i.e., “desensitizing” the agonist-receptor response. β-Arrestin recruitment to GRK-phosphorylated β-adrenergic receptors simultaneously targets them to clathrin-coated pits for endocytic receptor internalization and degradation, resulting in receptor “downregulation.” These two mechanisms, desensitization and downregulation, markedly diminish receptor-mediated signaling under conditions of chronic agonist stimulation.

There are seven mammalian GRKs (11), the prototype of which is GRK2, originally designated as the β-adrenergic receptor kinase (β-ARK) (12, 13). The most highly abundant GRKs in myocardium are GRK2 and GRK5 (159, 243), both of which are recruited to and can desensitize agonist-occupied β-adrenergic receptors. Despite their similarities in cardiac expression and targeting of adrenergic receptors, GRK2 and GRK5 are structurally distinct members of separate GRK subfamilies (242), which are as follows: 1) GRK1 and GRK7 (retinal opsin kinases), 2) GRK2 and GRK3 (also known as β-ARK1 and -2), and 3) GRK4, -5, and -6. An important distinguishing feature between GRK2 and GRK5 is different areas of intracellular localization at baseline and in response to receptor activation. GRK2 is normally cytosolic and actively translocates to membrane-bound active receptors by binding to Gβγ subunits of active G proteins. This unique characteristic was the basis for development of a truncated peptide, the β-ARKct, which can interfere with GRK2 phosphorylation of β-adrenergic receptors and is undergoing consideration as a form of inotropic therapy to prevent receptor desensitization in heart failure (reviewed in Refs. 65, 66). In contrast, GRK5 is constitutively membrane bound and uniquely targeted to nuclei (142, 201, 295).

In clinical heart failure, the vicious cycle of sympathetic stimulation leading to β-adrenergic receptor dysfunction can be interrupted with pharmacological β-adrenergic receptor blockade, which prolongs survival in heart failure (see Fig. 2) (210, 231). An experiment of nature suggests that increased adrenergic receptor desensitization by a gain-of-function GRK5 polymorphism can provide some of the same benefits seen with β-blocker treatment, i.e., that the GRK5 polymorphism is analogous.
to “genetic β-blockade.” A comprehensive screening for coding polymorphisms of GRK2 and GRK5 found no common nonsynonymous (i.e., amino acid changing) 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 carboxy terminus, not corresponding to any known functional domain; and nucleotide 1624 C/G changes proline to alanine at amino acid 542 within the carboxy-terminal calmodulin-binding domain (177). The amino acids encoded by three of the four major GRK alleles are completely conserved within members of the same GRK subfamily, and Gln at amino acid 41 is conserved in all nonretinal opsin human GRKs (including each of the splice variants of GRK4 and GRK6), and across all mammalian species in GRK5 (177). GRK5 variation at amino acids 304, 425, and 542 was rare (i.e., <2% allele frequency) in a diverse human cohort. In contrast, the GRK5 Leu41 allele, which is also rare in Caucasians (allele frequency 0.01–0.02), is common among African Americans (allele frequency 0.20, prevalence of heterozygous carriers of 0.35 and homozygous of 0.05). This means that ~40% of African Americans carry one or two polymorphic GRK5 Leu41 alleles.

The functional consequences of the GRK5 position 41 polymorphism were evaluated by recombinant expression in cultured cells. As might have been anticipated for a polymorphism within the regulatory domain, as opposed to the catalytic domain, GRK5 Leu41 had similar intrinsic in vitro kinase activity as GRK5 Gln41, measured by its ability to phosphorylate rhodopsin. However, when the pharmacological properties of GRK5 Leu41 were compared with those of GRK5 Gln41 in intact cells, a distinct effect of the polymorphism was found on adrenoreceptor signaling. GRK5 Leu41 and Gln41 were coexpressed at equivalent levels along with either human β1-adrenergic receptors (177) or β2-adrenergic receptors (325) in cultured CHO cells. Cells expressing GRK5 Leu41 showed enhanced agonist-promoted desensitization of β-adrenergic receptor-stimulated adenylyl cyclase activity for both β-adrenergic receptor subtypes, with increased agonist-mediated receptor phosphorylation and internalization. Improved GRK5 Leu41-mediated desensitization of a PKA phosphorylation site β2-adrenergic receptor mutant further showed that PKA, which is activated by β-adrenergic receptors and can phosphorylate them to induce a different form of negative signaling feedback called heterologous desensitization, was not involved. Absence of an effect of the GRK5 position 41 polymorphism in vitro phosphorylation studies, but increased receptor desensitization afforded by GRK5 Leu41 in intact cell systems, is consistent with the polymorphism affecting GRK regulation by one or more intracellular factors. It is interesting, therefore, that an important calmodulin binding domain spans GRK5 amino acids 20–39 (244). Binding of calcium-bound calmodulin inhibits GRK5 catalytic activity, alters its membrane-binding properties, thereby increasing its ability to phosphorylate soluble (nonreceptor) substrates, and alters the pattern of GRK5 autophosphorylation (94, 269). GRK5 has a much greater affinity for calcium-bound calmodulin than GRK2 (IC50 of ~40 nM vs. 2 μM), suggesting that increases in intracellular calcium may specifically inhibit GRK5. Currently, the impact of these interactions on GRK5 Leu41 function must be considered speculative, as the specific effect of the polymorphism on GRK5-calmodulin interactions has not yet been determined. However, the in vitro and cell-based studies have proven that the GRK5 Leu41 polymorphism enhances β-adrenergic receptor phosphorylation, desensitization, and internalization, accelerating receptor uncoupling from adenylyl cyclase, and more efficiently terminating β-adrenergic signaling.

To assess the consequences of the GRK5 position 41 polymorphism on cardiac function, cardiac-specific transgenic mice expressing equivalent levels of either human GRK5 Gln41 or GRK5 Leu41 were created and subjected to comparative analyses of cardiac phenotypes at baseline and after chronic isoproterenol infusion (177). Overexpression of human GRK5 at low levels (~5-fold endogenous levels) in these studies produced no baseline phenotype, which contrasted with a previous report of decreased basal and catecholamine-stimulated ventricular contractile performance in transgenic mice expressing higher (30-fold endogenous levels) levels of bovine GRK5 (41). In both GRK5 Gln41 and GRK5 Leu41 transgenic mice, the concentration-response curve for isoproterenol stimulation of contractility right-shifted in isolated perfused hearts, consistent with decreased sensitivity of GRK5-modified cardiac β-adrenergic receptors to this β1- and β2-adrenergic receptor agonist. Most importantly, desensitization of isoproterenol-stimulated cardiac contractility was significantly accelerated in the GRK5 Leu41 transgenics, compared with GRK5 Gln41 overexpressors. This finding is similar to the accelerated isoproterenol-mediated β-adrenergic receptor desensitization observed (and described above) in GRK5-expressing CHO cells.

Both GRK5 and GRK2 can desensitize myocardial β-adrenergic receptors. Since our previous studies in mice had demonstrated that genetic ablation of GRK2 in the heart, and the consequent impairment of normal desensitization mechanisms, exacerbates catecholamine cardiomyopathy in a chronic isoproterenol infusion model (204), we predicted that improved β-adrenergic receptor desensitization and more rapid termination of toxic β-adrenergic receptor signaling by GRK5 Leu41 could have the opposite effect. In other words, we antic-
ipated that compared with GRK5 Gln41, the more active polymorphic GRK5 Leu41 would protect mice subjected to chronic isoproterenol infusion against heart failure. Indeed, when left ventricular dilation and contractile function were examined at increasing time intervals after isoproterenol mini-pump implantation, pharmacological β-blockade with propranolol and the GRK5 Leu41 transgene conferred similar protection from the adverse consequences of chronic β-adrenergic receptor stimulation. In contrast, mice overexpressing wild-type GRK5 Gln41 developed the characteristic dilated catecholamine cardiomyopathy in response to isoproterenol, although they were still protected by propranolol (177).

The results of cell-based and transgenic mouse studies suggested that, biochemically and functionally, cardiac GRK5 Leu41 expression is similar to being treated with β-blockers. 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, Ref. 85), we next performed a preliminary evaluation of the effects of GRK5 position 41 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 from an ongoing longitudinal study of heart failure. We assessed outcome (time from heart failure onset to death or cardiac transplantation) as a function of the interaction between GRK5 genotype and β-blocker treatment status. There was no difference in GRK5 Leu41 allele frequency in nonaffected (control) African Americans versus those with dilated or ischemic cardiomyopathy. This demonstrates that the GRK5 Leu41 polymorphism is not an independent risk factor for the development of heart failure. This conclusion is consistent with the known actions of GRKs, which are stimulated by ligand occupation of receptors; hyperactivation of cardiac catecholaminergic signaling occurs as a compensatory response after the onset of heart failure, rather than as a factor contributing to its development. (For this reason, β-blockers are used to treat heart failure, and not as primary prevention in patients without other risk factors.) Indeed, we observed that African American heart failure subjects who carried one or two GRK5 Leu41 alleles and were not treated with β-blockers had significantly longer transplant-free survival times than their homozygous GRK5 Gln41 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 (HR 0.28, 95% confidence interval, 0.12–0.66; P = 0.004) that was similar to the degree of protection afforded by pharmacological β-blockade in GRK5 Gln41 homozygous subjects (HR 0.19, 95% confidence interval, 0.10–0.34; P < 0.001).

As a clinical study, the initial survey of the GRK5 position 41 polymorphism in heart failure had significant limitations. It was performed in a relatively small number of subjects recruited from a single referral center. There were only a small number who achieved the study end point (cardiac transplant or death). The decision to treat or not with β-blockers was not randomized, making the results subject to unknown factors possibly relating to patient tolerance. Thus these early results needed to be replicated in a larger, multicenter trial.

Because the strong concordance between GRK5 Leu41 effects in recombinant cell culture systems, in transgenic mice, and in this initial study of human heart suggested that gene-drug interactions could significantly impact interindividual variability of β-blocker treatment response, we included this polymorphism in a subsequent two-center study of the most clinically compelling adrenergic pathway polymorphisms in heart failure, the β1-adrenergic receptor Arg389Gly polymorphism and the GRK5 Gln41Leu polymorphism. Given the different allele frequencies for these two polymorphisms between populations of European versus African origin, we were specifically interested in whether they might contribute to differences in β-blocker protection against heart failure mortality reported between Caucasians and African Americans (340). While an overall mortality benefit for β-blocker therapy has been repeatedly demonstrated, the results of subgroup analyses of African Americans have been inconsistent. In part this relates to study design and power, but we considered that racial differences in the prevalence of the β1-adrenergic receptor Arg389Gly and GRK5 Gln41Leu polymorphisms that alter heart failure outcome could also play a role. To test this hypothesis, we examined mortality as a function of β1-adrenergic receptor and GRK5 genotype in a bicenter heart failure cohort of 2,460 subjects that included 711 African Americans (54). Within this cohort, 82% of subjects were treated with β-blockers (almost all with either carvedilol or metoprolol), permitting us to assess outcome as a function of genotype and (nonrandomized) β-blocker treatment status.

When analyzed as a whole (i.e., combined races), β-blocker treatment significantly improved mean survival time (P = 0.0008) and protected against heart failure mortality (age- and sex-adjusted hazard ratio = 0.78, P = 0.003). Subgroup analysis according to race confirmed the β-blocker survival benefit in Caucasians (P = 0.0004), but it was not significant in African Americans (P = 0.327). This finding is similar to previous large trials that failed to identify a significant β-blocker treatment benefit in African Americans, when the benefit was clear in Caucasians (reviewed in Ref. 340). We examined reasons for this apparent difference in β-blocker effect by comparing out-
comes in Caucasians and African Americans within the untreated or β-blocker-treated groups. In subjects not treated with β-blockers, survival times were similar between African Americans and Caucasians, whereas in subjects taking β-blockers, African Americans had shorter survival times than Caucasians ($P = 0.0005$). Thus the absence of a significant β-blocker treatment effect in this African American heart failure study cohort was attributable to a greater mortality benefit from β-blocker therapy in Caucasians, and not to any difference in outcome in untreated subjects.

We then compared heart failure survival times as a function of β$_1$-adrenergic receptor 389 or GRK5 41 genotype, in addition to race and β-blocker treatment status. β$_1$-Adrenergic receptor Gly389 genotype was associated with shorter survival times in β-blocker untreated Caucasians only ($P = 0.03$), consistent with previously reported modest outcome benefits for the β$_1$-adrenergic receptor Arg389 genotype (reviewed in detail in section IV). Also, as in most previous studies, our results showed that the β$_1$-adrenergic receptor position 389 genotype does not impact the mortality benefit from carvedilol or metoprolol. For GRK5 Leu41, which is almost exclusively expressed in individuals of African ancestry, the numbers of Caucasian carriers were too small to power a comparative analysis between races. However, within African Americans with heart failure, GRK5 Leu41 was associated with significantly longer survival times in subjects not being treated with β-blockers ($P = 0.01$), which is consistent with the previous report (177). Thus, in this heart failure cohort, the β$_1$-adrenergic receptor 389 polymorphism was associated with altered outcome only in Caucasians, and the GRK5 41 polymorphism, which is observed almost exclusively in African Americans, also altered outcome. Since (like all the major trials of β-blocker therapy in heart failure) our original analysis that showed apparent differences in β-blocker effect based on population ancestry did not incorporate these genetic factors, we reexamined our data comparing heart failure outcome between Caucasians and African Americans only in subjects matched for β$_1$-adrenergic receptor Arg/Arg389 and GRK5 Gln/Gln41 genotype (i.e., eliminating subjects with the confounding functional polymorphic alleles). When survival was matched for genotype in this manner, the mortality benefit for β-blocker therapy was significant in both Caucasians ($P = 0.005$) and African Americans ($P = 0.037$), and the previously observed racial differences in survival times for β-blocker-treated subjects disappeared. These results demonstrate the importance of accounting for relevant genotypes when examining drug effect. The totality of these data indicate that enhanced β-adrenergic receptor desensitization in the face of catecholamine excess, which is observed in the β$_1$-adrenergic receptor Arg389 and GRK5 Leu41 polymorphisms, is a key functional characteristic protecting against early mortality in β-blocker untreated heart failure.

In a small study with unanticipated results, Spinelli et al. (298) recently described an association between the GRK5Leu41 polymorphism and left ventricular apical ballooning syndrome, which is the prototypical morphological feature of stress-related or Takotsubo cardiomyopathy, a disease caused by chronic catecholamine excess (194, 333). In this intriguing study, 22 hospitalized Takotsubo cardiomyopathy subjects were genotyped, and the incidence of common polymorphisms of β$_1$- and β$_2$-adrenergic receptors, the G$_s$ to which the receptors are coupled, and GRK5, which desensitizes them, were compared with 740 controls. The GRK5 Leu41 polymorphism was significantly more common in the Takotsubo patients than in controls (21 vs. 6%). In another cohort, GRK5 Leu41 subjects had significantly lower resting heart rates, consistent with enhanced β-adrenergic receptor desensitization. Although this is a very small study of a relatively rare condition, the data indicate that the GRK5 Leu41 polymorphism may predispose to cardiomyopathy caused by repetitive catecholamine surges.

Genetic polymorphisms of GRK4, the genitourinary specific GRK, have been indirectly associated with cardiovascular disease. In a very unexpected finding, Felder et al. (86) described an association between GRK4 Ala142Val polymorphism and essential hypertension, and in cell-based and transgenic mouse studies defined a mechanism, GRK-mediated phosphorylation of renal dopamine D1 receptors, that impairs the natriuretic and diuretic effects of dopamine. Because of ethnic differences in GRK4 allele frequency and haplotype structure (192), it is important that subsequent studies have not only confirmed the GRK4 SNP association with hypertension, but have done so in different ethnic groups. A Japanese group described a multilocus association between three GRK4 polymorphisms, Arg55Leu, Ala142Val, and Ala486Val, and salt-sensitive hypertension (270). An Australian group confirmed the association between the Val142 GRK4 polymorphism and essential hypertension in a case-control study of 168 Caucasian hypertensive subjects and 312 normotensive controls (296). A case control study of ~1,000 Chinese showed an association between the GRK4 position 486 polymorphism and essential hypertension (110, 326). In contrast, a study from the Netherlands found no association between the GRK4 Ala142Val polymorphism and blood pressure or renal sodium excretion (299). Finally, a pharmacogenetic interaction was recently suggested between the GRK4 polymorphisms and antihypertensive effects of metoprolol in African Americans (15). In aggregate, the data strongly suggest that one or more GRK4 polymorphisms can impact homeostasis of blood pressure control and renal sodium excretion, and thereby contribute to altered risk for hy-
pertension or differential responses to antihypertensive agents.

D. CYP2D6 Polymorphisms

The above information has dealt directly with the consequences of polymorphic gene variants of adrenergic receptors, G proteins to which they couple, and a G protein kinase that posttranslationally modifies the receptors. In many instances, the consequences of these polymorphisms have been evaluated on β-blocker treatment effect, and in some cases, the data suggest that individual genetic profiling may be useful to personalize β-blocker therapy. These are genetic influences on pharmacodynamics, i.e., on drug target response. However, it is important also to recognize the possible effects of genetic polymorphisms on β-blocker pharmacokinetics, i.e., drug availability and elimination. Lipophilic β-blockers, such as metoprolol (but not bisoprolol), undergo extensive hepatic metabolism and are cleared from the body by cytochrome P-450 enzymes, especially CYP2D6 (see Fig. 3) (172). These metoprolol metabolites are functionally inactive at the concentrations achieved in vivo (254). Therefore, the rate of metoprolol metabolism by CYP2D6 is a primary determinant of active drug levels. To date, over 80 allelic variants of CYP2D6 have been described (http://www.cypalleles.ki.se/); functionally significant CYP2D6 polymorphisms interact to confer distinct human pharmacokinetic phenotypes. Scoring systems that relate complex genotypes to metabolizer phenotype exist that account for the relative function of different allelic variants and their combinatorial interactions (152). For the purposes of this review, the traditional system will be used, in which pharmacokinetic profiles assessed by the ability to metabolize specific CYP2D probe drugs have been broadly related to genotype (46, 267), classified as ultrarapid metabolizers (~5% of Caucasians), extensive metabolizers (~70% of Caucasians), intermediate metabolizers (~15% of Caucasians), and poor metabolizers (~10% of Caucasians) (247). Ultrarapid and extensive metabolizers have two fully functional CYP2D6 alleles, intermediate metabolizers have the equivalent of one functional allele (in many instances representing 2 hypofunctional alleles), and poor metabolizers have no functional alleles (343). Altered drug clearance as a result of CYP2D6 polymorphisms has striking consequences on metoprolol pharmacokinetics (99), with the elimination half-life ranging from 2.8 h in extensive metabolizers to 7.5 h in poor metabolizers (172). Indeed, multiple studies in normal subjects, hypertensive patients, and individuals with heart failure have found a strong relationship between CYP2D6 genotype, metabolizer phenotype, and metoprolol concentrations, even after chronic treatment at standard doses (252, 277). Accordingly, the possible consequences of altered metabolic clearance include altered drug efficacy (because of insufficient drug levels in extensive metabolizers or increased drug levels in poor metabolizers) or increased toxicity (because of high drug levels in poor metabolizers).

The preponderance of evidence supporting an effect of CYP2D6 genotype on metoprolol treatment effect derives from three recent studies, two in hypertension and one after myocardial infarction (16, 105, 253). Clinical efficacy of metoprolol was assessed as the change in heart rate and diastolic or mean arterial blood pressure after treatment. In each of these studies, patients with fully functional CYP2D6 alleles (i.e., poor metabolizers) exhibited enhanced negative chronotropism and, in the case of the hypertensive cohorts, a greater hypotensive effect after metoprolol. Poor metabolizers represent only a small fraction of the general population (7–10% of the Caucasian population), so it is understandable that these findings have not been reproduced in smaller studies that were insufficiently powered to independently examine this subgroup. When these types of analyses have compared metoprolol effect in subjects with two functional CYP2D6 alleles (i.e., extensive metabolizers, 70% of population) versus combined intermediate and poor metabolizers (15 and 10% of the population, respectively), differences have not been significant (277). Thus, while there is little doubt that CYP2D6 genotype can determine metabolizer status and affect metoprolol levels, increased drug effect is clearly significant only in poor metabolizers who represent a small fraction of the population. Since evaluating metoprolol efficacy is as simple as recording pulse and blood pressure during dose titration, there appears to be little indication for antecedent genotyping to gauge optimal drug dosing in this instance.

The other potential application for CYP2D6 genotyping is to identify poor metabolizers who, due to impaired drug clearance, may develop metoprolol side effects at standard doses. Support for this approach was provided in a retrospective study describing increased prevalence of poor metabolizers (38%) among patients who experienced adverse effects of metoprolol that required its discontinuation (336). Subsequent reports have failed to establish an unambiguous relationship between CYP2D6 genotype/phenotype and adverse events from metoprolol treatment, despite showing strong associations with plasma levels (99, 311, 348). However, the poor metabolizer group was underrepresented in these studies, and this is the group that displays a higher incidence of side effects from other drugs cleared in a CYP2D6-dependent manner (28, 220, 328). Given the wide therapeutic window and safety profile for metoprolol and other lipophilic β-blockers, any decision regarding the utility of genetic testing to identify poor metabolizers in the functionally brittle heart failure population who could receive alter-
nate drug dosing and increased surveillance requires properly powered prospective studies in this disease.

VI. SUMMARY AND CONCLUSIONS

After 10 years of concerted effort, developing guidelines for clinical genetic testing of adrenergic signaling factors in cardiovascular disease is a work in progress. One reason is the complexity of the problem. The human genome is far more variable than originally suspected. I think the human genome is even more variable than it is now considered to be. With 3 billion bases in the genome, and over 5 billion people on the planet (that is 10 billion genomic copies), the mathematical probability is that every nucleotide in the genome is polymorphic in at least one living individual. Contributing to the complexity of the physiological impact of genetic variation in adrenergic signaling are the multiple factors that intersect functionally to determine end-organ response. The most reductionist conceptual paradigm of adrenergic signaling, which ignores all factors determining endogenous agonist production and intrinsic target protein response, must integrate the function of receptor, G protein, and all downstream effectors, plus the impact of modulating G protein receptor kinases and regulators of G protein signaling proteins. At a biochemical level, the net effect of all genetically induced changes on expression and function of each of these individual components must be considered to gauge the overall impact in any individual. This is a formidable undertaking, requiring not only genotyping for the known common polymorphisms in each of the signaling factors, but comprehensive gene resequencing to identify rare or private gene mutations that may have even more functional significance in a particular individual. Finally, because the cardiovascular syndromes of interest are multifactorial, with only a minor genetic contribution by multiple genes, and with major contribution by environmental or sporadic factors, a meaningful analysis that can relate individual genotype and phenotype requires vast numbers of study subjects, and ultimately prospective evaluations that have not yet been attempted.

Notwithstanding these challenges, the data are sufficient to conclude at least that adrenergic receptor gene polymorphisms can impact cardiovascular function and modify heart disease. The relationship between the β1-adrenergic receptor Arg389 polymorphism and cardiac function and heart failure outcome has been reproduced sufficiently to accept. It is also easier to do this because of the compelling laboratory data demonstrating specific and quantifiable consequences of this polymorphism. If we determine that this polymorphism provides proof of principle that gene variations within adrenergic signaling pathways have meaningful pathophysiologic consequences, then it is reasonable to assume that other functionally significant polymorphisms will do the same. Again, what is required is to increase confidence for specific polymorphisms is much larger studies with confirmation in independent cohorts. Small exploratory clinical studies are not needed; as described in this review, there are abundant hypotheses and suggestive trials that deserve to be rigorously tested and independently validated in large cohorts. It is also important to note that small, underpowered studies that fail to replicate a specific finding only contribute to confusion, and so validation studies must be designed with adequate statistical power.

Even in the face of clear results that support a measurable clinical impact of a particular polymorphism (e.g., β1-adrenergic receptor Arg389), arriving at a consensus as to how best to employ this information in clinical decision making requires a different approach, prospective interventional trials that randomly assign study groups to treatments that are either blind to, or guided by, available genetic information. Only in this manner can the clinical utility of genetic information be determined. Fortunately, the technology for rapid and high-throughput gene resequencing is now available, so it should be possible to initiate these types of trials.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: G. W. Dorn II, Philip and Sima K Needleman Professor, Associate Chair for Translational Research, Director, Center for Pharmacogenomics, Dept. of Internal Medicine, Washington University School of Medicine, 660 S Euclid Ave., Campus Box 8220, St. Louis, MO 63110 (e-mail: gdorn@dom.wustl.edu).

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants R01-HL-87871 and RC2-HL-102222.

REFERENCES


Hahnlow IN, Koopmans RP, Michel MC. The beta2-adrenocep- tor gene and hypertension: is it the promoter or the coding region or neither? *J Hypertens* 24: 1003–1007, 2006.


200. Masuo K, Katsuya T, Fu Y, Rakugi H, Ogihara T, Tuck ML. Beta(2) and beta3-adrenergic receptor polymorphisms are related to the onset of weight gain and blood pressure elevation over 5 years. Circulation 111: 3428–3436, 2005.


