The Ile164 β_2-Adrenergic Receptor Polymorphism Adversely Affects the Outcome of Congestive Heart Failure

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Abstract

The β_2-adrenergic receptor (β_2AR), an important modulator of cardiac inotropy and chronotropy, has significant genetic heterogeneity in the population. Because dysfunctional βARs play a role in the pathogenesis of the failing ventricle, we tested the hypothesis that β_2AR polymorphisms alter the outcome of congestive heart failure. 259 patients with NYHA functional class II-IV heart failure due to ischemic or dilated cardiomyopathy were genotyped and prospectively followed, with the endpoint defined as death or cardiac transplantation. The allele frequencies between this group and those of 212 healthy controls also were compared and did not differ between the groups. However, those with the Ile164 polymorphism displayed a striking difference in survival with a relative risk of death or cardiac transplant of 4.81 ($P < 0.001$) compared with those with the wild-type Thr at this position. Age, race, gender, functional class, etiology, ejection fraction, and medication use did not differ between these individuals and those with the wild-type β_2AR, and thus the β_2AR genotype at position 164 was the only clear distinguishing feature between the two groups. The 1-yr survival for Ile164 patients was 42% compared with 76% for patients harboring wild-type β_2AR. In contrast, polymorphisms at amino acid positions 16 (Arg or Gly) or 27 (Gln or Glu), which also alter receptor phenotype, did not appear to have an influence on the course of heart failure. Taken together with cell-based and transgenic mouse results, this study establishes a paradigm whereby genetic variants of key signaling elements can have pathophysiologic consequences within the context of a disease. Furthermore, patients with the Ile164 polymorphism and heart failure may be candidates for earlier aggressive intervention or cardiac transplantation. (*J. Clin. Invest.* 1998. 102: 1534–1539.) **Key words: β-adrenergic receptor • adenylyl cyclase • cyclic AMP • mutation • polymorphism • genetics • heart failure • cardiomyopathy**

Introduction

Congestive heart failure remains a significant health problem worldwide with a 5-yr mortality of ~ 50% (1–3). The most common forms of congestive heart failure, such as idiopathic dilated cardiomyopathy and ischemic cardiomyopathy, affect 4.8 million individuals in the United States. The factors that lead to progression of heart failure from mildly symptomatic to severely decompensated, leading to death or the need for cardiac transplantation, are not well defined, and substantial interindividual variability in progression is observed (4, 5). Cardiac inotropy and chronotropy are modulated in part by β_1-adrenergic (β_1AR)[1] and β_2-adrenergic (β_2AR) receptors, which are both expressed in the human heart (6). A hallmark finding that occurs with progression of failure is a markedly depressed contractile response to β-agonist inotropes (7, 8). Concomitant with this hyporesponsiveness, a decrease in expression and/or function of cardiac β_1AR and β_2AR has been observed (6, 8).

Recently, we have elucidated genetic heterogeneity in the structure of the β_2AR in the human population (9–12). Polymorphisms within the coding block of the gene result in differences in encoded amino acids in the amino-terminus of the receptor and in the fourth transmembrane-spanning domain (see GenBank accession numbers AF022953, AF022954, and AF022956). Using site-directed mutagenesis and recombinant expression techniques, we have shown that these polymorphisms each have distinct functional characteristics (10, 11), which are summarized in Fig. 1. The most impaired polymorphic receptor is due to a Thr to Ile switch at amino acid 164 in the fourth transmembrane-spanning domain. This receptor displays a small decrease in binding affinity for catecholamines and certain βAR antagonists, a substantial decrease in basal and epinephrine-stimulated adenylyl cyclase activities due to defective coupling of the receptor to the stimulatory G protein G_s, and impaired agonist-promoted sequestration (10). Furthermore, in transgenic mice generated to express either the wild-type (Thr164) receptor or the Ile164 receptor in the heart, the Ile164 mice display depressed resting and agonist-stimulated contractile function in vivo compared with the Thr164 mice (12). The other two functionally significant polymorphisms occur at amino acid position 16 where Arg ("wild-type") or Gly can be present or at position 27 where Gln ("wild-type") or Glu can be found. The Gly16 and Glu27 receptors do not display altered functional coupling to adenylyl cyclase but rather differ in the extent to which they undergo agonist-promoted downregulation as compared with wild-type

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1. *Abbreviations used in this paper:* β_1AR, β_1-adrenergic receptor; β_2AR, β_2-adrenergic receptor; CI, confidence interval; LVEF, left ventricular ejection fraction; RR, relative risk.

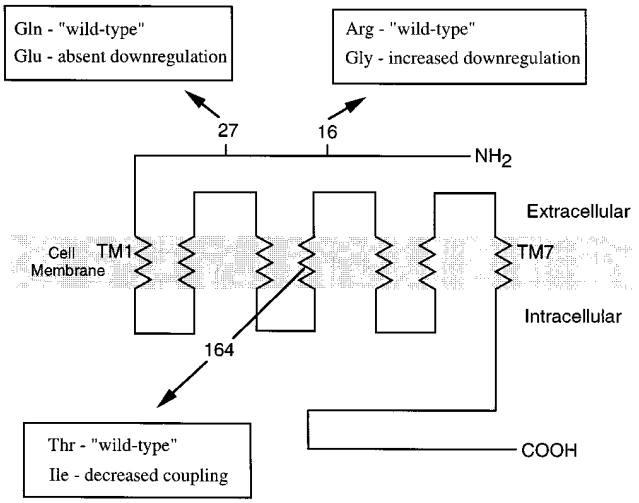

Figure 1. Functional polymorphisms of the human β_2-adrenergic receptor. Schematic of the proposed seven transmembrane topography of the receptor with the location (identified by amino acid number) of polymorphic sites. In the boxes, the phenotype of each variant, as determined in cells (10, 11, 13) and transgenic mice (12) is indicated.

receptor (11, 13). Although this is a more subtle phenotype, it is nevertheless potentially relevant in conditions such as heart failure, where levels of catecholamines are elevated and could act to differentially regulate β_2AR based on genotype.

β_2AR are also extensively expressed in the lung where they act to relax airway smooth muscle and thus modulate bronchomotor tone. Recent studies have shown that β_2AR polymorphisms alter the clinical characteristics of asthma, including severity (9), the extent of bronchial hyperresponsiveness (14), the expression of certain asthmatic phenotypes (15, 16), and responsiveness (17) or tachyphylaxis (18) to β-agonists. Since it is apparent that polymorphisms of the β_2AR alter receptor coupling or regulation in vitro and in vivo in cells and transgenic mice, and in patients with asthma, we prospectively tested the hypothesis that these genetic variants may alter the outcome of congestive heart failure.

Methods

Patients. The study was approved by the Institutional Review Board of the University of Cincinnati College of Medicine. The disease group consisted of 259 unrelated sequential patients with ischemic or idiopathic dilated cardiomyopathy (New York Heart Association functional class II-IV) who were referred to the University of Cincinnati Heart Failure/Transplant Program for evaluation between February 1, 1994 and June 1, 1997, and who gave informed consent. At the time of entry into the study, peripheral blood was drawn to determine β_2AR genotype (see below), and the patients were followed prospectively with the primary endpoint defined a priori as death or transplantation. The patients were aggressively treated with heart failure therapies including, but not limited to, cardiac transplantation. Investigators managing the heart failure therapy were blinded as to the β_2AR genotypes. Medications included digoxin, diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and antiarrhythmics. Patients deteriorating with conventional therapy were hospitalized and treated with intravenous inotropes (dobutamine as first-line therapy and milrinone for those patients who did not respond to dobutamine). Outpatient inotropic therapy was not used. As indicated below, ~ 20% of patients were treated with β-blockers (> 95% being the β_1AR specific antagonist metoprolol). The heart failure group had a mean age of 49.9 yr; 72% were male and 80% were Caucasian, with the remainder African American. The etiology of heart failure was classified as ischemic cardiomyopathy or idiopathic dilated cardiomyopathy. Individuals with preexisting hypertension were considered in the latter classification if they lacked significant epicardial coronary artery disease. The control group consisted of 212 healthy unrelated individuals recruited specifically for this study from a blood donor pool. Controls were without chronic disease, medication use, or family history of early cardiovascular disease, and consented to β_2AR genotyping. This normal population had a mean age of 33.3 yr; 61% were male and 91% were Caucasian.

β_2AR genotyping. Genomic DNA isolated from peripheral blood was subjected to three polymerase chain reactions (PCRs) amplifying portions of the β_2AR coding block. For the given polymorphic site, the sense and antisense primers were (5′-3′): codon 16 (nucleotide 46) ATGGGGCAACCCGGGAACGG and ACGATGAGAGACATGACGATG; codon 27 (nucleotide 79) CCGGGAACGGCAGCGC and ATGGCCAGGACGATGAGAGAC; codon 164 (nucleotide 491) TTACTTCACCTTTCAAGTACCAGAGC and CATAGCAGTTGATGGCTTCCTG. The reaction conditions consisted of 70–100 ng genomic DNA, 400 μM each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 0.17 ng/μl bovine serum albumin, 0.5 μM each primer, 0.025 U/μl Taq DNA polymerase and 0.55 μg/μl TaqStart antibody (Clontech, Palo Alto, CA) in a final volume of 30 μl. PCR conditions were initial denaturation of 94°C for 2 min, then 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min, with a final extension at 72°C for 7 min.

The polymorphisms were identified by the genetic bit analysis technique, which provides for rapid colorimetric detection in 96-well plates (19). In brief, the procedure involves immobilization of an additional primer to the plates, digestion of the PCR products to single-stranded template, and hybridization of these templates to the immobilized primers. After hybridization, one of two polymerase extension mixes, each including fluorescein- and biotin-labeled ddNTPs, are added to the 96-well plates. Detection of the labeled ddNTPs incorporated during extension involved two separate antibody/colorimetric assays. The fluorescein-labeled ddNTP was detected with an anti–fluorescein-AP conjugate (Boehringer-Mannheim, Indianapolis, IN) with the OD at 405 nm determined. After washing, the biotin-labeled ddNTP was detected with antibiotin horseradish peroxidase (VWR, Bridgeport, NJ) with the OD at 620 nm determined.

Statistical methods. A total of 259 patients with congestive heart failure were enrolled in the study. The genotypes at all three β_2AR loci were obtained for 98.8% of the study population. The objective of the primary data analysis was to determine whether the polymorphisms found at amino acid positions 16, 27, and 164 were related to the rate of death or transplantation. A Cox proportional hazards model (20) was fitted separately for each of the three polymorphic loci. The study population was followed from the date of entry in the study until death, heart transplant, or completion of the study, which was October 1, 1997 for the purpose of this analysis. Thus the minimal follow-up time was 3 mo; the mean follow-up time for all patients in the study was 672 d. The survival models also considered possible confounders such as age, age at onset of symptoms, race, gender, functional class, etiology (idiopathic dilated cardiomyopathy or ischemic cardiomyopathy), medication use, cause of death (sudden death or progressive failure), and left ventricular ejection fraction. Effect modification was assessed by examining interactions between the potential confounders and each polymorphism. Plots of the Kaplan-Meier estimates (21) for the survival curves for each type of polymorphism were also computed. Survival at 1 yr was derived from the Kaplan-Meier estimates as described (21). In addition, we compared the study group with a group of 212 healthy individuals with respect to the frequencies of each polymorphism by using analysis of covariance.

Table I. Frequencies of β_2AR Polymorphisms in Normal Subjects and Patients with Heart Failure

Position	Genotype	Frequency (%)	
		Normal	Heart failure
16	Arg* homozygous	14.6	19.2
	Arg/Gly	46.6	42.0
	Gly homozygous	38.8	38.8
27	Gln* homozygous	31.6	31.5
	Gln/Glu	52.9	48.6
	Glu homozygous	15.5	19.8
164	Thr* homozygous	97.1	96.1
	Thr/Ile	2.9	3.9
	Ile homozygous	0	0

*Considered the wild-type β_2AR at this locus (see text).

Table II. Clinical Characteristics at Time of Enrollment of Patients Having the Wild-type β_2AR or the Ile164 Polymorphism

	Ile164	Wild type
n	10	247
Age (yr)	47.2±3.7	49.9±0.7
Sex (% male)	50.0	73.7
Race (% Caucasian)	70.0	81.8
Etiology (%)		
Idiopathic dilated cardiomyopathy	80.0	54.7
Ischemic cardiomyopathy	20.0	45.3
Functional class (% III/IV)	80.0	66.4
LVEF (%)	22±3	22±0.6
Medication usage (%)		
ACE inhibitor	100	94.4
Digoxin	100	95.3
Diuretics	100	97.4
β-blockers	20.0	24.6
Antiarrhythmics	20.0	19.8
Ca^{2+} channel blockers	0	16.8

Results

We first delineated the frequencies of the three β_2AR polymorphisms in a group of normal individuals and in our heart failure cohort. The frequencies of the β_2AR polymorphisms in our control population were similar to what we (9, 15) and others have reported (17). Furthermore, they were identical between the normal subjects and the heart failure subjects (Table I). This suggests that these β_2AR polymorphisms are unlikely to be major causative factors in the development of heart failure.

Potential relationships between the pathophysiologic characteristics of the patients with heart failure and the β_2AR polymorphisms were then explored. We hypothesized that individuals bearing the substantially impaired Ile164 receptor would undergo rapid progression to death or transplant. 10 individuals in the cohort were found to be heterozygous for this polymorphism; no homozygous Ile164 individuals were found. The survival curves comparing patients with the Ile164 receptor to those with the wild-type (Thr164) receptor are shown in Fig. 2. The unadjusted relative risk estimated from the Cox proportional hazards model for the Ile164 patients to the Thr164 patients was 3.69 ($P = 0.002$). When adjusting this comparison for age at onset of symptoms, functional class and left ventricular ejection fraction, the relative risk was 4.81 (95% confidence interval [CI] = 2.0–11.5), $P < 0.001$. There was no interaction noted for this relationship when considering age, race, gender, cause of death (sudden death or progressive failure), medication use (including β-blockers), or etiology of heart failure (i.e., idiopathic dilated or ischemic cardiomyopathy). The 1-yr

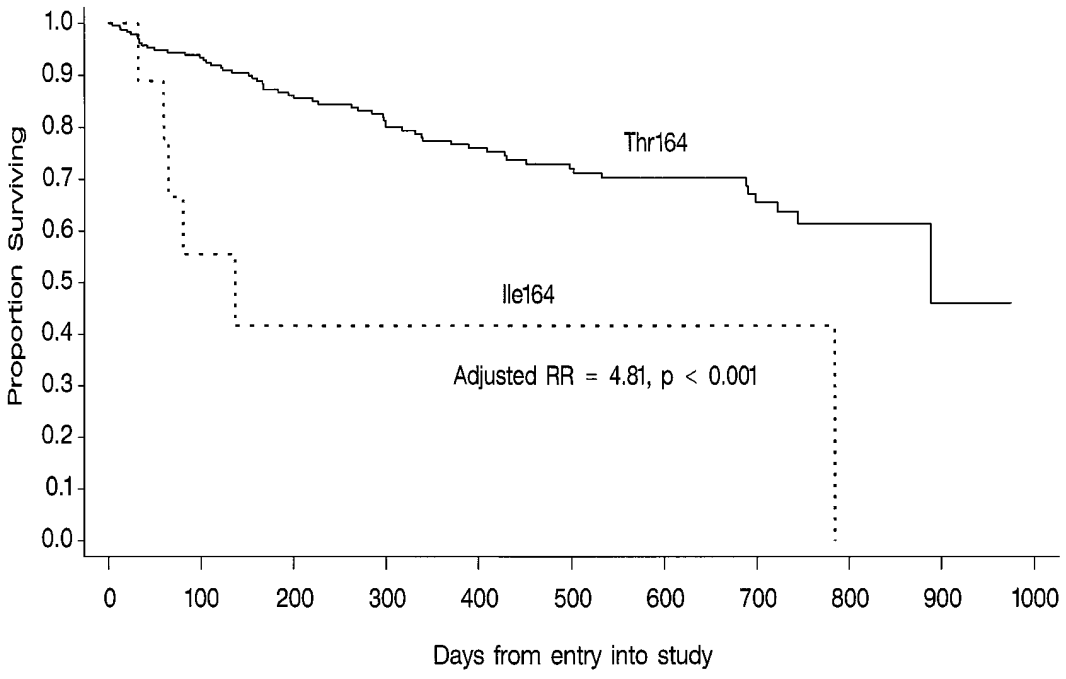

Figure 2. Kaplan-Meier survival curves for patients with congestive heart failure harboring the wild-type β_2AR or the Ile164 polymorphism. The Ile164 polymorphism was found only in the heterozygous state. The survival function is the proportion of patients who have not died or have not undergone cardiac transplantation. As is shown, individuals with the Ile164 polymorphism ($n = 10$ patients) had an increased risk of death or transplant compared with those with the Thr164 (wild-type) receptor ($n = 247$ patients).

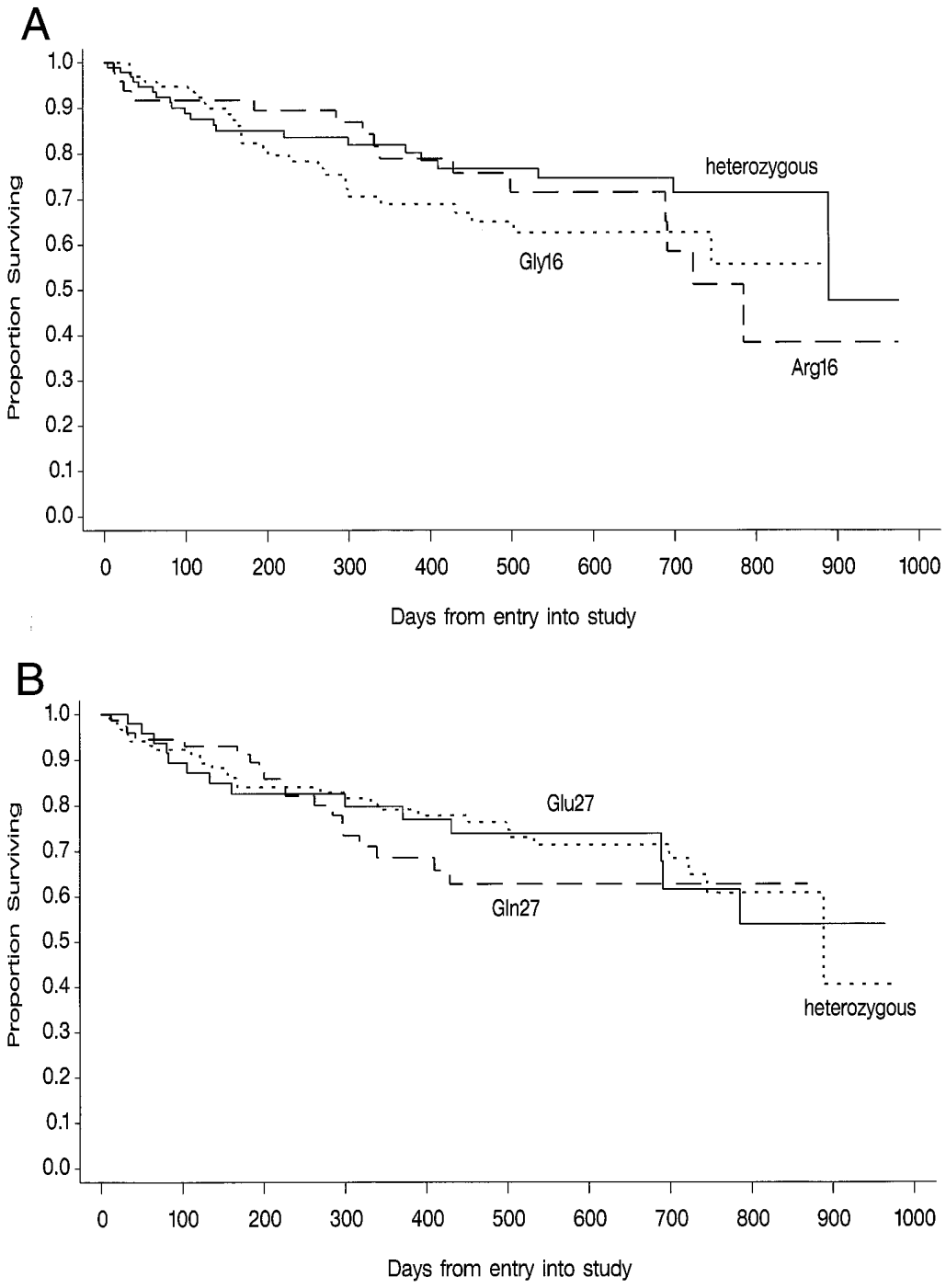

Figure 3. Kaplan-Meier survival curves for patients with congestive heart failure harboring polymorphic β_2AR at amino acid positions 16 (*A*) and 27 (*B*). The survival function is the proportion of patients who have not died or have not undergone cardiac transplantation. The apparent decreased survival of those with Gly16 (adjusted RR = 0.965, 95% CI = 0.5–1.86) or with Gln27 (adjusted RR = 1.16, 95% CI = 0.58–2.31) was not statistically significant.

follow-up survival for individuals with the Ile164 receptor was 42%, as compared with 76% for those with the wild-type (Thr164) receptor ($P = 0.019$). These findings were independent of the presence of other polymorphisms at the other two loci. The characteristics of patients with the Ile164 vs. Thr164 receptor are shown in Table II. At the time of enrollment, there was no statistically significant clinical characteristic (age, sex, race, etiology of heart failure, medication use, functional class, or left ventricular ejection fraction [LVEF]) that distinguished the Ile164 individuals from those with the wild-type receptor. As shown, the medications used for treatment of the patients with the Ile164 polymorphism were typical of the heart failure population as a whole. In particular, the frequency of β-blocker usage amongst the two groups (20.0 vs. 24.6%) was not different. The survival of those with the Ile164 polymorphism was not due to the use of β-blockers, as in fact none of the survivors were taking these agents.

The survival function curves for individuals harboring the homozygous and heterozygous forms of the receptor at loci 16 and 27 are shown in Fig. 3, *A* and *B*. Within the current follow-up period, we are unable to detect significant differences in survival for either of these genotypes. However, patients who

were homozygous for the Gln27 receptor (which undergoes downregulation as compared with the Glu27 polymorphism that does not) did appear to have decreased survival (Fig. 3 *B*), although this was not statistically significant (Gln27 vs. Glu27 adjusted relative risk (RR) = 1.16, 95% CI 0.58–2.31). A similar apparent decrease in survival was noted for those with the Gly16 receptor compared with the Arg16 receptor (Fig. 3 *A*), but again this was not statistically significant (RR = 0.965, 95% CI = 0.5–1.86). Since the polymorphisms at loci 16 and 27 can occur together, nine different combinations are possible. The survival analysis was thus performed on each haplotype, but again we found no significant differences in survival (data not shown) nor was there any confounding or interaction with age, age of onset, sex, race, etiology, medication usage, cause of death, functional class, or LVEF. Finally, we also compared the number of hospitalizations for heart failure exacerbations (without and with correction for length of follow-up) and found no association with genotype at these two loci.

Discussion

It has been known for decades that adrenergic receptor function is dynamically regulated by a host of factors and that there is substantial variation in receptor expression and function within a given population (22). Regulatory processes that have been identified to be relevant to heart failure include receptor phosphorylation by G protein coupled receptor kinases (such as the βAR kinase) and protein kinase A, internalization of receptors, and downregulation of receptor expression (23, 24). Only recently has a potential genetic basis for regulation of receptor function been realized for the β_2AR (10–12, 25). Three functionally significant polymorphisms have been identified in the human population at amino acids 16, 27, and 164 of the β_2AR. (To maintain a consistent reference sequence, Arg16, Gln27, and Thr164 are denoted as the wild-type alleles, although it is clear from the frequencies of the polymorphisms at positions 16 and 27 that there is no consensus wild-type sequence.) The functional relevance of these variations has been extensively evaluated in transfected cells (10, 11), cells endogenously expressing various polymorphic receptors (13), and in transgenic mice (12). In cell culture studies, each receptor has a unique phenotype as compared with wild type, with the Gly16 receptor displaying enhanced agonist-promoted loss of receptor expression (downregulation), the Glu27 form being resistant to downregulation, and the Ile164 form displaying markedly depressed coupling to the stimulation of cAMP (Fig. 1). The latter receptor has also been studied within the context of cardiac function by the use of targeted transgenesis in mice (12), where we found a very significant impairment of receptor function in vivo.

We found that there was no difference in the frequency of any polymorphism in the heart failure group vs. the normal population. Having established, then, that β_2AR polymorphisms do not appear to predispose to heart failure, we focused upon potential modifying roles of these genetic variants in the progression and outcome of congestive heart failure. If maintenance of β_2AR function were beneficial, one would hypothesize based on the aforementioned studies that those with the Ile164 polymorphism might display a more rapid decline in ventricular function than those with the wild-type β_2AR. Despite the small number of patients with the Ile164 polymorphism, the estimated increase in relative risk of death or transplant was highly statistically significant ($P < 0.001$) and highly elevated, i.e., almost a fivefold higher risk. Polymorphisms at the other two sites affect the downregulation of the receptor by agonist. While it is possible that such differences might also be reflected in the indices that we examined in our heart failure patients, this did not turn out to be the case within the length of the current follow-up. Interestingly, those patients with the Gln27 receptor (which is also potentially hypofunctional since it downregulates as compared with the Glu27 receptor) do show a trend towards decreased survival (Fig. 3 *B*). Also, those with the Gly16 receptor, which downregulates to a greater extent than the Arg16 receptor, appear to have decreased survival. Neither of these trends are statistically significant. However, with additional follow-up time, we may find that survival is indeed impacted by the position 16 and 27 polymorphisms as well. On the other hand, it may be that the most pertinent effect of these two polymorphisms is in the early stages of the disease (i.e., < 600 d based on Fig. 3). Larger study populations will likely be necessary to test this hypothesis.

The mechanisms by which the hypofunctional Ile164 β_2AR substantially alters the progression and outcome of patients with heart failure is presently unknown. In fact, several observations might lead one to conclude that the more "active" βAR in heart failure would predispose individuals to decompensation. First, it is well recognized that some patients with heart failure favorably respond to administration of β-blockers (26). These studies, however, have been carried out largely with β_1AR subtype–specific antagonists (e.g., metoprolol) or nonselective βAR antagonists. Virtually nothing is known about the effects of a purely β_2AR subtype–selective antagonist on the course of human heart failure. Second, activation of βAR, as well as inhibition of phosphodiesterases both lead to increased cAMP, and clinical trials with phosphodiesterase inhibitors have shown an increase in mortality in patients with heart failure (27). However, it appears that cAMP is compartmentalized in the cardiomyocyte (28, 29) and that the pools affected by β_1AR or β_2AR activation may be different than those affected by inhibition of phosphodiesterases. Also, it should be noted that the above observations are based on interventions typically initiated at some point in time after the onset of the disease. Thus, this is fundamentally different from a genetically based alteration in receptor function that is present at birth, at the onset of the disease, and throughout the course of the disease. In addition, recent studies have shown that β_2AR efficiently couple to the Na^+/H^+ exchanger regulatory factor, which regulates the activity of the Na^+/H^+ exchanger type 3 (30). Deficient β_2AR coupling to this pathway in the failing myocyte may result in potential effects on intracellular pH and important functional consequences. Finally, it should be noted that as the β_1AR undergoes extensive downregulation of receptor number in progressive failure, the heart has been thought to become more reliant on the β_2AR for maintenance of catecholamine mediated function (31). A genetically dysfunctional β_2AR due to the Ile164 polymorphism, then, may not have the level of function necessary to provide this compensatory role. It is interesting to note recent studies whereby cardiac function or myocyte signaling has been shown to be improved by expression of β_2AR either via transgenesis (32), direct injection of β_2AR cDNA (33), or infection with viral constructs (34). It is thus conceivable that gene therapy with the β_2AR may be useful in human heart failure. Perhaps

those with the Ile164 receptor may be particularly relevant candidates, since their endogenous β_2AR are inherently defective.

We conclude that individuals with heart failure harboring the Ile164 polymorphism of the β_2AR are at significant risk for rapid progression. This observation may prove useful in the intensity of follow-up, the selection and timing of conventional and/or experimental pharmacologic therapies, and in the prognostic evaluation and selection of such individuals for orthotopic cardiac transplantation. Also, it appears that additional studies are indicated to assess the role of this polymorphism in the onset and early evolution of hypertrophy or failure in high risk populations.

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