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

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

G-protein-coupled receptors (GPCRs) are the most common protein target among currently marketed drugs. As a result, they were among the first group of proteins whose genetic variability was studied in order to extend pharmacogenomics beyond drug-metabolizing enzymes. Among the best studied of the GPCRs are the adrenergic receptors (ARs). Among the most commonly used drugs that target a GPCR are the β-adrenergic receptor blockers.

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

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of conditions for which they are useful and the fact that their protein target is affected by genetic variation, with documented in vitro and cellular effects, make the β-blockers logical targets for pharmacogenomics research.

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

**ARs AND ASSOCIATED PROTEINS**

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

The ARs are GPCRs that represent principal components of the sympathetic nervous system and use the endogenous hormone/neurotransmitters epinephrine and norepinephrine as agonists. Almost every cell type or organ expresses one or more of the nine AR subtypes, and ARs are critical for the maintenance of cellular, organ, and whole-body homeostasis at “rest,” during normal physiological stress such as exercise, and during pathologic stress such as during decreased end-organ perfusion. The distribution of the three α1-AR subtypes, the three α2-AR subtypes, and the three β-AR subtypes have been described elsewhere. As highlighted in the schematic in Figure 1, α1-ARs couple to the heterotrimeric G protein GαS, with Gβγ activating phospholipase C, thereby converting phosphatidylinositol 4,5-biphosphate to the second messengers inositol trisphosphate and diacylglycerol. β-ARs couple primarily to Gαi, with Gβγ activating adenyl cyclase, which converts adenosine-5′-triphosphate to the second messenger cyclic adenosine monophosphate. α2-ARs couple to Gαi, with Gβγ inhibiting adenylyl cyclase and cyclic adenosine monophosphate production. The βγ-subunits of these heterotrimer G proteins also act to evoke signals that can be highly dependent on cell type. Furthermore, these receptors also appear to signal via other mechanisms independent of G-protein interaction. In this review, we concentrate on the β1-AR, β2-AR, and α2C-AR, in view of the nature of their polymorphisms, and the results from clinical studies to date.

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

**ARs IN HEART FAILURE AND HYPERTENSION**

Both β1-AR and β2-AR are expressed on human cardiomyocytes (Figure 1) and promote increased cardiac inotropy and
chronotropy, with the β1-AR serving a dominant role in this regard. In heart failure arising from almost any cause, catecholamines (particularly norepinephrine) are elevated, representing the system's attempt to increase cardiac output via cardiac β1-ARs. Although such chronic stimulation is highly effective in an acute setting such as traumatic hypovolemia, in the case of a heart with limited metabolic and physiological reserves, it appears to worsen ventricular function, leading to a vicious circle. Therefore, β-blockers, judiciously titrated to partially antagonize the “toxic” effects of norepinephrine at the cardiomyocyte, can promote cellular remodeling of the heart toward improved hemodynamics. The β2-ARs are expressed to a lesser extent than the β1-ARs and are distributed at the cell surface in discrete regions that are thought to facilitate specialized signaling, such as signaling to Gs. Both β-AR subtypes appear ultimately to signal to certain common events, as well as to events that are distinct to a given subtype. For example, prolonged β1-AR stimulation is associated with a more pro-apoptotic state, whereas β2-AR has several antiapoptotic signaling consequences. Although these properties might suggest that, in the high-catecholamine state of heart failure “β1-AR-specific” antagonists might have a better treatment effect, this has not been evident in clinical trials. Nevertheless, trials are under way to explore the benefits of administering a β1-AR antagonist with a β2-AR agonist in heart failure, in an attempt to produce the most favorable β-AR status.

The antihypertensive effect of β-blockers results primarily from antagonism of β1-ARs expressed on juxtaglomerular cells of the kidney, thereby decreasing the release of renin. Additionally, there is typically a lowering of heart rate during therapy, which, in some individuals, may contribute to the overall antihypertensive effect. α2A-ARs expressed in the central nervous system provide important regulation of peripheral sympathetic output, and α2-AR agonists such as clonidine decrease blood pressure (BP) through these central α2A-ARs. α2-ARs are also expressed on presynaptic sympathetic peripheral nerve endings, and norepinephrine released within the cleft acts through a negative feedback mechanism to inhibit subsequent release. Consequently, the status of these cardiac presynaptic nerve α2-ARs (α2A- and α2C-AR subtypes) indirectly affects responsiveness to β-blockers, given that these α2-ARs partially control the release of the endogenous agonist for β1-AR. Presynaptic β2-ARs have also been described, and these appear to promote the release of norepinephrine.

**GENETIC POLYMORPHISMS OF THE ARs**
The first discrete, nonsynonymous polymorphism of any GPCR was described in 1993, when the β1-AR was shown to have several single-nucleotide polymorphisms (SNPs). Subsequent studies showing that these SNPs have functional effects were published in 1993 and 1994, spurring the publication of a substantial number of reports over the years related to variations in this receptor and other GPCRs and clinical association studies related to disease risk, disease modification, or predictors of drug response. Since then, nonsynonymous polymorphisms have been defined for genes encoding most of the ARs.

**Table 1** Coding region variations of adrenergic signaling genes

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Common name</th>
<th>Nucleotide variability</th>
<th>Amino acid variability</th>
<th>MAF (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ED</th>
<th>AD</th>
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<tbody>
<tr>
<td>ADRB1</td>
<td>β1-AR</td>
<td>145 (A/G)</td>
<td>49 (Ser/Gly)</td>
<td>15 13</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>1,165 (C/G)</td>
<td>389 (Arg/Gly)</td>
<td>27 42</td>
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<tr>
<td></td>
<td></td>
<td>1,166 (G/T)</td>
<td>389 (Arg/Leu)</td>
<td>&lt;0.1 0.9</td>
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<td></td>
</tr>
<tr>
<td>ADRB2</td>
<td>β2-AR</td>
<td>46 (G/A)</td>
<td>16 (Gly/Arg)</td>
<td>40 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>79 (C/G)</td>
<td>27 (Gln/Glu)</td>
<td>43 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>491 (C/T)</td>
<td>164 (Thr/Ile)</td>
<td>&lt;4 &lt;4</td>
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<td></td>
</tr>
<tr>
<td>ADRB3</td>
<td>β3-AR</td>
<td>387 (T/C)</td>
<td>64 (Trp/Arg)</td>
<td>20 15</td>
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<td></td>
</tr>
<tr>
<td>ADRA1A</td>
<td>α1A-AR</td>
<td>1,039 (C/T)</td>
<td>347 (Arg/Cys)</td>
<td>57 30</td>
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<tr>
<td>ADRA2A</td>
<td>α2A-AR</td>
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<td>251 (Asn/Lys)</td>
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<tr>
<td>ADRA2B</td>
<td>α3B-AR</td>
<td>901–909</td>
<td>INS/Del301–303</td>
<td>31 12</td>
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<tr>
<td>ADRA2C</td>
<td>α2C-AR</td>
<td>964–975</td>
<td>INS/Del322–325</td>
<td>3.8 40</td>
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<td></td>
</tr>
<tr>
<td>GRK4</td>
<td>GRK4</td>
<td>448 (G/T)</td>
<td>65 (Arg/Leu)</td>
<td>35 47</td>
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<td></td>
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<tr>
<td></td>
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<td>679 (C/T)</td>
<td>142 (Ala/Val)</td>
<td>40 60</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>1,711 (C/T)</td>
<td>486 (Ala/Val)</td>
<td>40 19</td>
<td></td>
<td></td>
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<tr>
<td>GRK5</td>
<td>GRK5</td>
<td>122 (A/T)</td>
<td>41 (Gln/Leu)</td>
<td>1.3 23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Ad, African descent; AR, adrenergic receptor; ED, European descent; MAF, minor allele frequency.
<sup>b</sup>The most common allele in the general US population (including 15% African Americans) is the first listed. Allele frequencies as reported in the literature and public databases show some variance, probably due to differences in racial admixture.

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

**Genetic polymorphisms of the β1-AR**
The β1-AR has two commonly variable sites, at positions 49 and 389. At the latter position, Arg or Gly may be found, with Arg being more common in individuals of European descent, and Gly occurring with approximately the same frequency as Arg in those of African descent. This polymorphism is within the intracellular eighth helix, which is known to be involved in coupling to G<sub>α</sub>. In membrane adenylyl cyclase assays from recombinantly expressing cells, Arg389 had greater basal and agonist-stimulated activity as compared with Gly389, consistent with the concept of enhanced coupling (Figure 2a). This phenotype gave us the initial idea that, in a clinical setting, those with the Arg389 receptor may be more responsive to β-blockers, given that there is more “room” to move from the higher activity of Arg389 to some lower level. Several additional studies in recombinant cells, as well as in transgenic mouse and human
hearts, showed that this interaction is more complex. First, we showed that Arg389 underwent a greater degree of agonist-promoted desensitization relative to Gly389 in recombinant cells. These results indicated that the conformation of Arg389 is more favorable for phosphorylation by GRKs and is consistent with the notion that the more “active” coupling conformation is also the most favorable for GRK-mediated desensitization. Therefore, the phenotype of Arg389 appeared to have the capacity to undergo greater modification under conditions of elevated levels of catecholamines.

In order to ascertain the potential physiologic significance in relation to the heart, transgenic mice were generated using the α-myosin heavy chain promoter overexpressing the human Arg or Gly389 receptor on cardiomyocytes to an equal extent. Using a perfused ex vivo model, hearts were studied at baseline and in response to the agonist isoproterenol. Contractility (+dP/dt) and heart rate were the primary outcomes. In 3-month-old mice, baseline contractility was slightly elevated as compared with Gly389, and the maximal stimulated contractility was also increased by ~30% (Figure 2b). Heart rate at baseline and for several concentrations of the agonist were also higher in Arg389 mice but, at maximally tolerated doses, appeared to converge with those in Gly389 mice. In the same model, Arg389 mice showed a marked decrease in +dP/dt with infusion of propranolol, which was not observed in Gly389 hearts (Figure 2c). With chronic dosing of propranolol in the drinking water, only Arg389 mice showed a decrease in heart rate as determined by echocardiography (Figure 2d).

These data were the first to suggest that these β₁-AR polymorphisms may alter the effectiveness of β-blocker therapy in...
a physiologic setting. In normal hearts from Arg389 individuals, a substantially greater contractile force was generated by agonists as compared with hearts from Gly389 carrier individuals (Figure 2e). The same phenotype was also observed in failing hearts, although the difference between the force generated by hearts from Arg389 individuals vs. those from Gly389 was less than in normal hearts (Figure 2f). This is consistent with a dampening of the effect size of the Arg389, but there was no evidence of an actual switch between the phenotypes of Arg389 and Gly389 in progressive heart failure such as has been observed in transgenic mouse models. 13,14

In the amino terminus of the β1-AR lies a polymorphism at amino acid position 49, where Ser (the major allele) or Gly can be found. 15 This region of the receptor is not involved with agonist binding or coupling to Gs; however, in analogy with some other GPCRs, it may play a role in membrane insertion, expression, trafficking, and agonist-promoted downregulation. In our laboratory, we expressed the two polymorphic versions of the human β1-AR on the basis of the position 49 SNPs in human embryonic kidney 293 cells and Chinese hamster fibroblasts and found no differences in agonist-promoted stimulation of adenyl cyclase activity. Levin et al. 16 performed similar experiments in human embryonic kidney 293 cells, and reported increased basal and agonist-promoted adenyl cyclase activities in the Gly49 variant, although the mechanism for this unexpected finding remains unclear. Both groups noted an enhanced agonist-promoted downregulation of the Gly49 receptor. 15,16 Given that the downregulation phenotype has been demonstrated by two independent groups, it is probable that the Gly49 receptor will alter cardiovascular responses to β-blockers because of its enhanced downregulation status.

**Polymorphisms of the β2-AR**

Although β1-ARs are the primary targets of β-blockers in the treatment of heart failure, cardiac ischemia, and hypertension, the source of catecholamines for stimulating these receptors is the cardiac presynaptic sympathetic nerve. As mentioned, α2A-ARs, α2C-ARs, and β2-ARs are expressed on the cardiac presynaptic nerve endings, with the α2-ARs acting to inhibit norepinephrine release. 7 The most extreme phenotype 17 of the polymorphisms within these receptor genes is found to carry the α2C-Del322–325. This receptor has an in-frame deletion of 12 nucleic acids, with a loss of Gly-Ala-Gly-Pro within the third intracellular loop of the receptor. In recombinant cells expressing wild-type α2C-Del322–325 at equivalent levels, agonist-promoted coupling to inhibition of adenylate cyclase was severely blunted with the deletion mutant (Figure 3). 17 Two other intracellular consequences of α2C-AR activation—inositol trisphosphate production and extracellular signal-regulated kinases 1/2 phosphorylation—were also severely impaired in the presence of the α2C-Del322–325 receptor. 17 Taken together, this phenotype points toward a potential loss of regulation of norepinephrine release in humans with this α2C-AR polymorphism, leading to enhanced norepinephrine release in these individuals and a greater potential for β1-AR stimulation. Because of these findings, the α2C-Del322–325 polymorphism was considered to be a potential pharmacogenomic locus for predicting the response to β-blockers.

**Polymorphisms of the α2C-AR**

The β1-AR polymorphisms may be more applicable to β-agonist treatment such as for asthma, 18 but these receptors are expressed on virtually all cell types including cardiomyocytes and vascular smooth muscle, and certain β-blockers are not selective for the β1-AR subtype. Three nonsynonymous polymorphisms have been shown to exist in the coding region of the β2-AR, at amino acid positions 16, 27, and 164. 10 The latter polymorphism is rare (Ile164, heterozygous frequency ~0.03), but agonist-promoted coupling to stimulation of adenylate cyclase is markedly lower in Ile164-expressing cells as compared with wild-type (Thr) ones. 13 This uncoupling was also demonstrated in transgenic mice in the form of a depressed agonist-promoted cardiac contractility in Ile vs. Thr164 hearts. 10 Therefore, this severe phenotype may have importance in a small number of subjects. The common β2-AR polymorphisms (Arg or Gly at 16; Gln or Glu at 27) are in the amino terminus and appear to affect agonist-promoted loss of receptor expression (downregulation). 19 In the original report, 19 all four possible haplotypes were studied in recombinant cells: Gly16/Glu27, Gly16/Gln27, Arg16/Gln27, and Arg16/Glu27. However, the latter haplotype is rarely, if ever, found. 20 Of the
three main haplotypes, the two receptors with the Gly16 allele (regardless of the position 27 genotype) were found to undergo enhanced agonist-promoted downregulation as compared with Arg16/Gln27. For this genotype, (under the conditions of a stably transfected cell line, and using an artificial promoter), downregulation is associated with the allele at position 16, with the allele at position 27 being irrelevant. However, SNPs in the 5′ promoter region and the 3′ untranslated region of the intronless β2-AR gene also have an impact on baseline expression or agonist-promoted downregulation.21 Therefore, genotyping with haplotype-tagging SNPs (which might include position 27) may be useful to further refine predictive value. Indeed, Glu at position 27 uniquely identifies β2-AR haplotype II-1, which includes several unique SNPs in the 5′-flanking region and 7Cs in the poly-C tract of the 3′ untranslated region.21 This haplotype displays a relatively higher baseline expression and less agonist-promoted downregulation than other haplotypes when studied using a whole-gene transfection system.21 Together, β2-AR SNPs at amino acid positions 16 and 27 (or nucleotide positions outside the open reading frame), have potential direct or indirect predictive power with regard to variability in receptor function or response to therapy.

**Polymorphisms of the GRKs**

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

In the coding region of GRK5, there is one nonsynonymous polymorphism at amino acid position 41, where the major allele results in Gln and the minor encodes for Leu.22 This polymorphism is localized to the amino terminus near a calmodulin binding domain. Leu41 is almost exclusively found in individuals of African descent (Table 1). In a transfected cell model (Figure 4a), the Leu41 variant suppressed β1-AR signaling to cyclic adenosine monophosphate, indicating that this variant is a gain of function, potentially acting as a “genetic β-blocker.” Transgenic mice were generated expressing Gln and Leu41 GRK5 in myocytes and were studied in the context of a mouse model of heart failure (isoproterenol infusion over 6 days). In Gln41 mice, left ventricular end diastolic dimension increased with isoproterenol infusion, indicative of a dilated cardiomyopathy (Figure 4b). When the β-blocker propranolol was included in the drinking water, cardiomyopathy was completely blocked, as expected. In contrast, only a small increase in left ventricular end diastolic dimension was observed in Leu41 mice, and there was no effect of β-blockade (Figure 4b). This suggested that GRK5–Leu41 polymorphism partially blocked the effect of isoproterenol infusion, thereby protecting the heart from β1-AR-mediated cardiomyopathy. The lack of β-blocker effect is consistent with a “common” mechanism, that is, attenuation of β1-AR function.

**ADRENERGIC RECEPTOR SIGNALING, GENETIC POLYMORPHISMS, AND CLINICAL DRUG RESPONSES**

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

**β-BLOCKER PHARMACOGENOMICS IN HEART FAILURE**

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

The β-blocker class includes drugs that are relatively selective for the β1-AR, nonselective (block both β1-AR and β2-AR),
Figure 4  G-protein-coupled receptor kinase (GRK) 5-Leu41 properties in transfected cells and transgenic hearts. (a) Agonist-promoted cyclic adenosine monophosphate (cAMP) production over time by β1-AR is dampened by coexpression of GRK5-Leu41 or -Gln41, but the greatest desensitization is observed with the Leu41 variant. (b) Protection from heart failure and lack of β-blocker effect in transgenic mice expressing GRK5-Leu41. Mice expressing equivalent levels of the two GRK variants were treated with isoproterenol (ISO) (or saline control) by implanted mini-pump for 6 days in the absence or presence of the β-blocker propranolol in the drinking water. Gln41 mice displayed a pathologic increase in the cardiac left ventricular end diastolic dimension (LVEDD) in response to ISO (as shown by the red arrow), which was blocked by propranolol (green arrow). In contrast, Leu41 mice had a nonsignificant increase in LVEDD from ISO (red arrow) and no change in dimension with concomitant β-blockade (green arrow). (c–e) Kaplan–Meier curves for time from diagnosis of heart failure to death or cardiac transplantation. (c) Comparison of GRK5-Q41-only subjects with and without β-blocker use; (d) comparison of GRK5-L41-only subjects with and without β-blocker use; (e) comparison of GRK5-Q41-only subjects treated with β-blockers and GRK5-L41 carrier subjects without β-blocker use. From ref. 22 with permission.

and/or possess other ancillary properties. As noted, in the case of β-blockers in heart failure, it seems clear that the most important pharmacological property is β1-AR blockade, although it is possible that other properties (e.g., β2-AR blockade, vasodilator effects) might be important as well.

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

**β-Blocker pharmacogenomics in heart failure:** studies of LVEF

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

Studies of ADRB2 have generally been negative (or not tested because the study’s focus was on a β1-selective blocker), and the only reported ADRA2C study suggested greater LVEF improvement in Del322–325 carriers treated with metoprolol controlled release/extended release. It is of interest to note that this association is in contrast with the ADRA2C findings for bucindolol (see below), in which Del322–325 carriers had no benefit from bucindolol as compared with placebo. However, these differences may be attributable to the pharmacological differences between metoprolol and bucindolol. With metoprolol, the Del322–325 carriers are likely to experience a greater release of norepinephrine, making β1-AR blockade with metoprolol beneficial. In contrast, bucindolol has the ancillary property of sympatholysis, which has been shown in other studies to be detrimental in heart failure; this was shown to be more marked with bucindolol treatment in Del322–325 carriers than in noncarriers.

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

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

What is striking about the positive-versus-negative studies is the approach to analysis. In all the studies in which outcomes were compared among genotypes (i.e., Arg389Arg vs. Arg389Gly vs. Gly389Gly) in β-blocker-treated patients, no significant associations have been noted (with the exception of an ADRB2 study). In contrast, all the positive associations with ADRB1, ADRA2C, and GRK5 arise from analyses in which β-blocker-treated patients are compared with untreated (or low-dose-treated; <50% target) individuals within a genotype. These findings are consistent with the polymorphism in question having an effect on outcomes (i.e., a risk allele, or main effect) and the β-blocker reducing the risk associated with that allele. There are several lines of evidence to support the hypothesis that in heart failure, patients with ADRB1 Arg389Arg, ADRA2C Del322-325, and GRK5 Gln41Gln22 genotypes have worse outcomes.

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

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

In summary, the literature suggests that, when compared with those treated with a low-dose β-blocker or no β-blocker at all,
Table 3  β-Blocker pharmacogenomics studies of adrenergic signaling genes in patients with heart failure: associations with clinical outcomes

<table>
<thead>
<tr>
<th>Study type</th>
<th>β-Blocker</th>
<th>N</th>
<th>Duration</th>
<th>Gene(s) studied</th>
<th>Outcome(s)</th>
<th>Results</th>
<th>P value</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Various</td>
<td>375</td>
<td>37–60 Months</td>
<td>ADRB1, ADRB2</td>
<td>D, HT</td>
<td>Gly49 carrier was associated with the longer survival rate than Ser49Ser (HR = 0.31, 95% CI 0.12–0.79)</td>
<td>0.014</td>
<td>30</td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>207</td>
<td>2.8 years</td>
<td>ADRB1</td>
<td>D</td>
<td>Arg398Arg carriers showed significant benefit from bucindolol (vs. placebo) (HR 0.62; 95% CI 0.40–0.96). No bucindolol benefit over placebo in Gly389 carriers (HR 0.90, 95% CI: 0.62–1.30)</td>
<td>0.03</td>
<td>14</td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>201</td>
<td>3.3 Years</td>
<td>ADRB1</td>
<td>D</td>
<td>Arg398Arg carriers showed significant benefit from bucindolol (vs. placebo) (HR 0.66; 95% CI: 0.50–0.88)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>227</td>
<td>Median 2.8 years</td>
<td>ADRB1, ADRB2</td>
<td>D, HT</td>
<td>Del322–325 carriers had no benefit from bucindolol (vs. placebo) (HR 1.09; 95% CI 0.57–2.08; P = 0.80). Ins322–325Ins carriers had significant benefit from bucindolol (HR 0.70; 95% CI: 0.51–0.96; P = 0.025)</td>
<td>0.025</td>
<td>27</td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>201</td>
<td>3.3 Years</td>
<td>ADRB1</td>
<td>D</td>
<td>Arg398Arg carriers showed significant benefit from bucindolol (vs. placebo) (HR 0.66; 95% CI: 0.50–0.88)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Metoprolol Carvedilol</td>
<td>637</td>
<td>2.8 Years</td>
<td>ADRB1, ADRB2, ADRA2C</td>
<td>D, HT</td>
<td>No association noted for ADRB1, ADRB2, ADRA2C</td>
<td>NS</td>
<td>33</td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>810</td>
<td>(HF)</td>
<td>GRK5</td>
<td>D, HT</td>
<td>African Americans: Gln41Gln had significant survival prolongation with β-blocker (P = 7.96e-8); Leu41 carriers had no benefit from β-blocker (P = 0.53) due to similar survival in untreated Leu41 carriers and Gln41Gln β-blocker treated (P = 0.31). Inadequate power in Caucasians</td>
<td>8e-8</td>
<td>22</td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>822</td>
<td>(ACS)</td>
<td>GRK5</td>
<td>D, HT</td>
<td>Replication cohort of ACS patients: interaction between Leu41 and β-blocker use relative to death outcome (P = 0.023)</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Various</td>
<td>2,460</td>
<td>3.8 Years</td>
<td>ADRB1, GRK5</td>
<td>D</td>
<td>No association noted by Arg389Gly genotype in β-blocker treated (P = 0.80); untreated Gly389 carriers had borderline difference in survival (P = 0.0587)</td>
<td>0.03</td>
<td>31</td>
</tr>
</tbody>
</table>

Δ Change before and after treatment; ADRA2C, c,-adrenergic receptor gene; ADRB1, β,-adrenergic receptor gene; ADRB2, β,-adrenergic receptor gene; CI, confidence interval; D, death; GRK5, G-protein-coupled receptor kinase 5 gene; HR, hazard ratio; HT, heart transplantation; Hos, hospitalization; N, sample size; NS, not significant; P, prospective study designed specifically to test pharmacogenetics hypotheses; R, retrospective study conducted on an existing data set or patient cohort (in some cases, the cohorts were prospectively enrolled and followed, but the genetic/pharmacogenetic questions were posed retrospectively); RCT, randomized, placebo-controlled clinical trial.
carriers of ADRB1 Arg389Arg and GRK5 Gln41Gln derive the greatest survival benefit from β-blocker treatment. Also, perhaps because of the unique sympatholytic effects of bucindolol, carriers of ADR2C Ins322–325Ins also derive greater benefit. The flip side of the coin is that those with the other genotypes may derive minimal benefit from β-blocker therapy and might be better served with alternative treatment approaches.

**β-Blocker pharmacogenomics in hypertension**

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

In contrast to β-blockers in heart failure, for which properties beyond β1-AR blockade might be beneficial, the bulk of the antihypertensive effect of β-blockers is believed to derive from β1-AR inhibition, given that β1-AR-selective and nonselective β-blockers have similar antihypertensive efficacies. It is not surprising, therefore, that the majority of pharmacogenomics studies in hypertension center on the nonsynonymous ADRB1 SNPs, ADRB1 and ADRB2. Several studies, some involving healthy volunteers and others involving patients with hypertension, have tested the association of the ADRB1 Ser49Gly and Arg389Gly on the heart rate– and BP-lowering effects of β-blockers, and many of these have been previously summarized. Consistent with the *in vitro* and *ex vivo* data and the heart failure association data summarized above, several studies have shown that the more active forms of the β1-AR (Ser49 and Arg389) are associated with more pronounced BP lowering. Two small but carefully conducted prospective studies involving patients with hypertension had nearly identical findings, showing that Ser49 homozygotes and Arg389 homozygotes experienced greater BP-lowering effects with metoprolol than did the carriers of other variants, with the magnitude of the effect being greater for the codon 389 than for the codon 49 SNP. It is interesting to note that one of these studies was conducted in the United States and the other in China; this suggests a consistency of effect across populations. However, findings from other studies, including two recent, larger prospective studies from Europe did not observe a significant association with ADRB1 genotype, although in one of these, Ser49Ser homozygotes had numerically greater responses, with each Gly49 allele leading to a lesser BP response. Whether the lack of association in these studies was due to differences in study design, the drugs and/or doses investigated, differences in how the BP response was measured, or lack of a strong genetic effect of ADRB1 on BP response to β-blockers is unclear. Therefore, the literature at present is not completely clear with respect to associations between ADRB1 genotype and BP lowering with β-blockers. Like the literature related to heart failure, all the studies with positive associations show the Arg389Arg genotype (and in some studies, Ser49Ser or the haplotype) to be associated with the greatest extent of BP lowering. ADRB2 was studied in some of the studies, and overall the data do not suggest an important association between ADRB2 genotype and antihypertensive response. This is perhaps not surprising, given that nearly all the β-blockers tested in hypertension pharmacogenomics studies are β1-selective blockers.

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

Two studies have tested the association of the ADRB1 and ADRB2 SNPs with cardiovascular outcomes in patients with hypertension receiving treatment with β-blockers. In the genetics substudy of INVEST, a randomized, controlled clinical trial of atenolol vs. verapamil in patients with hypertension plus coronary artery disease, nearly 6,000 patients were genotyped for the ADRB1 and ADRB2 nonsynonymous SNPs. ADRB1 Ser49/Arg389 haplotype carrier status was associated with a main effect of an increased risk of the primary outcome (death, nonfatal myocardial infarction, or nonfatal stroke), driven by a greater-than-threefold risk for death. Evaluation of the death outcome by treatment showed that the risk associated with this allele was attenuated by treatment with atenolol but not with verapamil. Specifically, as shown in Figure 5, there was significant protective effect with atenolol (vs. verapamil) in carriers of Ser49–Arg389 (hazard ratio 0.64; 95% confidence interval 0.41–0.98) but no differences in outcome by treatment in the noncarriers (hazard ratio 1.51; 95% confidence interval 0.27–8.51). These findings are consistent with the association with BP response and the literature related to heart failure, which suggest greater benefit with β-blockers in carriers of Ser49–Arg389 (or Arg389 alone). Also, as highlighted above in the context of heart failure (and as evident in Figure 5), there were no differences in outcome by genotype in the atenolol-treated individuals; the pharmacogenomic effect was evident only through comparisons within genotype of those treated with β-blocker vs. a contrasting therapy. Specifically, as shown in Figure 5, there were no differences by genotype among atenolol-treated individuals (groups B and C in Figure 5), but Ser49Gly389 carriers treated with verapamil SR (group D) had significantly worse outcomes than the same haplotype group.
treated with atenolol (group C). Again, this suggests the Ser49-Arg389 is a risk-associated allele for adverse outcomes, with the risk attenuated by β-blocker treatment. This is fully consistent with the literature on heart failure.

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

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

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

Other genes in the adrenergic signaling pathway

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

FUTURE DIRECTIONS

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

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

**Figure 5** All-cause mortality and mean on-treatment blood pressure by ADRB1 Ser49-Arg389 haplotype and atenolol/verapamil sustained-release (SR) therapy. S49-R389-Ser49-Arg389 haplotype. AT, atenolol, DBP, diastolic blood pressure; SBP, systolic blood pressure; VE, verapamil SR. From ref. 42.
That the literature suggests pharmacogenomic effects might be evident only when contrasting treatments are compared within one genotype could have important implications that extend well beyond β-blockers and cardiovascular disease. First, these data suggest that to rule out a pharmacogenomic effect, particularly for genes involved in the pharmacological pathway (vs. pharmacokinetic effect through drug metabolism or drug transport), it may be essential to design studies such that data on contrasting treatments (or placebo) are available for analysis. This is likely to be most important when a main effect is present but could also occur in the absence of a main effect, as we have seen in other hypertension pharmacogenomics studies. Second, it highlights how carefully the literature must be analyzed when making conclusions about the clinical relevance of pharmacogenomic targets. Finally, this suggests that there are certain genotype groups who may derive minimal benefit from a given therapy. This could lead to therapy guided by genetic information, which is the ultimate goal of pharmacogenomics research.

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

It is also clear that basic studies of signal transduction in reductionist systems (such as transfected cells or genetically modified mice), human-derived tissues, and human physiologic studies, can lead to insights into potential mechanisms and can deliver new biological insights relevant to drug response. It is well recognized that the results from these types of studies may not correlate with a global clinical outcome, consistent with the notion that we do not know all that we think we do about a given disease or the mechanism of action of a drug. However, the studies will lead to additional insights. Finally, there is a need for more prospective and adequately powered clinical trials. We urge consideration of primary and secondary end points as important outcomes so as to maximize the gain from such trials. We also suggest that add-in polymorphisms be included without the antiquated notion of requiring a sacrosanct a priori set of hypotheses prior to enrollment of the first patient. In essence, we propose that as the tools at the level of discovery, genotyping, high-throughput compound screening, and statistical analysis have undergone a revolution, so should the trial design and approval processes, so that personalized medicine can realize its full potential.

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CONFLICT OF INTEREST
The authors declared no conflict of interest.

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