Pharmacogenomics of β₁-Adrenergic Receptor Polymorphisms in Heart Failure

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β₁-ADRENERGIC RECEPTOR CODING POLYMORPHISMS

The coding block of the intron-less β₁-adrenergic receptor (β₁AR) gene has two common nonsynonymous single nucleotide polymorphisms (SNPs) at nucleotides 145 and 1165.1,2 Although some literature suggests many other SNPs, careful examination shows that the frequencies of these are often less than 0.01 (1%), have been found in only a few individuals, or have never been found in the homozygous state. These findings all indicate that these variants are either spurious or rare and therefore unlikely to impact public health.

The location of these two SNPs within the 7-transmembrane spanning β₁AR and the approximate allele frequencies are shown in Fig. 1. Concerning the amino acid position 389 polymorphism, Gly at position 389 was the originally cloned receptor and has often been denoted as the wild-type. However, Arg is more common in Caucasians, whereas Arg and Gly are almost equally common in African Americans.3 Therefore, it seems best not to designate “wild-type” versus “polymorphism” but to simply state the allele; in the case of the β₁AR, these would be Ser49 or Gly49, and Arg389 or Gly389.

APPROACH FOR STUDYING β₁AR POLYMORPHISMS

Fig. 2 indicates the approach the author took for assessing the β₁AR polymorphisms (and others found in different genes)4–6 in terms of signaling characteristics and clinical effect. The author initially concentrated on signal transduction in transfected cells to understand, in the most reductionist way, the potential differences between polymorphic receptors. In some cases, this was followed through creating myocyte-targeted transgenic mice, which provides measurements of cellular and physiologic signaling of the cell type of interest under the conditions of the organ of interest.

Small physiology-based clinical studies were also performed, again building a wider base of knowledge about a given polymorphism within the context of the disease. When possible, the author also used human tissue (in this case right ventricular trabeculae) from normal and failing hearts to further address phenotype under normal and diseased conditions. The pharmacogenetic clinical trials used all the results from these studies to formulate a hypothesis based on biologic plausibility. Finally, new drug or SNP targets can be derived from pathway analysis in ancillary studies, such as expression arrays performed on the human or transgenic mouse hearts.7

THE β₁AR ARG389 AND GLY389 SIGNALING PHENOTYPE

The cDNAs for these two receptors were used to stably transfect Chinese hamster fibroblasts (CHW-1102 cells), and the major results that define

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the phenotype are shown in Fig. 3. Agonist completion binding studies showed no effects of the GTP analog GppNHp in Gly389 membranes (see Fig. 3A) compared with Arg389 (see Fig. 3B). In the former, regardless of the presence of GppNHp, Gly389 curves could be fit to a single site (see Fig. 3B). In contrast, in the absence of GppNHp, Arg389 curves could be resolved into high- and low-affinity binding sites, and in the presence of GppNHp, only a low-affinity site. The ratio of the low- to high-affinity sites essentially represents the potential change in Gibb’s free energy that the agonist-bound receptor conformation can convert, which represents the signal-transduction capacity.

These studies suggested that Arg389 would have a greater agonist-promoted coupling to Go/adenyl cyclase than Gly389, which (see Fig. 3C) it in fact shows. Isoproterenol-stimulated adenyl cyclase activation was approximately threefold greater for Arg than Gly, which was also the finding for basal levels, consistent with the fact that receptors can “toggle” to the active state in the absence of agonist.

Transgenic mice were made using the α-myosin heavy chain promoter, which targets the transgene (the human β1AR Arg389 or Gly389 cDNA) to the cardiomyocyte. At equivalent expression levels, basal contractility (+dP/dt) was greater for 3-month-old Arg389 mice than Gly389 mice, as was maximal dobutamine stimulated contraction (see Fig. 4A). At 6 months of age, mice showed marked desensitization (essentially a total loss of responsiveness) of the Arg389 response, and approximately 15% desensitization of the Gly389 response, whereas basal (no agonist) contractility remained greater for Arg versus Gly389 (see Fig. 4B). Furthermore, by 9 months of age, Arg389 mice had frank heart failure with depressed fractional shortening and increased mortality.

These results can be interpreted several ways. One is that Arg389 is a risk factor for heart failure. However, the author has shown that, as a single
variant, Arg389 and Gly389 allele frequencies are equivalent in patients who have heart failure and those who do not, indicating that alone this polymorphism does not represent a significant risk for human heart failure.

Another interpretation is that age causes a gradual change in the receptor phenotypes, so that the Gly389 receptor becomes the hyperfunctional receptor near end-stage failure. An additional consideration is that the nature of the experiment (overexpression of both receptors) promotes long-standing enhanced contractility, eventually leading to enhanced desensitization of the Arg389 hearts, and heart failure, from these chronic stimulation. In fact, elements of both of these interpretations were found in the human tissue.

The first indication of a potential differential response to β-blockers based on the 389 genotype came from studies of 3-month-old transgenic mice. As shown in Fig. 4C, acute ex vivo administration of propranolol promoted a significant, and dose-dependent, decrease in 1dP/dt in Arg389 hearts, but had minimal effect (and only at the highest concentration) in Gly389 hearts. In vivo, the author examined heart rates through echocardiography in matched 3-month-old mice treated with carrier or propranolol in the drinking water for 1 month. The Arg389 mice showed a significant decrease in heart rate, whereas Gly389 mice did not (see Fig. 4D).

Ex vivo studies of nonfailing and failing human right ventricular trabeculae further defined the phenotype of Arg versus Gly389. Fig. 5A shows that in normal hearts, contractility in response to isoproterenol was greater for Arg389 homozygous hearts than Gly389 carrier hearts. These results were consistent with the aforementioned studies of transfected cells and young transgenic mice. This same phenotype was seen in the failing hearts (see Fig. 5E), but the scale difference should be noted. Therefore, compared with the normal hearts, some narrowing of the phenotype occurred, but Arg389 hearts in fact had greater contractility than Gly389 hearts even at end-stage heart failure.

Additional studies were performed with bucindolol (a β-blocker for which DNA was available from a clinical trial) and carvedilol. Carvedilol acted as a neutral antagonist, with no significant change noted in contraction with either genotype. In contrast, for the Arg389 trabeculae, bucindolol acted as an inverse agonist (ie, decreased contractile force), whereas at Gly389 bucindolol acted as a neutral antagonist (see Fig. 5C).

THE β1AR SER49 GLY49 SIGNALING PHENOTYPE

As indicated in (see Fig. 1), Gly is clearly the minor allele at position 49. This region of the receptor is in the extracellular amino terminus and has not been subjected to extensive structure or function studies. A single N-linked glycosylation site is present on the human β1AR at amino acid position 15. The author expressed the Ser49 and Gly49 receptors in CHW-1102 cells and found no differences in basal or agonist-promoted adenylyl cyclase activities. Binding affinities for isoproterenol, norepinephrine, and metoprolol were not different between the receptors.

Another phenotype investigated was agonist-promoted down-regulation, but no loss of β1AR expression was found after 24 hours of 10^{-5} M isoproterenol exposure in the culture medium. The author then stably transfected HEK-293 cells, actually finding an up-regulation of Ser49 and a down-regulation of Gly49. To eliminate new receptor synthesis, cells were treated with cycloheximide and then vehicle or 10^{-5} M isoproterenol for 18 hours. Ser49 underwent a down-regulation of approximately 36% whereas Gly49 underwent approximately 55%.

Short-term exposure (30 min) to agonist resulted in equivalent degrees of receptor internalization.
Additional studies showed that Ser49 and Gly49 may have differences in glycosylation. A high molecular weight band found with Ser49-solubilized membranes on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was not observed with Gly49 membranes. The band was sensitive to in vivo exposure to the glycosylation inhibitor tunicamycin, and to N-glycosidase treatment in vitro. O-glycosidase treatment had no effect. How N-glycosylation at position 15 could be affected by a polymorphism at position 49 is unclear. It is now apparent that β1ARs can form homodimers, so the high molecular weight species could represent a minimally glycosylated dimer.

Additional studies are required to understand the molecular basis of this phenotype. However, Levin and colleagues\(^\text{11}\) also reported the enhanced agonist-promoted down-regulation of the Gly49 receptor. However, this group also reports differences in basal and agonist-promoted stimulation of adenylyl cyclase, which the author of this article did not find. Nevertheless, a difference in agonist-promoted down-regulation is a clinically relevant phenotype in heart failure, and therefore the Gly49 variant may have a role in risk, progression, or therapeutic response to β-blockers in heart failure.

ASSOCIATION STUDIES OF β-BLOCKER EFFICACY AND β₁AR POLYMORPHISMS

The most promising candidate polymorphisms for distinguishing responses to β-blockers are the position 389 variants. The Arg389 receptor is hyperfunctional, which may be equated with a state that is more amenable to being

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**Fig. 4.** Physiologic characterization of the β₁-Arg389 and -Gly389 receptors expressed in transgenic mice. (A) Contractile response to dobutamine in 3-month-old mice. (B) Contractile response to dobutamine in 6-month-old mice. (C) Response to acute ex vivo administration of propranolol. (D) Response to chronic administration of propranolol over a 1-month period in young mice. (Data from Mialet-Perez J, Rathz DA, Petrashevskaya NN, et al. β₁-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med 2003;9(10):1300–5.)
antagonized by β-blockers. This finding seemed to be the case in the transgenic mice, in which only Arg389 hearts responded acutely (decreasing contractility) or chronically (decreased heart rate) to propranolol. Therefore, the author hypothesized that Arg389 was associated with improved outcomes in chronic heart failure treated with β-blockers.

It was also considered that in the absence of β-blockers, the SNP may have some effect on survival (as in the transgenic mice); therefore, an ideal clinical trial would have a placebo arm so that the response could be stratified by genotype and treatment status, comparing patients who had Arg389 hearts taking placebo with those taking β-blockers, and patients who had Gly389 hearts in the same manner.

One study12 that provided these groups was the Beta-blocker Extends Survival Trial (BEST). The DNA substudy of this trial consisted of archived DNA from 1040 patients who had been followed up for up to approximately 5 years: 525 on placebo and 515 on bucindolol.

An additional genotype-dependent effect was noted with bucindolol (see Fig. 5C) in the ex vivo failing-heart studies, in which this drug was an inverse agonist at Arg389 but not Gly389. Thus, particularly for bucindolol, the Arg389 genotype was hypothesized to be associated with a greater response than Gly389. The author therefore genotyped these samples at the 389 locus, with the primary hypothesis that, compared with placebo, patients who were homozygous for Arg389 would experience an improvement in end points (survival, hospitalizations, or the combined end point) whereas those carrying the Gly389 polymorphism would have experienced no significant advantage with taking bucindolol over placebo.10 The subjects were well matched in ethnicity, cause of heart failure, left ventricular ejection fraction, blood pressure, and other parameters.10

The salient results from these studies are shown in Fig. 6, which are Kaplan-Meyer curves of end points stratified by treatment and genotype. Cox-proportional hazards modeling was performed adjusting for age, sex, and ethnicity.10 The comparisons, adjusted for age, sex, and race, showed that patients homozygous for Arg treated with bucindolol had increased survival compared with those treated with placebo (hazard ratio [HR], 0.62; 95% CI; 0.40–0.96; P = .03), which represented a 38% improvement.

However, this same comparison in Gly carriers showed no difference in survival curves (HR, 0.90; 95% CI; 0.62–1.30; P = .57), indicative of no statistically significant treatment response to bucindolol. The β-AR genotype also had an apparent influence on the heart failure exacerbations during bucindolol treatment, as measured by time to first hospitalization because of heart failure (Arg389: HR, 0.64; 95% CI; 0.46–0.88; P = .006, and Gly389: HR, 0.86; 95% CI; 0.64–1.15; P = .30). For the combined outcome of time to first heart-failure hospitalization or death, a favorable treatment effect was evident for bucindolol compared with placebo in patients carrying the Arg polymorphism (HR, 0.66; 95% CI, 0.50–0.88; P = .004), but not in Gly389 carriers (HR, 0.87; 95% CI, 0.67–1.11; P = .25).

Taken together, these data indicate the Arg389 locus as a predictor of the response to bucindolol.10 The original BEST study suggested that the drug may not have been as beneficial in African Americans. Gly389 is more common in African Americans than Caucasians from the United States (see Fig. 1); however, this study’s findings with this polymorphism were not simply from Gly being a marker of African ancestry. First, in the
Cox-proportional hazards model, results were adjusted for race. Secondly, in an analysis of the white patients only, the beneficial effect of Arg389 was observed in patients receiving bucindolol versus placebo, but not found in Gly389 carriers, essentially mirroring what was found in the entire DNA substudy group.

The author is aware of only one other double-blind placebo-controlled trial of a β-blocker in heart failure in which patient DNA was collected: the MERIT-HF DNA substudy. However, the published information from this study showed a relatively high prevalence of American Heart Association class II patients compared with BEST, the patients were followed up for an average of 1 year, and the patients experienced few events and therefore death or hospitalization was the primary end point. Ascertaining a pharmacogenetic effect from this report is difficult, however, because the metoprolol and placebo groups were combined.

Recently, Sehnert and colleagues examined potential relationships with several polymorphisms, including β₁-389, in 637 patients from a retrospective study of subjects recruited from cardiac catheterization laboratories. The β-blockers were carvedilol or metoprolol, and no placebo (or no β-blocker) group was included. They compared outcomes between those who had Arg389 and Gly389 polymorphisms, finding no associations. However, this study is limited by its retrospective nature, the lack of uniform dosing, and a potential bias from recruiting patients who were undergoing a cardiac catheterization. Nevertheless, the discrepancy between this study and the BEST DNA substudy involving bucindolol may be caused by the pharmacologic differences among bucindolol, metoprolol, and carvedilol. This possibility raises question of whether the associations found in BEST are a class effect or are specific to bucindolol.

**SUMMARY**

Many issues remain regarding the β₁AR polymorphisms and their potential influence on β-blocker response in heart failure. First, the promoter region of the β₁AR gene is now known to be highly polymorphic and that multiple haplotypes (specific combinations of polymorphisms) are present in the human population. In whole-gene transfection studies, the author found that these alter β₁AR expression and can be placed into at least three expression groups. Therefore, these other polymorphisms, or the haplotypes, should be considered because this approach may improve the predictive power.

Second, the sympathetic signaling network of the heart is well known to involve other adrenergic receptors and other nonreceptor components. Studies with the α₂C-AR deletion polymorphism and the G-protein–coupled receptor kinase 5 polymorphism highlight the need to consider multiple polymorphisms in multiple genes in the context of

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**Fig. 6.** Kaplan-Meyer curves showing survival in patients stratified by genotype and drug treatment in the BEST DNA substudy. The patients who had Arg389 polymorphism receiving the active drug (bucindolol) had improved survival compared with those receiving placebo. Patients who had Gly389 polymorphisms treated with bucindolol and placebo had equally poor outcomes. (Adapted from Liggett SB, Mialet-Perez J, Thaneemit-Chen S, et al. A polymorphism within a conserved β₁-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure. Proc Natl Acad Sci U S A 2006;103(30):11288–93.)

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β-blocker pharmacogenomics. The statistical analysis of these potential complex interactions is not trivial and may require some new approaches.

Finally, the issue of specific drug versus class effects must be resolved. Many β-blockers for heart failure treatment are known to have unique (or different) properties. Therefore, they each may have a distinct group of polymorphisms that are most predictive of response. However, some primary-effect polymorphisms may be useful for predicting response for all β-blockers.

REFERENCES