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β_1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure

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Catecholamines stimulate cardiac contractility through β_1-adrenergic receptors (β_1-ARs), which in humans are polymorphic at amino acid residue 389 (Arg/Gly). We used cardiac-targeted transgenesis in a mouse model to delineate mechanisms accounting for the association of Arg389 with human heart failure phenotypes. Hearts from young Arg389 mice had enhanced receptor function and contractility compared with Gly389 hearts. Older Arg389 mice displayed a phenotypic switch, with decreased β-agonist signaling to adenylyl cyclase and decreased cardiac contractility compared with Gly 389 hearts. Arg389 hearts had abnormal expression of fetal and hypertrophy genes and calcium-cycling proteins, decreased adenylyl cyclase and $G\alpha_s$ expression, and fibrosis with heart failure This phenotype was recapitulated in homozygous, end-stage, failing human hearts. In addition, hemodynamic responses to β-receptor blockade were greater in Arg389 mice, and homozygosity for Arg389 was associated with improvement in ventricular function during carvedilol treatment in heart failure patients. Thus the human Arg389 variant predisposes to heart failure by instigating hyperactive signaling programs leading to depressed receptor coupling and ventricular dysfunction, and influences the therapeutic response to β-receptor blockade.

Despite recent treatment advances, mortality from heart failure is ~50% within 5 years[1]. A potential role for common genetic variants in susceptibility, progression and response to treatment is suggested by familial clustering of phenotypes, reduced penetrance in familial cardiomyopathies and marked interindividual variations in progression and treatment outcomes[1–3]. β_1-ARs are the predominant cardiac receptors for the catecholamines norepinephrine and epinephrine; along with a smaller contribution by β_2-ARs, they represent the major mechanism whereby cardiac output is increased by the sympathetic nervous system. However, prolonged activation of β_1-ARs ultimately worsens ventricular function regardless of the initial cause of failure. This is the presumed basis for the therapeutic efficacy of β-blockers[1] in heart failure[4,5].

We identified nonsynonymous polymorphic variations in the human β_1-AR gene (*ADRB1*) at nucleotide 1165 of the coding region, resulting in either Arg or Gly as amino acid 389 (ref. 6). Although Gly is the minor allele, with a frequency of ~25–45% (ref. 7), it has been the reference receptor for recombinant functional studies. Based on the crystal structure of bovine rhodopsin[8], this nonconservative variation is predicted to lie within a conformationally sensitive G_s coupling domain in the intracellular helix between transmembrane domain 7 and the palmitoylated cysteine(s) in the intracellular tail of the receptor. Initial studies with human β_1-ARs expressed in fibroblasts support this notion[6]. We recently reported that the Arg389 β_1-AR, when present with a polymorphic α_{2c}-AR that regulates presynaptic norepinephrine release, is a risk factor for human heart failure[9]. In addition, β_1-AR variants at this position are proposed to be associated with intermediate heart failure phenotypes[10] and hypertension[11] (which itself can predispose to heart failure). As such, this polymorphic variation may be useful for risk assessment and tailoring therapy. However, a molecular and physiologic basis for the aforementioned observations is lacking, so the specific pathogenic roles of the β_1-AR variants are not known and a treatment strategy cannot be formulated based on genotype. Here we report targeted transgenic overexpression of both β_1-AR variants in mouse ventricles, revealing an allele-specific cardiac phenotype that was recapitulated in human heart failure.

RESULTS

Arg389 β_1-AR causes an early hypercontractile state

To assess the potential role of these β_1-AR variants in the development of heart failure, transgenic mice were made to express human Arg389 or Gly389 β_1-ARs in the ventricles, under the control of the α-myosin heavy-chain promoter. This overexpression approach has been a useful model for examining adrenergic signaling in the heart because it provides persistent subtype- and cell-type-specific activation of a specific signal transduction pathway[4,12,13]. We generated several mouse lines expressing various levels of these two receptors, as determined by radioligand binding. Except where noted, we used two lines with equivalent expression of the two receptors in the heart (1.0 ± 0.17 and 1.2 ± 0.21 pmol receptor per mg protein, respectively).

To examine cardiac function under consistent loading conditions independent of systemic neurohumoral influences, work-performing

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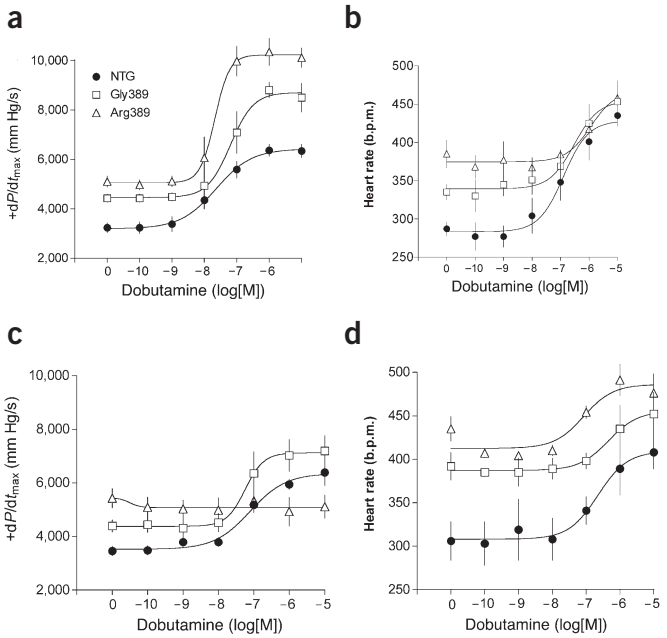

Figure 1 Functional consequences of polymorphic β_1-AR expression in transgenic mouse hearts. (**a**,**b**) Work-performing heart studies at 3 months of age reveal enhanced contractility (+dP/dt_{max}) **a**) and heart rates (**b**) at baseline, and an enhanced contractile response to the β_1-AR agonist dobutamine ($n = 5$, $P < 0.01$ for Arg389 compared with Gly389 β_1-AR transgenic mice). (**c**,**d**) Studies at 6 months of age of contractile (**c**) and heart rate (**d**) responses reveal a loss of contractile responses to agonist in Arg389, but not Gly389, β_1-AR transgenic mice ($P < 0.01$, $n = 5$). NTG, nontransgenic. Symbols in **b**–**d** are same as in **a**.

isolated heart preparations were studied in 3- and 6-month-old mice (**Fig. 1**). At 3 months of age, both Arg389 and Gly389 transgenic hearts showed increased cardiac contractility (+dP/dt_{max}) compared with those of nontransgenic littermates (**Fig. 1a**). However, Arg389 hearts had significantly greater ($P < 0.01$) basal and β_1-agonist-stimulated contractility compared with Gly389 hearts. Baseline heart rates were also higher for Arg389 mice, although both ultimately reached the same maximal agonist-stimulated rates (**Fig. 1b**). Agonist-stimulated adenylyl cyclase activity was determined in ventricular membranes (**Fig. 2**). At 3 months of age, activity measured in Arg389 hearts showed enhanced stimulation by the partial β_1-AR agonist GW805415, compared with Gly389 hearts; a similar trend was noted for the full agonist isoproterenol (**Fig. 2a**). Expression of the fetal, calcium-cycling and hypertrophy-associated genes encoding myosin α- and β-heavy chains, atrial natriuretic factor, sarcoplasmic endoplasmic reticulum calcium ATPase-2 (SERCA-2a) and phospholamban (PLN) was examined using dot blots of ventricular mRNA (**Fig. 3**). At 3 months of age, no differences in transcript expression were noted in Arg389 or Gly389 hearts, as compared with nontransgenic hearts (**Fig. 3a**). In addition, histopathologic sections of the ventricles (data not shown) were normal in both sets of transgenic mice at this early age.

Age-dependent contractile failure in Arg389 mice

By 6 months of age, a marked contractile impairment of Arg389 hearts to β-agonist stimulation became evident. Although baseline dP/dt_{max} remained greater for the Arg389 mice, a positive inotropic response to the β-agonist dobutamine was no longer observed (**Fig. 1c**). Despite this, the Arg389 mice showed a small but consistent chronotropic response to dobutamine (**Fig. 1d**). This loss of cardiac β-AR inotropic responsiveness to agonist is a hallmark of human heart failure[14] and is related to ventricular function as well as the improvement in function after treatment with β-blockers. To address these issues, we conducted studies in transgenic mice and in humans with various β_1-AR genotypes (**Fig. 4**). We used noninvasive echocardiography to longitudinally assess *in vivo* function in the mice (**Fig. 4a**). By 9 months of age (**Table 1**), the Arg389 mice had markedly reduced fractional shortening at rest compared with Gly389 mice (26 ± 2% versus 42 ± 4%, respectively; $P = 0.02$). Cardiac gene expression profiles also revealed allele-specific changes in the older mice (**Fig. 3b**,**c**). At 6 and 9 months, the fetal pattern of expression of the myosin α- and β-heavy chain genes was apparent in ventricles expressing the Arg389, but not the Gly389, variant. In addition, atrial natriuretic factor was increased and SERCA-2a and PLN were decreased, again only in the Arg389 mice. Histopathology of sections from 9-month-old mice revealed myocyte loss with replacement fibrosis in the Arg389 mice, which was not apparent in the Gly389 or nontransgenic mice (**Fig. 4b**). No evidence of apoptosis in ventricles from either Gly389 or Arg389 mice was detected (data not shown). The phenotype associated with the Arg389 genotype was explored in additional transgenic

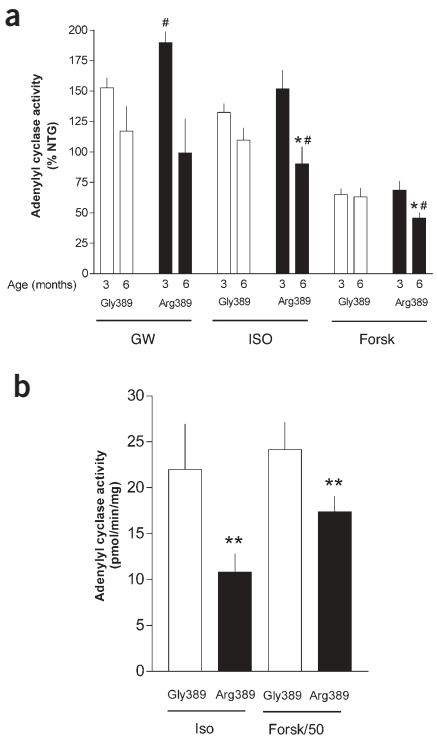

Figure 2 Phenotypic switching of Arg389 and Gly389 β_1-AR signaling in mouse and human ventricles. (**a**) β-AR stimulation of adenylyl cyclase activity by the agonists GW805415 (GW) and isoproterenol (Iso) undergoes a marked decrease between the ages of 3 and 6 months, in Arg389 ventricular membranes of mice. Similarly, forskolin (Forsk)-stimulated activities are reduced in an age-dependent fashion in Arg389, but not Gly389, mice. *, $P < 0.02$ for 3 months compared with 6 months, within genotype; #, $P < 0.02$ between genotypes of the same age ($n = 5$–9). (**b**) Membranes from homozygous β_1-AR variant human end-stage failing hearts show depressed function of the Arg389 variant. Stimulation of Arg389 membranes with isoproterenol and forskolin (Forsk/50, values divided by 50) show decreased signaling compared with Gly389 membranes. **, $P < 0.01$ for Arg389 versus Gly389. ($n = 6$ in each group).

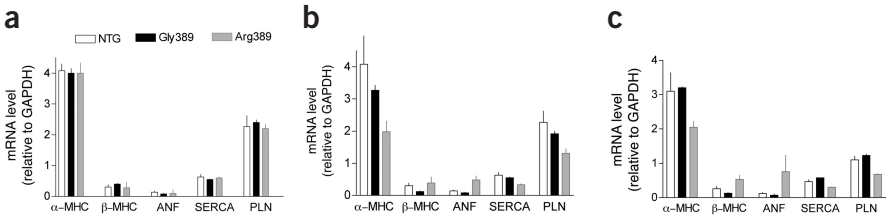

Figure 3 Cardiac gene expression profiles from polymorphic β_1-AR transgenic mice. (**a**–**c**) RNA dot blot analysis showed no differences in transcript expression between Arg389, Gly389 and nontransgenic hearts at 3 months of age (**a**). At 6 months (**b**) and 9 months (**c**) Arg389, but not Gly389, hearts showed differences in transcript expression compared to nontransgenic hearts of fetal, calcium-cycling and hypertrophy-associated genes ($P < 0.05$, $n = 4$–6). α- and β-MHC, α- and β-myosin heavy chains; ANF, atrial natriuretic factor. NTG, nontransgenic.

mouse lines, where a gene-dose response was evident. At expression levels of 2.8 ± 0.05 pmol/mg of the Arg389 variant, young mice had markedly elevated ventricular atrial natriuretic factor transcript expression and died of massive cardiomegaly. In mice expressing 1.0 ± 0.17 pmol/mg of the Arg389 variant, the enhanced early responsiveness was followed by a decline observed at 6 months, as shown in **Figure 1a,c**. However, in mice expressing the Arg389 or Gly389 variants at ~0.4 pmol/mg, enhanced $+dP/dt_{max}$ at baseline and in response to dobutamine was observed at 3 months but did not progress to a depressed functional response by 6 months (maximal dobutamine-stimulated $+dP/dt_{max}$ was 10,500 ± 700 for Arg389 and 8,020 ± 600 mm Hg/s for Gly389).

Altered force-frequency and calcium-cycling proteins

Work-performing isolated heart studies examining the force-frequency relationships in 6-month-old Arg389 and Gly389 mice were carried out to assess non-receptor-mediated contractile parameters. For these experiments, hearts were paced from baseline rates up to 800 beats per minute (b.p.m.), with $+dP/dt_{max}$ determined at multiple intervals. Neither transgenic heart showed an ascending limb (early increase in contractility with increased heart rate) because their baseline heart rates and $+dP/dt_{max}$ were already increased. At higher-paced rates, however, the threshold for a decrease in $+dP/dt_{max}$ (descending limb) occurred at 475 ± 50 b.p.m. for Arg389 mice, in contrast to 725 ± 70 b.p.m. for Gly389 mice (the threshold for nontransgenic mice was 600 ± 80 b.p.m.). These results suggested aberrant calcium cycling, potentially because of altered expression of PLN or SERCA-2a. Western blots were done using ventricular lysates, to assess expression of calcium-cycling proteins and β-AR signaling elements (**Fig. 5**). The phosphorylated form of PLN was markedly decreased in 6-month-old Arg389 mice compared with Gly389 mice, with total PLN levels being slightly lower (**Fig. 5a**), consistent with decreased β-AR responsiveness in intact hearts. SERCA-2a levels were also depressed in an allele-specific manner in 6-month-old mice. In contrast, only a small decrease (~15%) in SERCA-2a was observed at 3 months in the Arg389 ventricles compared with Gly389, with no other allele-specific changes in phosphorylated PLN or total PLN protein expression (data not shown).

Phenotypic switching of Arg389 and Gly389 β_1-ARs

The decreased cardiac β_1-AR-mediated stimulation observed in human heart failure is caused by a downregulation of receptor expression and by a functional desensitization of receptor–adenylyl cyclase coupling[15]. These events can be considered adaptive in nature, as they may protect the failing heart with limited contractile reserves from persistent inotropic stimulation by elevated norepinephrine levels. Agonist-promoted downregulation of β_1-AR is the result of decreased transcription and increased receptor protein degradation[16]. In hearts from Gly389 mice, a ~74% loss of β_1-AR expression was observed over

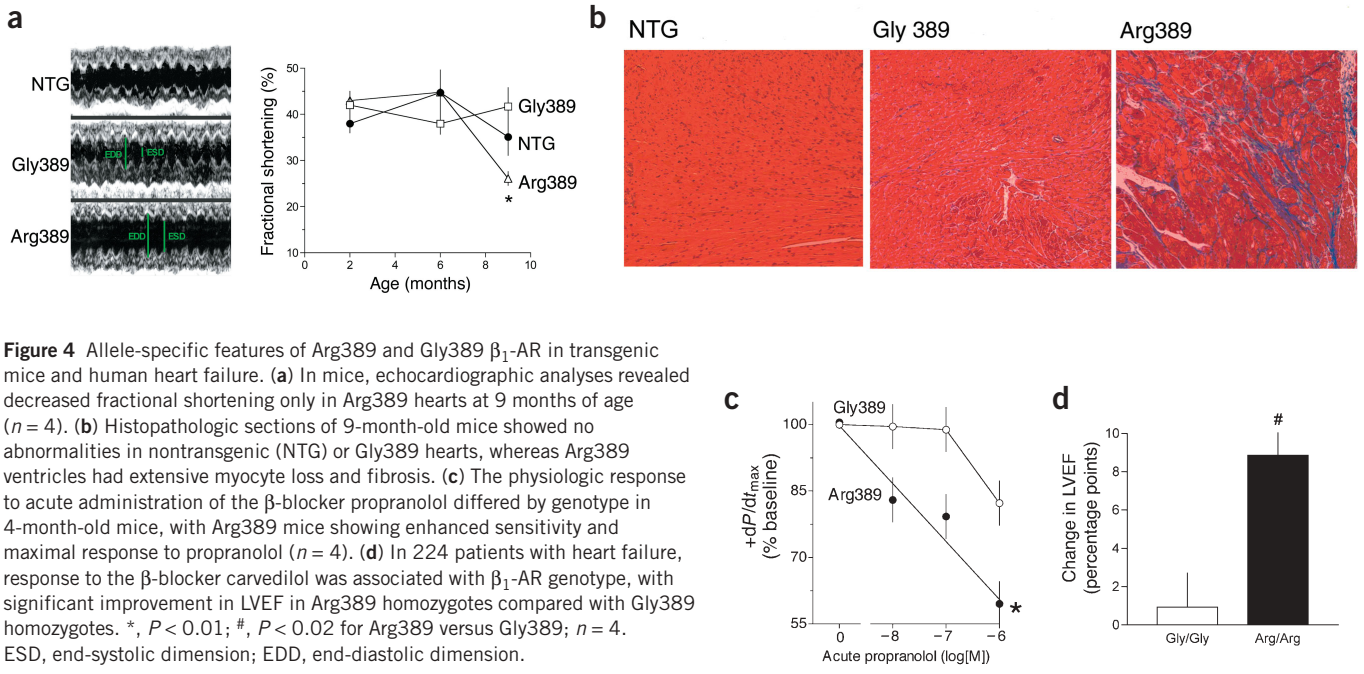

Figure 4 Allele-specific features of Arg389 and Gly389 β_1-AR in transgenic mice and human heart failure. (**a**) In mice, echocardiographic analyses revealed decreased fractional shortening only in Arg389 hearts at 9 months of age ($n = 4$). (**b**) Histopathologic sections of 9-month-old mice showed no abnormalities in nontransgenic (NTG) or Gly389 hearts, whereas Arg389 ventricles had extensive myocyte loss and fibrosis. (**c**) The physiologic response to acute administration of the β-blocker propranolol differed by genotype in 4-month-old mice, with Arg389 mice showing enhanced sensitivity and maximal response to propranolol ($n = 4$). (**d**) In 224 patients with heart failure, response to the β-blocker carvedilol was associated with β_1-AR genotype, with significant improvement in LVEF in Arg389 homozygotes compared with Gly389 homozygotes. *, $P < 0.01$; #, $P < 0.02$ for Arg389 versus Gly389; $n = 4$. ESD, end-systolic dimension; EDD, end-diastolic dimension.

Table 1 Echocardiographic indices of 9-month-old mice

	NTG	Gly389	Arg389
LVEDD (mm)	3.34 ± 0.13	3.25 ± 0.14	4.23 ± 0.12*
LVESD (mm)	2.16 ± 0.12	1.19 ± 0.19	3.12 ± 0.04*
Rate (b.p.m.)	442 ± 58	484 ± 17	500 ± 53
H/R	0.34 ± 0.01	0.47 ± 0.04	0.24 ± 0.02*
Vcf (circ/s)	6.1 ± 0.85	8.23 ± 0.86	5.21 ± 0.34*
FS (%)	35.1 ± 4.04	41.7 ± 4.05	26.1 ± 1.52*

LVEDD and LVESD, left-ventricular end-diastolic and end-systolic dimensions, respectively; H/R, ratio of wall thickness to left-ventricular radius; Vcf, velocity of circumferential fiber shortening; FS, fractional shortening; NTG, nontransgenic littermates; circ, circumference. *, $P < 0.02$ compared with Gly389.

time (from 1.19 ± 0.21 at 3 months to 0.31 ± 0.04 pmol/mg at 6 months). In contrast, the Arg389 β_1-AR underwent only ~44% downregulation over the same time period (1.02 ± 0.17 at 3 months versus 0.56 ± 0.04 pmol/mg at 6 months; $n = 4$, $P < 0.01$ for Arg389 compared with Gly389). These data indicate that the Arg389 variant is impaired in this important protective event, and that the absence of contractile responsiveness to a β-agonist, observed at 6 months, is not caused by a paucity of receptors. β-AR coupling was assessed by agonist-stimulated adenylyl cyclase assays in ventricular membrane preparations. At 3 months, both types of transgenic mice had enhanced stimulation of cardiac adenylyl cyclase compared with nontransgenic mice (**Fig. 2a**). By 6 months, however, a marked decrease in agonist-stimulated activity was observed in Arg389 mice compared with 3-month-old Arg389 mice, despite having relatively preserved receptor expression (**Fig. 2a**). In comparison, Gly389 mice showed little age-dependent decrease in β-AR signaling to adenylyl cyclase. This desensitization of the Arg389 receptor was true for both the partial (GW805415) and full (isoproterenol) agonists used in the assay. Forskolin-stimulated activities, a measure of expression and function of adenylyl cyclase, were not different between 3-month-old and 6-month-old Gly389 mice. In contrast, Arg389 mice showed an age-dependent decrease of ~30% in forskolin-stimulated activities over the same period of time (**Fig. 2a**). Western blots showed that type V adenylyl cyclase was ~40% less abundant in 6-month-old Arg389 ventricles compared with Gly389 ventricles (11,073 ± 890 versus 15,697 ± 653 pixels; $P < 0.01$; **Fig. 5b**). Similarly, $G\alpha_s$ expression was lower in Arg389 ventricles compared with Gly389 ventricles, but only at 6 months of age (12,008 ± 738 versus 22,607 ± 119 pixels; $P < 0.01$; **Fig. 5b**). Sodium fluoride–stimulated adenylyl cyclase activities were ~30% less in 6-month-old Arg 389 ventricular membranes, compared with those from Gly389 mice (62 ± 2.2 versus 88 ± 11.7 pmol/min/mg, respectively; $P < 0.05$), consistent with allele-specific downregulation of $G\alpha_s$. We also examined expression of G-protein-coupled receptor kinase-2 (GRK-2) and $G\alpha_i$, known to contribute to β-AR dysfunction in various rodent models of hypertrophy or heart failure, and in the human syndrome[17–21]. We found a marked increase in the expression of both proteins, but this increase was not different between Arg389 and Gly389 hearts (**Fig. 5b**). Taken together, the loss of agonist-promoted contractile responsiveness is partially explained by decreased function of β-AR in an allele-specific fashion, as well as decreases in the abundance of $G\alpha_s$ and type V adenylyl cyclase, the next two elements in the signaling pathway. This physiologic desensitization of Arg389 hearts at 6 months also involves distal components, as indicated by alterations in calcium cycling and PLN and SERCA-2a expression that are only observed in mice with that genotype.

Because enhanced agonist-promoted function is evident in Arg389 β_1-AR-transfected cells[6], intact hearts (**Fig. 1a**) and ventricular membranes (**Fig. 2a**) of young (3-month-old) Arg389 transgenic mice, the temporal events leading to depressed function suggest a 'phenotypic switch'. We explored whether this reversed phenotype is also present in end-stage human heart failure, using explanted hearts from idiopathic dilated cardiomyopathy patients (six of each homozygous genotype). As expected, ventricular β_1-AR was markedly reduced in Arg389 hearts compared with Gly389 hearts (12.0 ± 5.2 and 9.6 ± 3.8 fmol/mg, respectively). This trend was of the same magnitude and direction as in the transgenic mouse data, but was not statistically significant. However, functional responses showed a substantial difference when stratified by receptor genotype. Agonist-stimulated adenylyl cyclase activity in ventricular membranes from hearts of patients bearing the Arg389 β_1-AR variant were markedly lower compared with the Gly389 variant (**Fig. 2b**). In addition, and also consistent with the mouse data, forskolin-stimulated activities were lower in membranes from hearts with the Arg389 variant.

Arg389 predicts β-blocker response in mice and humans

β-AR antagonists are used in the treatment of human heart failure, where they are thought to partially relieve the compromised heart of the forced inotropic and chronotropic effects of elevated norepinephrine. However, there is marked interindividual variability in the response to β-blockade[2], which may be based on genetic variability in β_1-AR, the target of these agents. We sought to ascertain whether a differential response to β-blockade, in terms of sensitivity or maximal responsiveness, was present in the transgenic mice. Acute and chronic responses to the β-AR antagonist propranolol were assessed. Acute responses were studied in 4-month-old hearts by measuring the decrease in $+dP/dt_{max}$ and heart rate with increasing concentrations of infused propranolol. Gly389 hearts showed no decrease in contractility except at the highest dose of propranolol (**Fig. 4c**). In contrast, Arg389 hearts were highly sensitive to acute β-blockade, displaying a steep linear response and a greater decrease in contractility (**Fig. 4c**). The heart-rate response in these acute studies also indicated a greater sensitivity to propranolol for Arg389 compared with Gly389 hearts (slopes were −45 ± 1.8 versus −26 ± 4.7, respectively; $P < 0.01$; data not shown). To study the chronic response, heart rates were determined in young mice by echocardiography, before and at the end of a 5-week period, in untreated mice and mice treated orally with propranolol. In the Gly389 mice, there was no difference in heart rates between those treated with chronic propranolol and the untreated controls (541 ±

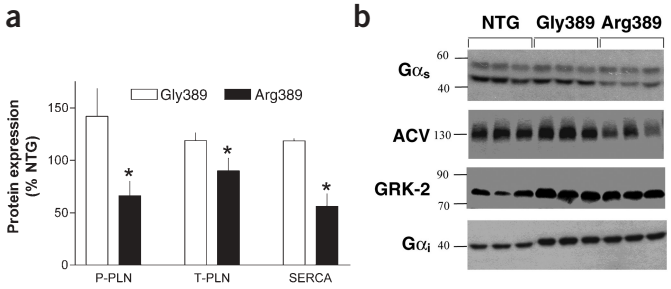

Figure 5 Altered expression of calcium-cycling and β-AR signaling proteins in 6-month-old Arg389 hearts. (**a**) Phosphorylated PLN (P-PLN), total PLN (T-PLN) and SERCA-2a were all reduced in ventricular membranes from Arg389 compared with Gly389 transgenic mice. (*, $P < 0.01$, $n = 3–5$) (**b**) Expression of ventricular $G\alpha_s$ and type 5 adenylyl cyclase (ACV) was reduced in Arg389 compared with Gly389 transgenic hearts ($P < 0.01$, $n = 4$). GRK-2 and $G\alpha_i$ were increased in hearts from both lines of transgenic mice, with no evidence of differential regulation by β_1-AR genotype. NTG, nontransgenic.

45 versus 534 ± 23 b.p.m., respectively; $n = 4$). The Arg389 mice, however, showed a ~165-b.p.m. decrease in heart rate in response to propranolol, compared with untreated Arg389 mice (415 ± 21 versus 580 ± 31 b.p.m., respectively; $n = 4$, $P < 0.005$).

This allele-specific response suggested that the protective effect of β-blockade against chronic catecholamine stimulation of cardiac β_1-ARs in human heart failure would be more likely to occur in patients expressing the Arg389 variant. This would result in a greater therapeutic response from Arg389 patients, as represented by improvement in left-ventricular function (LVEF). To address this, 224 patients with heart failure were treated with a standardized dosing regimen of the β-blocker carvedilol[22] and genotyped at codon 389 of the β_1-AR locus. Ventricular function before treatment was not different between Arg389 and Gly389 homozygotes (LVEF of 26 ± 8.6% versus 25 ± 0.9%, respectively); nor was the final, stable dose of carvedilol different between the two groups (54 ± 2.9 versus 56 ± 7.4 mg/d, respectively). However, β_1-AR genotype was associated with improvement in LVEF during β-blockade (**Fig. 4d**). Arg389-homozygous patients showed a substantially greater improvement in LVEF (8.7 ± 1.1%) compared with Gly389-homozygous patients (0.93 ± 1.7%; $P < 0.02$). Heterozygotes showed an improvement similar to that of Arg389-homozygous patients (7.02 ± 1.5%).

DISCUSSION

These studies reveal that Arg389 β_1-AR, as compared with Gly389 β_1-AR, the other allelic variant found in the human population, causes enhanced signaling at an early age, but evokes a program that ultimately leads to decreased signaling and heart failure. In young mice, the Arg389 genotype does not cause abnormalities in hypertrophy or fetal gene expression, proximal or distal signaling elements, or histology. By 6 months of age, however, multiple alterations are evident only in Arg389 mice, representing potentially adaptive and maladaptive events. At this age, Arg389 hearts lack contractile responsiveness to β_1-AR stimulation (**Fig. 1**). Depressed β_1-AR stimulation *in vivo*, *ex vivo* and *in vitro* are well-established features of human heart failure[14,15,23]. Downregulation of β_1-AR is also evident in the human syndrome, although there is marked interindividual variability in both β_1-AR desensitization and downregulation[15], suggesting that there may be a genetic basis for such variations in phenotype.

We show here that mice overexpressing either the Arg389 or Gly389 β_1-AR undergo age-dependent downregulation of receptor density. This has not been observed in hearts of β_2-AR-overexpressing mice, and this subtype-specific response has also been observed in human heart failure[15,23]. In the current study, however, we found a significant difference in the extent of downregulation between the two polymorphic β_1-ARs, with Arg389 mice showing less downregulation than Gly389 mice. Despite this greater expression at 6 months, β_1-AR signaling to adenylyl cyclase is depressed at this age in Arg389 mice, which correlates with decreases in $G\alpha_s$ and type V adenylyl cyclase expression. The greater propensity for the Arg389 receptor to undergo desensitization by GRKs[24], as well as the increase in GRK-2 expression, may further contribute to these proximal signaling perturbations. At more distal elements of the agonist-contractile response pathway, phosphorylated PLN, total PLN and SERCA-2a protein expression is reduced, and the fetal-like pattern of myosin heavy-chain gene expression is observed. By 9 months, Arg389 mice develop depressed ventricular function and pathologic fibrosis, which are not observed in Gly389 mice. Taken together, these results suggest that the Arg389 polymorphism may predispose an individual to heart failure. In support of this concept, several studies have shown associations between Arg389 β_1-AR and cardiovascular phenotypes. These include a synergistic gene-gene interaction with the α_{2c}-AR, which modulates presynaptic norepinephrine release[9], and hypertension in patients in the absence[11] or presence[10] of heart failure. Prior to the current study, the molecular basis for such observations was not clear, nor was the time-dependent phenotypic switch a consideration. We also show here that the two lines of transgenic mice respond differently to the β-blocker propranolol. In the human syndrome, the therapeutic response to β-blockade shows a high degree of interindividual variability that is not readily accounted for by clinical status[2]. In our study, we found that propranolol has a greater effect in Arg389 compared with Gly389 mice, in the settings of acute and chronic administration. This suggests that heart-failure patients with the Arg389 β_1-AR variant may represent those more likely to respond to β-blockade. Indeed, in our retrospective study of 224 heart failure patients, we report a clinically significant LVEF enhancement in Arg389, but not Gly389, homozygotes during long-term β-blockade. Thus, the risk-factor allele also seems to be associated with a favorable clinical response, but a prospective trial will be necessary to confirm this.

The current work in transgenic mice also indicates a time-dependent component, suggesting that phenotypes may have 'windows' when clinical characteristics or responses to therapy in patients with heart failure can be predicted and acted upon. Early in the syndrome, patients with Arg389 may have improved function and exercise tolerance, or fewer symptoms, compared with those with Gly389. In later stages, however, these phenotypes seem to be reversed. This age-dependent phenotypic switch (which was also present in transplanted human hearts; **Fig. 2b**) may define a period in which functional status or response to therapy in human heart failure could also change. The Arg389 variant, which seems to predispose an individual to heart failure, could ultimately be associated with a better prognosis because of its acquired 'auto-β-blocked' state that develops over time as a result of counter-regulatory events. If these phenotypic associations are borne out with additional human studies, an argument could be made for early β_1-AR genotyping of all heart failure patients (or healthy individuals with other risk factors) for assessing risk or prognosis, or for individual tailoring of pharmacologic therapy.

METHODS

Transgenic mice. The cDNAs encoding the human Arg389 or Gly389 β_1-ARs (GenBank accession nos. AF169007 and J03019, respectively) were subcloned into the full-length mouse α-myosin heavy-chain promoter. Briefly, the intronless β1-AR cDNAs, including 94 base pairs of the 5′ and 51 base pairs of the 3′ flanking sequences, were subcloned into the *Sal*I site of the polylinker of the α-myosin heavy-chain promoter construct[12]. Transgenic mice (FVB/N strain) were created at the University of Cincinnati Transgenic Core using methods similar to those described in detail elsewhere[12]. The study was approved by the Animal Use and Care Committee of the University of Cincinnati. Genomic incorporation of the transgene was identified by Southern blot analysis of tail-clip DNA using a ^{32}P-labeled probe that spans the junction of the promoter and the β_1-AR open reading frame. Selected F_1 mice were killed and cardiac β_1-AR expression was determined by radioligand binding (see below). Mouse lines with equivalent expression of the Arg389 or Gly389 variants were propagated.

Cardiac physiology. M-mode echocardiography was done in sedated mice as previously described[25]. In some studies, echocardiography was done before and after 4-month-old mice were treated with 0.5 mg/ml of propranolol in their drinking water for 5 weeks. For W-mode work-performing heart studies, mice were administered a lethal dose of pentobarbital and heparinized, and their hearts were removed. The heart was rapidly excised and the aorta cannulated with a 20-gauge needle, followed by retrograde perfusion with a modified Krebs-Henseleit solution (118 mM NaCl, 2.5 mM $CaCl_2$, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 0.5 mM Na_2EDTA, 25 mM $NaHCO_3$ and 11 mM glucose). The buffer was equilibrated with 95% O_2 and 5% CO_2, with a pH of

7.4, and maintained at 37 °C. A PE-50 catheter was inserted into the left atrium and advanced into the left ventricle, and the tip was advanced through the ventricular apex. The proximal end of the catheter remained in the left ventricle, with the distal end connected to a pressure transducer. Antegrade perfusion was established and studies were carried out with a workload of 250 mm Hg ml/min, which was achieved using a custom micrometer-controlled venous return of 5 ml/min and an aortic pressure of 50 mm Hg, as described[26]. Contractility and relaxation were assessed as derivatives of intraventricular pressure. After establishment of the baseline, responses to infusion of the β_1-AR agonist dobutamine by a microperfusion pump (MasterFlex) were measured at the indicated concentrations for 2 min. For the β-blockade studies, hearts that had not been exposed to agonist were perfused with varying concentrations of propranolol for 10 min. Heart rate, left-ventricular pressure (systolic, diastolic and end-diastolic), left-atrial pressure and mean coronary perfusion pressure were continuously monitored and digitized; the maximal response was determined using BioBench software (National Instruments).

Cardiac gene expression and histology. Total RNA was prepared from ventricles using Triazol (Gibco-BRL), and 5 μg were loaded onto a dot-blot apparatus for quantitative expression of the indicated genes using ^{32}P-labeled probes, as described in detail elsewhere[12]. Results were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. Paraffin-embedded heart sections fixed in 10% formalin were stained with Masson trichrome and examined by light microscopy. Apoptosis was determined by a TUNEL and fluorescence apoptosis detection system (Promega).

β-AR expression and adenylyl cyclase activation. Mice were killed, hearts were extracted and ventricular membranes were prepared as described[13]. For human hearts, a left-ventricular section was taken from explanted hearts during cardiac transplantation and frozen in liquid nitrogen, and membranes were prepared at the time of the assays. Receptor expression was determined by radioligand binding using [^{125}I]cyanopindolol, with nonspecific binding determined with 10 μM alprenolol[13]. Adenylyl cyclase activities were determined as described[27] by incubating membranes with 2 mM Tris (pH 7.4), 0.8 mM EGTA, 5 mM $MgCl_2$, 2.8 mM phosphoenolpyruvate, 0.06 mM GTP, 0.12 mM ATP, 0.1 mM cyclic adenosine monophosphate (cAMP), 4 U/ml myokinase, 10 U/ml pyruvate kinase, 0.1 mM ascorbic acid and 3×10^6 c.p.m. [α-^{32}P]ATP. Activities were determined in the presence of water (basal), 10 μM isoproterenol, 10 μM GW805415 (Glaxo Wellcome), 10 mM NaF or 100 μM forskolin at 37 °C for 10 min. [^{32}P]cAMP was separated by chromatography over alumina columns and recovery was normalized to a [^{3}H]cAMP tracer.

Western blots. Cardiac lysates were prepared as described[27]. The following antibodies were used for western blots by enhanced chemiluminescence: AS/7 and RM/1 (1:1,000; New England Nuclear); adenylyl cyclase-5/6 and GRK-2 (1:200; Santa Cruz Biotechnology); T-PLN and SERCA-2a (1:1,000; Affinity Bioreagents); and phosphorylated PLN (1:500, Upstate Biotechnical).

Carvedilol response by genotype in human heart failure. The study was approved by the University of Cincinnati Institutional Review Board and patients gave informed consent. We examined 224 patients (ages 40–65) with ischemic or dilated cardiomyopathy and LVEF < 35%. The patients were treated with the β-blocker carvedilol using a standard up-titration dosing regimen described in detail elsewhere[22]. LVEF was determined by radionuclide ventriculography before initiation of drug treatment and after a maintenance period of >6 months on a stable dose. The change in LVEF was reported as the difference between values before and after drug treatment. Patients were genotyped at the β_1-AR 389 locus as described[28]. The distribution of genotypes (16 Gly389 homozygotes, 95 Gly389/Arg389 heterozygotes and 144 Arg389 homozygotes) was in Hardy-Weinberg equilibrium. After stratification of patients by genotype, the change in LVEF was compared by nonparametric tests. Potential effects of age, sex, race, etiology, baseline LVEF or carvedilol dose were assessed by multiple regression techniques[29].

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COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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