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GENETIC VARIATION IN THE β_2-ADRENERGIC RECEPTOR: IMPACT ON INTERMEDIATE CARDIOVASCULAR PHENOTYPES

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Abstract

Genetic variation in drug targets (e.g. receptors) can have pronounced effects on clinical responses to endogenous and exogenous agonists. Polymorphisms in the gene encoding the β_2-adrenergic receptor (β_2-AR) have been associated with altered expression, down-regulation, and altered cell signaling in vitro. Because β_2-ARs play a crucial role in the regulation of the cardiovascular system, the functional importance of genetic variation in the β_2-AR on cardiovascular responses to physiological or pharmacological stimuli has gained widespread attention. The objective of this review is to characterize these intermediate cardiovascular phenotypes and their influence on cardiovascular disease and adrenergic drug responses.

Two common single nucleotide polymorphisms, encoded at codon 46 ($Gly^{16}Arg$) and 79 ($Gln^{27}Glu$) of the β_2-AR gene, have been studied intensively. They have been shown to be associated with altered vasodilator responses to regional and systemic administration of β_2-agonists, altered cardiovascular responses to sympathoexcitatory maneuvers, and altered myocardial function. Importantly, these intermediate physiological patterns may influence the development of and the outcomes associated with hypertension and other cardiovascular diseases. As recently reported, β_2-AR gene variation can risk-stratify patients receiving β-blocker therapy and may predict β-blocker efficacy in patients post acute coronary syndrome or in patients with heart failure.

Further studies will advance our understanding of the link between β_2-AR genotypes, intermediate cardiovascular phenotypes, and clinical phenotypes. In the long term, reassessment of the benefits of β-blocker-therapy within genotype groups should be carried out with the ultimate goal to design the optimal therapeutic regimen for the individual patient.

Keywords

β2-adrenergic receptor; polymorphism; genotype; haplotype; phenotype

INTRODUCTION

It is a common clinical problem that the same drug leads to variable responses in different patients. Modern clinical trials often average the treatment effect for thousands of individuals, summarized by a single statistical value; however, important individual differences are lost, and the drug effect may even be dangerous in some patients [Kent, D. and Hayward, R. 2007]. Although many non-genetic factors – including age, organ function, concomitant therapy / drug interactions, and the nature of the disease – may influence drug effects, there is increasing evidence that inter-individual differences in drug response may also be caused by

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sequence variants in genes encoding drug-metabolizing enzymes, drug transporters, or the drug targets themselves [Evans, W. E. and McLeod, H. L. 2003]. It is estimated that genetic variation can account for 20 to 95 percent of the variability in drug disposition and effects [Kalow, W. *et al.* 1998].

The ultimate promise of pharmacogenetics is the possibility that knowledge of a patient's DNA might be used to maximize drug efficacy, to give drugs only to those patients that are likely to respond, and to predict risks and thus avoid adverse drug reactions [Weinshilboum, R. and Wang, L. 2004]. Pharmacogenetic research began with a focus on drug metabolism, and after years of fundamental research based on understanding the functional consequences of polymorphisms in genes encoding drug-metabolizing enzymes, individualized therapy using genetic analyses is entering clinical practice. Examples include testing for lack of the enzyme thiopurine S-methyltransferase (TPMT) to prevent severe toxicity to thiopurine drugs [Weinshilboum, R. and Wang, L. 2004], or testing for CYP2D6 deficiency to determine tamoxifen's effectiveness in adjuvant breast cancer therapy [Borges, S. *et al.* 2006].

Interest in drug target genetic variation and the potential for pronounced effects on endogenous and exogenous agonist responsiveness [Evans, W. E. and McLeod, H. L. 2003] is relatively recent when viewed within the longer history of pharmacogenetic research. Because of the pivotal role in the regulation of cardiovascular function, genetic variation in the β_2-adrenergic receptor (β_2-AR) has been studied intensively during the last decade. From the initial differentiation of adrenergic receptors in 1948 to the description of the β_2-AR gene sequence in 1987, the "pre-genomic" era was likely under the assumption that β_2-AR's are similar in all individuals (Fig. 1). In 1993, Green et al. published the first *in-vitro* functional characterization of a naturally occurring variant in the human β_2-AR, showing that it alters ligand binding and the functional properties of the receptor [Green, S. A. *et al.* 1993]. In these formative stages of the "genomic era," candidate gene approaches determined that the β_2-AR gene may be associated with hypertension [Kotanko, P. *et al.* 1997], but over time incrimination of specific polymorphisms has been inconclusive probably due to the multifactorial and polygenic nature of hypertension. Nevertheless, the relationship between β_2-AR gene variation and β_2-AR function became a fundamental physiologic question, and "physiological bridge building" strategies emerged as a means to determine whether variation in the β_2-AR gene influences intermediate physiological traits relevant to the pathogenesis of cardiovascular disease.

The objective of this review is to summarize and discuss studies that focus on characterizing these intermediate phenotypes and on exploring their influence on cardiovascular disease and adrenergic drug response. Growing evidence suggests that β_2-AR gene variation influences blood pressure regulation, adrenergic drug response, and clinical outcome, and the implications from these findings may be important in achieving the ultimate goal to individualize drug therapy based on patient's genotype.

β_2-AR GENE VARIATION

β_2-ARs are expressed in numerous tissues including vascular and bronchial smooth muscle cells, kidneys and the heart [Kirstein, S. L. and Insel, P. A. 2004]. The gene encoding the β_2-AR is intronless, located on chromosome 5q31-32 and contains several polymorphic sites (Fig. 2): At least 51 variants have been identified so far across the span of 5.3 kilobases [Hawkins, G. A. *et al.* 2006; Kirstein, S. L. and Insel, P. A. 2004]. Five of these are non-synonymous single nucleotide substitutions in the β_2-AR coding region, occurring at positions 46 (extracellular amino terminus; $Gly^{16}Arg$), 79 (extracellular amino terminus; $Gln^{27}Glu$), 100 (1^{st} transmembrane-spanning domain; $Val^{34}Met$), 491 (4^{th} domain; $Thr^{164}Ile$), and 659 (5^{th} domain; $Ser^{220}Cys$). The single nucleotide polymorphisms (SNPs) at position 46 ($Gly^{16}Arg$) and 79 ($Gln^{27}Glu$) are the most common, with minor allele frequencies in the white population

around 0.4 (Arg^{16}, Glu^{27}), whereas the Thr^{164}Ile SNP is rare (minor allele frequency around 0.02) and the Val^{34}Met and Ser^{220}Cys variants are extremely rare [Hawkins, G. A. *et al.* 2006; Kirstein, S. L. and Insel, P. A. 2004]. Another common SNP (Cys^{-19}Arg) at position -47 is within a short open reading frame, the 5'-leader cistron, which encodes a signaling peptide involved in regulation of β_2-AR translation [McGraw, D. W. *et al.* 1998; Scott, M. G. *et al.* 1999]; the minor allele frequency for this SNP is 0.44 [Hawkins, G. A. *et al.* 2006].

The polymorphisms are in strong linkage disequilibrium. Drysdale et al. have resequenced the β_2-AR in order to evaluate gene variation and haplotype structure [Drysdale, C. M. *et al.* 2000]. They selected 13 polymorphic sites in the promoter and coding region, which mathematically would produce 8192 inherited combinations in humans (see Fig. 3). However, only 12 haplotypes were found in their population. It is postulated that the reason behind so few numbers of chromosomally phased SNPs is that the human species is relatively young, and therefore combinations of individual polymorphisms are in linkage disequilibrium. For example, Glu^{27} homozygotes are nearly uniformly homozygous for Gly^{16} (haplotype 2 in Fig. 3), and this haplotype is the only one that encodes the Arg variant at position -47 in the 5'-leader cistron [Drysdale 2000; Hawkins 2006]. Further, Arg^{16} homozygotes are nearly uniformly Gln^{27} homozygotes (haplotype 4 in Fig. 3).

Significant differences in distribution of some haplotypes were noted in Caucasian, African-American, Asian, and Hispanic-Latino ethnic groups with >20-fold differences among the frequencies of the four major haplotypes [Drysdale, C. M. *et al.* 2000]. In a larger study by Hawkins et al., 31 polymorphisms from sequencing data from 429 whites and 240 African Americans were used to estimate haplotype frequencies, and a total of 24 haplotypes were observed [Hawkins, G. A. *et al.* 2006]. When analysis was limited to the polymorphisms studied by Drysdale et al. [Drysdale, C. M. *et al.* 2000], only haplotypes 2, 4, 6, 7, and 10 were observed in whites, whereas only haplotypes 1, 2, 4, 6, and 9, were observed in African Americans. Frequencies of haplotype 2 and 4 were higher in whites, whereas haplotype 6 was more frequent in African Americans (Fig. 3).

IN-VITRO STUDIES

In order to study the functional consequences of these polymorphisms, *in vitro* experiments in recombinant systems have been performed (for review see [Brodde and Leineweber 2005]). It was found that neither agonist binding nor G-protein coupling, resulting in stimulation of adenylyl cyclase activity, was altered by Gly^{16}Arg or Gln^{27}Glu polymorphisms. However, altered degrees of agonist-promoted down-regulation of receptor expression were described [Green, S. A. *et al.* 1994]. Specifically, the substitution of glycine for arginine at position 16 (Gly^{16}) was associated with enhanced agonist-induced desensitization as compared to Arg^{16}, and the substitution of glutamic acid for glutamine at position 27 (Glu^{27}) was associated with resistance to desensitization [Green, S. A. *et al.* 1995]. The resistance of the Glu^{27} receptor variant to agonist-promoted down-regulation has recently been confirmed by performing cell-signaling studies in two HEK293 cell lines over-expressing similar levels of the Gln^{27} or Glu^{27} variant: The Glu^{27} variant magnified the catecholamine-induced activation of ERK and p38, two mitogen-activated protein kinases that have been involved in myocyte hypertrophy [Iaccarino, G. *et al.* 2006].

In contrast to the two common polymorphisms, the rare Thr^{164}Ile polymorphism does alter ligand binding and G-protein coupling: In cells transfected with cDNAs that mimic this SNP, the Ile^{164} receptor displayed a lower binding affinity for epinephrine, and functional coupling to G_s - as determined in adenylyl cyclase assays - was significantly depressed as compared to the wild-type receptor [Green, S. A. *et al.* 1993]. The 5'- leader cistron polymorphism Cys^{-19}Arg leads to altered promoter activity in recombinant cells, with reduced β_2-AR

expression [McGraw, D. W. *et al.* 1998] and β_2-AR promoter-driven luciferase activity [Johnatty, S. E. *et al.* 2002] of the Arg^{-19} variant as compared to the wild type.

IN-VIVO STUDIES

Vascular Function (Local Infusion Studies)

The main effect of β_2-ARs in the vasculature is to evoke marked vasodilation. For many years the cAMP pathway in vascular smooth muscle cells was considered to be the main dilating pathway activated by the β_2-ARs in skeletal muscle vessels. More recently it has been shown that β_2-ARs can evoke nitric oxide (NO) release from the vascular endothelium, and this mechanism likely contributes (~30-50%) to the dilator responses to both β_2-agonist infusions and mental stress [Cardillo, C. *et al.* 1997; Cardillo, C. *et al.* 1997; Eisenach, J. H. *et al.* 2002]. Several studies using local infusions of β-agonists into the brachial artery have demonstrated differences in vascular response dependent on β_2-AR genotype. Cockroft et al. found greater arterial vasodilator responses in Glu^{27} versus Gln^{27} homozygotes, and a trend toward greater responses in the Gly^{16} versus Arg^{16} group [Cockcroft, J. R. *et al.* 2000]. A larger study at our institution controlled for normal dietary sodium intake for 5 days (150 mmol/day), and observed greater arterial vasodilator responses in the Gly^{16} homozygotes compared to the Arg^{16} homozygotes [Garovic, V. D. *et al.* 2003]. After inhibition of NO synthase with L-NMMA these differences in vasodilator responsiveness were no longer apparent (see Fig. 4), suggesting that the augmented vasodilator responses seen in the Gly^{16} subjects are mainly due to β_2-mediated release of NO from the vascular endothelium [Garovic, V. D. *et al.* 2003].

In isolated hand vein experiments, Dishy et al. observed that the maximal venodilatory responses to isoproterenol were greater in Gly^{16} than Arg^{16} homozygotes [Dishy, V. *et al.* 2001]. However, the difference was attributed to effects of the Glu^{27} variant rather than the Gly^{16} variant, because greater responses were present only in Gly^{16}+Glu^{27} homozygotes, not in Gly^{16}+Gln^{27} homozygotes, when compared to Arg^{16}+Gln^{27} homozygotes. In the same study, β_2-agonist-mediated desensitization in the vasculature was tested and interestingly, the findings differed from those obtained *in vitro* by Green et al. [Green, S. A. *et al.* 1995]: Namely, in healthy individuals, homozygosity for the Arg^{16} was associated with rapid agonist-mediated vascular desensitization, whereas homozygosity for the Glu^{27} polymorphism was associated with enhanced agonist-mediated vasodilation [Dishy, V. *et al.* 2001]. Along these lines, Bruck et al. reported that terbutaline-induced venodilation in the dorsal hand vein following 2 weeks of oral terbutaline treatment evoked the greatest desensitization in the Arg^{16} homozygotes [Bruck, H. *et al.* 2005]. Thus the Arg^{16} variant of the β_2-AR could be considered a loss-of-function mutation because of the shorter duration of stimulation, and the Glu^{27} variant could be considered a gain-of-function mutation because of the longer duration of stimulation.

The differences between *in vitro* and *in vivo* data may be explained by a dynamic model of receptor regulation, where receptor variants that are highly sensitive *in vitro* are ‘pre-desensitized’ *in vivo* by chronic exposure to endogenous agonists, e.g. catecholamines. These receptors may not become further desensitized by the challenge with an exogenous agonist, thereby producing an apparently paradoxical response [Liggett, S. B. 2002].

Vascular Function (Systemic Infusion Studies)

In contrast to the local infusion studies, results from systemic infusion studies of β_2-agonists *in vivo* paint a picture that is generally but not uniformly consistent with the cell signaling studies, with greater systemic vasodilation seen in Arg^{16} than Gly^{16} homozygotes [Gratze, G. *et al.* 1999; Hoit, B. D. *et al.* 2000; Snapir, A. *et al.* 2003] or no difference between genotype groups [Bruck, H. *et al.* 2003; Eisenach, J. H. *et al.* 2006]. Additionally, inhaled β_2-agonist

produced a greater decrease in diastolic blood pressure in asthmatics homozygous for Arg^{16}+Gln^{27} [Lee, D. K. *et al.* 2004]. However, the methods used to assess cardiac output and peripheral vasodilation in these studies have significant limitations, as no efforts were made to account for the potentially confounding effects of cardiovascular baroreflexes on the hemodynamic responses to the infusions [Shannon, J. R. *et al.* 1998; Wilkins, B. W. *et al.* 2007]. Because the response to a vasoactive drug is mediated by the net effect of vascular sensitivity to the drug and the counter-regulatory baroreflex actions, the contrasting results from local versus systemic infusion studies may be explained by counter-regulatory baroreflex activation, (over-)compensating for augmented β_2-mediated vasodilation in carriers of the Gly^{16} or Glu^{27} variant. Further studies – e.g. during disengagement of confounding compensatory baroreflex activity – will be needed to address this hypothesis.

Another group has focused on studying the time course of desensitization. They found that the Arg^{16}Gly and the Gln^{27}Glu variants of the β_2-AR do not alter the extent of terbutaline treatment-induced desensitization of cardiac β_2-AR-mediated increases in heart rate and contractility. However, subjects who were homozygous for Glu^{27} exhibited a slower time course of desensitization compared to subjects who were homozygous for Gly^{16} or homozygous for Arg^{16}+Gln^{27} [Bruck, H. *et al.* 2003]. This idea is generally consistent with studies suggesting that the Glu^{27} variant is associated with resistance to desensitization and thus enhanced responsiveness, and larger group sizes are needed to further characterize the Glu^{27} interaction with the polymorphism at position 16.

Cardiac Function

Several studies have determined the influence of β_2-AR gene variation and ventricular function [Eisenach, J. H. *et al.* 2005; Iaccarino, G. *et al.* 2002; Tang, W. *et al.* 2003]. In an echocardiographic analysis of a biethnic sample of normotensives, the Gly^{16} homozygotes displayed greater fractional shortening, ejection fraction, midwall shortening, and stress-corrected midwall shortening compared to the heterozygous and Arg^{16} homozygous groups, independent of age, sex, ethnicity, heart rate, body mass index, systolic blood pressure, LV end-diastolic dimension, and field center [Tang, W. *et al.* 2003]. These genotype-dependent differences in resting cardiovascular function have been confirmed in studies using other techniques to evaluate ventricular function: Individuals homozygous for Gly^{16} have a greater cardiac output (assessed via the open-circuit acetylene wash-in method) and stroke volume compared to Arg^{16} homozygotes at rest. Furthermore, during both low- and high intensity exercise, the Gly^{16} group continues to have a greater stroke volume and cardiac output compared to Arg^{16} [Snyder, E. M. *et al.* 2006]. These findings suggest that the Arg^{16} genotype is associated with reduced baseline receptor function or density compared to the Gly^{16} genotype, and that these baseline differences contribute to the observed differences during exercise.

The Gly^{16}Arg polymorphism also makes a difference in patients with heart disease: A collaborative group recently performed echocardiography in 95 heart failure patients (EF ≤ 40%) and found that Arg^{16} homozygotes had higher plasma norepinephrine and atrial natriuretic peptide levels, greater left atrial diastolic dimension, higher peak velocity of early/late diastolic filling ratio, shorter deceleration time, and reduced exercise tolerance when compared to Gly^{16} homozygotes [Wolk, R. *et al.* 2007].

Response to Sympathoexcitation

Laboratory-based measures of cardiovascular responses to sympathoexcitation provide intermediate physiologic characteristics of cardiac and vascular function that are useful determinants of broader phenotypes in humans, such as hypertension. Normotensive individuals who show a robust pressor response to sympathoexcitatory stimuli like mental

stress are at increased risk for developing hypertension [Cardillo, C. *et al.* 1998; Flaa, A. *et al.* 2006; Matthews, K. A. *et al.* 2004; Murphy, J. K. *et al.* 1994; Murphy, J. K. and McGarvey, S. T. 1994; Treiber, F. A. *et al.* 1994]. Furthermore, increased job strain has now been shown to be predictive of future incidence of hypertension [Markovitz, J. H. *et al.* 2004; Matthews, K. A. *et al.* 2004]. In this context, adrenergic receptors play an important role in the arterial blood pressure responses to mental stress and other sympathoexcitatory maneuvers like isometric handgrip to fatigue [Eisenach, J. H. *et al.* 2005; Eisenach, J. H. *et al.* 2004; Freyschuss, U. *et al.* 1988; Halliwill, J. R. *et al.* 1997; Hjemdahl, P. 2002]. For example, during mental stress some vascular beds undergo β_2-AR mediated vasodilation while others undergo α-adrenergic vasoconstriction, and the balance between these responses probably contributes to the magnitude of the overall rise in blood pressure [Eisenach, J. H. *et al.* 2005; Eisenach, J. H. *et al.* 2004; Freyschuss, U. *et al.* 1988; Halliwill, J. R. *et al.* 1997; Hjemdahl, P. 2002]. This means that if β_2-receptor mediated dilation is blunted, the pressor response to mental stress is likely to be increased. Consistent with these general ideas are a number of observations primarily from the pre-genomic era: 1) The pressor response to mental stress is heritable [Busjahn, A. *et al.* 2000; Carmelli, D. *et al.* 1985; Carmelli, D. *et al.* 1991; Li, G. H. *et al.* 2001; McCaffery, J. M. *et al.* 2002]; 2) β_2-mediated forearm vasodilator responses to mental stress are blunted in subjects thought to be at increased risk for hypertension and in individuals with mild hypertension [Cardillo, C. *et al.* 1998; Cardillo, C. *et al.* 1998; Hughes, J. W. *et al.* 2003]; 3) Vasodilator responses to brachial artery, or systemic infusions of β-agonists are blunted in subjects thought to be at increased risk for hypertension, and in mild hypertension [Dimsdale, J. *et al.* 1988; Feldman, R. D. 1987; Feldman, R. D. 1990; Ford, G. A. *et al.* 1992; Halliwill, J. R. *et al.* 1997; Lang, C. C. *et al.* 1995; Sherwood, A. and Hinderliter, A. L. 1993; Stein, C. M. *et al.* 1995; Watkins, L. L. *et al.* 1995].

During emotional stress there is an increase in forearm blood flow [Abramson, D. I. F., E.B. 1940; Grant, R. T. P., R.S.B. 1938], and this reaction in humans is similar to the defense reaction in animals [Bulbring, E. and Burn, J. H. 1935; Folkow, B. *et al.* 1948]. Studies have demonstrated that the most likely mechanisms involved in the vasodilator responses to sympathoexcitatory maneuvers including mental stress center around sympathetic withdrawal, local release of NO, and β_2-mediated stimulation (via circulating epinephrine) acting on receptors on the vascular endothelium and smooth muscle [Dietz, N. M. *et al.* 1994; Halliwill, J. R. *et al.* 1997; Joyner, M. J. and Halliwill, J. R. 2000; Liu, Z. *et al.* 2006; Reed, A. S. *et al.* 2000]. As a result of these multiple mechanisms, the pressor response and forearm vasodilator response to mental stress demonstrates marked inter-individual variability. The key questions are, what is the role adrenergic receptor gene variation on these responses, and will gene variation ultimately influence the cardiovascular phenotypes?

The contribution of the β_2-AR polymorphisms to differences in the pressor response to certain sympathoexcitatory maneuvers has been evaluated, and in comparison to other polymorphic sites in the β_2-AR gene, the $Arg^{16}Gly$ polymorphism has been suggested to exert a dominant effect [Busjahn, A. *et al.* 2000]. An analysis of German Caucasian twins determined that the $Arg^{16}Gly$ polymorphism was associated with systolic and diastolic blood pressure at rest, during mental arithmetic, and during the cold pressor test, as well as the increase in diastolic pressure during both maneuvers, which was higher in the Arg^{16} homozygotes [Li, G. H. *et al.* 2001]. The Pittsburgh Twin Study revealed a higher resting diastolic BP in Gly^{16} homozygotes, but no association of the $Arg^{16}Gly$ polymorphism with cardiovascular responses to mental stress [McCaffery, J. M. *et al.* 2002]. The absent genotype effect on the pressor response to mental stress in the latter study is not readily explained.

Regarding regional vasodilator differences during sympathoexcitation, a study of Brazilian women found that subjects homozygous for Gly^{16} and Glu^{27} demonstrated a greater forearm vasodilator response to mental stress and to isometric handgrip, an effect that was no longer

present following brachial artery infusion of a β-blocker [Trombetta, I. C. *et al.* 2005]. These findings were attributed to depend on position 27, as Gly^{16}+Glu^{27} subjects had a greater FBF response than Arg^{16}+Gln^{27} and Gly^{16}+Gln^{27} subjects. Two studies at our institution confirmed genotype-dependent differences in response to handgrip; individuals homozygous for Gly^{16} had greater increases in heart rate and cardiac output, and a tendency for lower systemic vascular resistance [Eisenach, J. H. *et al.* 2005; Eisenach, J. H. *et al.* 2004]. The higher heart rate and cardiac output response during handgrip in the Gly^{16} homozygotes may be due to greater ß$_2$-AR-mediated vasodilator effect was evoked by circulating catecholamines in Gly^{16} homozygotes. This means that to achieve the same pressor response, greater increases in heart rate were needed in the Gly^{16} subjects. This interpretation is also consistent with findings in the previous studies demonstrating greater ß$_2$-AR-mediated NO-dependent vasodilator responses during local arterial infusion of ß$_2$-agonists in Gly^{16} subjects [Cockcroft, J. R. *et al.* 2000; Garovic, V. D. *et al.* 2003]. These findings underscore the need for further characterization of the interaction between positions 16 and 27, as well as additional coding or noncoding polymorphic variants in the ß$_2$-AR gene that may contribute to inter-individual differences. Further characterization of additional DNA sequence variation in the ß$_2$-AR gene may help refine understanding of these relationships

Modulation of β_2-AR function by Dietary Sodium Intake

Dietary sodium intake influences the pathogenesis and treatment of hypertension [He, F. J. *et al.* 2005; Meneton, P. *et al.* 2005], and the blood pressure response to changes in dietary sodium aggregates in families and is heritable [Miller, J. Z. *et al.* 1987]. In normotensives and individuals with hypertension, dietary sodium intake influences β_2-AR mediated vascular function [Feldman, R. D. 1990; Feldman, R. D. 1990; Feldman, R. D. *et al.* 1987; Feldman, R. D. *et al.* 1983; Feldman, R. D. *et al.* 1984; Lang, C. C. *et al.* 1995; Naslund, T. *et al.* 1990; Stein, C. M. *et al.* 2000; Stein, C. M. *et al.* 1995]. Evidence linking salt sensitivity to the β_2-AR locus has emerged from a linkage analysis of both hypertensive and normotensive siblings of hypertensive individuals [Svetkey, L. P. *et al.* 1997].

The effects of sodium intake on sensitivity to β_2-AR mediated vasodilatation have established that a normal response to increased sodium intake, consisting of increased sensitivity to β_2-AR mediated vasodilatation, is decreased in hypertensive compared to normotensive individuals [Naslund, T. *et al.* 1990]. Furthermore, β_2-AR mediated responsiveness is reduced selectively in peripheral veins of borderline hypertensive subjects, an effect which potentially is reversible by a low sodium diet [Feldman, R. D. 1990]. In this context it seems reasonable to postulate that variation in the β_2-AR gene would influence vasodilation in the setting of dietary sodium manipulation. Our group recently demonstrated that compared to Arg^{16} homozygotes, the greater β_2-mediated forearm vasodilator responses in healthy normotensive Gly^{16} homozygotes following a normal sodium diet are no longer present following a low sodium diet [Eisenach, J. H. *et al.* 2006]. Furthermore, in the Gly^{16} group, dietary sodium restriction increased resting systemic vascular resistance, decreased cardiac output, and tended to decrease resting stroke volume, whereas these indices were essentially unaffected in the Arg^{16} group [Eisenach, J. H. *et al.* 2006]. A concomitant study at our institution administered an acute intravenous sodium load (normal saline) to healthy individuals and found that mean arterial pressure was greater and urinary sodium uptake was greater (less urinary sodium excretion) in the Gly^{16} subjects [Snyder, E. M. *et al.* 2006]. Taken together, these findings provide early evidence that sodium influences the Arg^{16}Gly β_2-AR genotype-dependent cardiovascular and renal intermediate phenotypes, and decreases in sodium balance may reduce ß$_2$-AR-mediated cardiovascular function for the Gly^{16} allele.

Interestingly, a recent multi-center trial examined blood pressure following a 2-week crossover of low (10 mmol/day) and high (200 mmol/day) dietary sodium intake. Genotype differences

were not seen among normotensives (n=48); however, among hypertensives (n=171), Arg^{16} and Gln^{27} – alone and in combination – displayed the greatest increase in mean arterial pressure from the low to the high sodium state, and the combination Arg^{16}+Gln^{27} was associated with low-renin hypertension, higher plasma aldosterone, lower renin, and lower potassium [Pojoga, L. *et al.* 2006]. Another investigation administered inhaled β_2-agonist to asthmatics and found a greater decrease in serum potassium in Arg^{16}+Gln^{27} homozygotes (n=8) vs. Gly^{16}+Glu^{27} homozygotes (n=8) [Lee, D. K. *et al.* 2004]. In view of these findings and the above findings from intravenous saline loading in healthy subjects [Snyder, E. M. *et al.* 2006], one may conclude that β_2-AR genotype-dependent intermediate physiologic traits may differ between healthy individuals and individuals with hypertension or asthma; furthermore, although unifying conclusions between these investigations are elusive, this nevertheless affirms the emerging theme that variation in the β_2-AR gene has powerful broad-based physiologic implications.

CLINICAL TRIALS / OUTCOME STUDIES

Evidence that β_2-AR gene variation has an important impact on clinical outcome is increasing; recent studies have suggested that the Gly^{16} and/or Glu^{27} alleles may actually be favorable in cardiovascular health. The Cardiovascular Health Study (CHS), a population-based prospective cohort study with 7 to 10 years of follow-up, evaluated 4,441 white and 808 black participants. Irrespective of race/ethnicity, Glu^{27} carriers had a lower risk of coronary events than Gln^{27} homozygotes, and there was a suggestion of decreased risk among Gly^{16} carriers compared with Arg^{16} homozygotes [Heckbert, S. R. *et al.* 2003]. A subsequent report from the CHS demonstrated a higher risk of sudden cardiac death in Gln^{27} homozygotes irrespective of race/ethnicity; similar findings were noted in the Cardiac Arrest Blood Study (CABS), presented in the same publication [Sotoodehnia, N. *et al.* 2006]. In another study, eight polymorphisms in the sympathetic nervous system and renin-angiotensin system were evaluated in 227 patients with heart failure. Of these, the Arg^{16}+Gln^{27} diplotype was the only genetic marker of increased risk of death or heart transplantation (see Fig. 5) [Shin, J. *et al.* 2007].

There is also growing evidence that genetic variation in the β_2-AR may predict the efficacy of therapeutic regimens [Iaccarino, G. *et al.* 2006; Kaye, D. M. *et al.* 2003; Lanfear, D. E. *et al.* 2005]. In eighty genotyped heart failure patients treated with carvedilol, individuals homozygous for Gln^{27} represented a significantly lower proportion of ‘good’ responders (improvement in left ventricular function) than individuals who were homozygous or heterozygous for the Glu^{27} polymorphism [Kaye, D. M. *et al.* 2003]. Another prospective cohort study followed patients with acute coronary syndrome: No mortality difference between genotypes was found among patients discharged without β-blocker therapy for neither the Arg^{16}Gly nor the Gln^{27}Glu polymorphism. However, among patients treated with β-blockers, both polymorphisms – independently as well as combined – were predictive of survival: Patients homozygous for Arg^{16}+Gln^{27} had the worst survival, whereas patients homozygous for Gly^{16}+Glu^{27} had the best survival (see Fig. 6) [Lanfear, D. E. *et al.* 2005].

Knowledge of the patient’s genotype may also be advantageous in choosing the optimal hypertensive therapy in patients with hypertension-induced left ventricular hypertrophy (LVH): Iaccarino et al. randomly assigned hypertensive patients with LVH to receive therapy with either a selective β_1-blocker (atenolol) or an ACE-inhibitor (enalapril) [Iaccarino, G. *et al.* 2006]. After 2-year follow-up, the patients carrying at least one Glu^{27} allele showed a larger reduction in LVH when treated with enalapril, but not with atenolol therapy. A possible explanation for these data is based on the finding that the Glu^{27} variant enhances the hypertrophic effect of the sympathetic system. Angiotensin-converting enzyme (ACE)-inhibitors are able to reverse LVH through the reduction of the hypertrophic effect of

catecholamines [Trimarco, B. *et al.* 1985]. This property may be particularly relevant in patients carrying the Glu^{27} allele, because by reducing sympathetic activation, it may prevent the more marked catecholamine-mediated hypertrophy stimulus induced by the Glu^{27} variant [Iaccarino, G. *et al.* 2006].

HAPLOTYPES AND INTERMEDIATE CARDIOVASCULAR TRAITS

As mentioned above and shown in Fig. 3, the original description of β_2-AR haplotypes demonstrated the importance of interactions among multiple SNPs within a haplotype to influence physiologic function at a greater predictive power than individual SNPs. Specifically, the authors found that asthmatic patients with haplotype pair 2/2 as shown in Fig. 3 had a 50% greater bronchodilator response than those with haplotype pair 4/4; furthermore, this correlated with β_2-AR mRNA expression in HEK cells transfected with haplotype vector [Drysdale, C. M. *et al.* 2000]. Comprehensive analysis of 5'-flanking, coding-region, and 3'-untranslated sites of variation with lung function in asthmatics has been subsequently characterized by these authors [Hawkins, G. A. *et al.* 2006].

At the time of this review, we are not aware of any similarly comprehensive genotype-phenotype analyses with intermediate cardiovascular traits. This is most likely due to the large number of subjects required to achieve sufficient power with these more extended haplotypes. However, because of linkage disequilibrium, further inspection of the haplotypes in Fig. 3 reveals that individuals with haplotype 2/2 are homozygous for Gly^{16}+Glu^{27}, and individuals with 4/4 are homozygous for Arg^{16}+Gln^{27}. Furthermore, haplotype 4 (containing Gly^{16}+Glu^{27}) also contains Cys^{19} (T-47C) in the 5'-leader cistron, which affects β_2-AR expression [McGraw, D. W. *et al.* 1998], and T-20C, which is completely concordant with Gln^{27}Glu. Taken together, these four sites generate haplotypes that account for approximately 94% of all haplotypes [Cerrone, G. E. *et al.* 2007;Herrmann, S. M. *et al.* 2002], and would explain why the majority of studies described in this review have examined SNP variation at positions 16 and 27, individually and in combination. Future investigations will take advantage of these tag SNPs to group patients according to haplotype and advance current understanding of the influence of β_2-AR haplotype on intermediate cardiovascular traits relevant to pharmacogenomic association studies.

PERSPECTIVES

Summarizing the clinical trials, the Arg^{16}Gly and Gln^{27}Glu polymorphisms appear to have powerful pharmacogenetic and disease-modifying implications, with growing evidence that the Gly^{16} and/or Glu^{27} alleles may be associated with favorable cardiovascular outcome. The causal contribution of each single polymorphism to the observed differences as studied in several *in vivo* studies is less clear so far; lack of statistical power and strong linkage disequilibrium between SNPs may account for inconsistencies in reported effects of isolated SNPs. The unique interactions of multiple SNPs within a haplotype ultimately can affect biologic and therapeutic phenotype, whereas individual SNPs alone may have poor predictive power as pharmacogenetic loci. Thus comprehensive haplotype analysis is needed to gain more precise information regarding the functional importance of genetic variation in the β_2-AR.

In the era of genome-wide association studies, it becomes increasingly clear that not only genetic variation within a single gene, but genetic variation throughout an entire pathway is important for pathophysiology and pharmacology and may have potential impact on clinical outcome. However, this makes defining clinically relevant polymorphisms much more complex. With increasing number of potentially important genetic subgroups (i.e. haplotypes, or even combinations of haplotypes of different genes), it will be increasingly difficult to perform randomized clinical trials with a sufficient number of participants to ensure appropriate power.

Nevertheless, more physiological studies are needed to answer the question how genetic variation in a crucial therapeutic target, the β_2-AR, affects cardiovascular responses, and more prospective randomized clinical trials are needed to find out the optimal therapy for a patient with a specific β_2-AR genotype. As recently reported, sequence variants in the β_2-AR can risk-stratify patients receiving β-blocker therapy and may predict β-blocker efficacy post-acute coronary syndrome [Lanfear, D. E. *et al.* 2005]. In the long term, reassessment of the benefits of β-blocker therapy within haplotype groups should be pursued, and extension of these findings into other disease states where β-blocker therapy or adrenergic stimulation is important (e.g. heart failure) should be considered. If future studies confirm a consistent and clinically relevant effect of genetic variation in the β_2-AR, then these findings could ultimately be used to tailor pharmacotherapy to the individuals` needs (personalized medicine).

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LIST OF ABBREVIATIONS

ACE
angiotensin converting enzyme

Arg
arginine

β_2-AR
β_2-adrenergic receptor

CABS
Cardiac Arrest Blood Study

CHS
Cardiovascular Health Study

Cys
cysteine

Gln
glutamine

Glu
glutamate

Gly
glycine

HEK
human embryonic kidney

Ile
isoleucine

LVH
left ventricular hypertrophy

Met
methionine

NO
nitric oxide

Ser
serine

SNP
single nucleotide polymorphism

Thr
: threonine

TPMT
: thiopurin-S-methyltransferase

Val
: valine

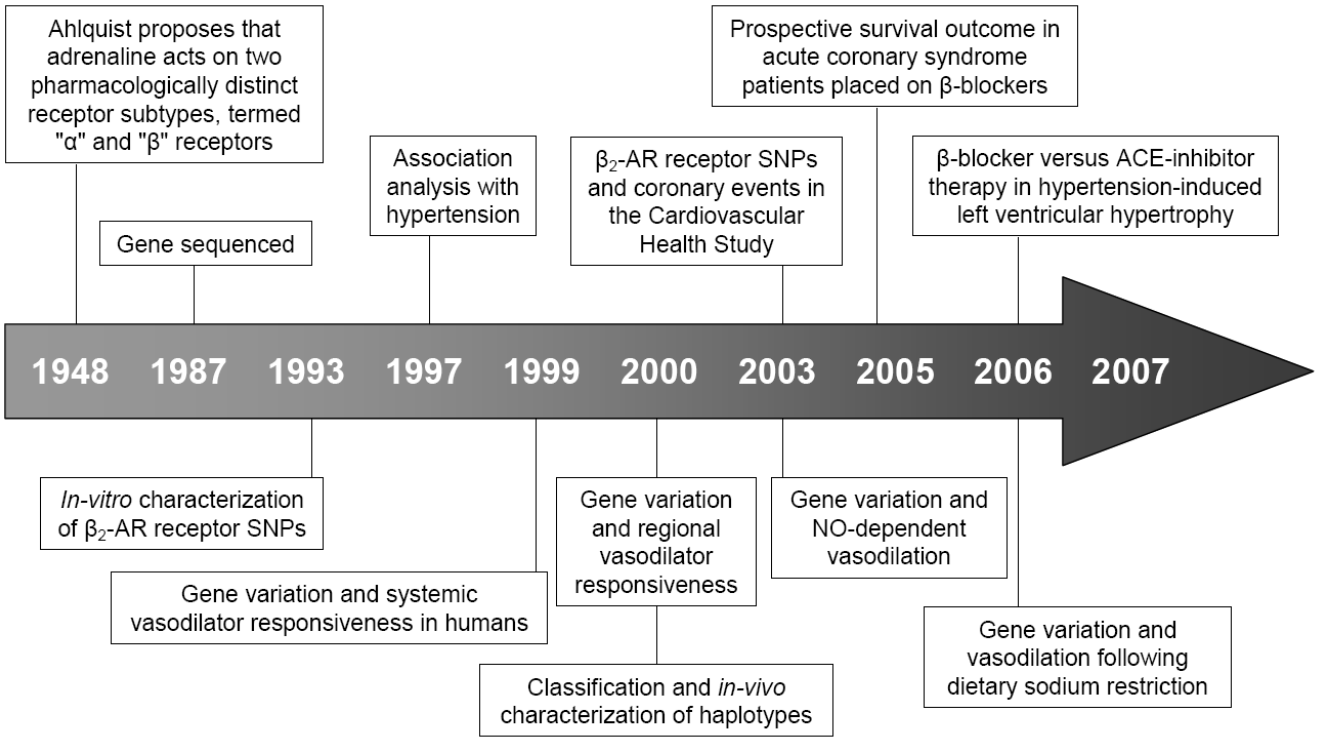

Figure 1.
Historical highlights in β_2-adrenergic receptor research.

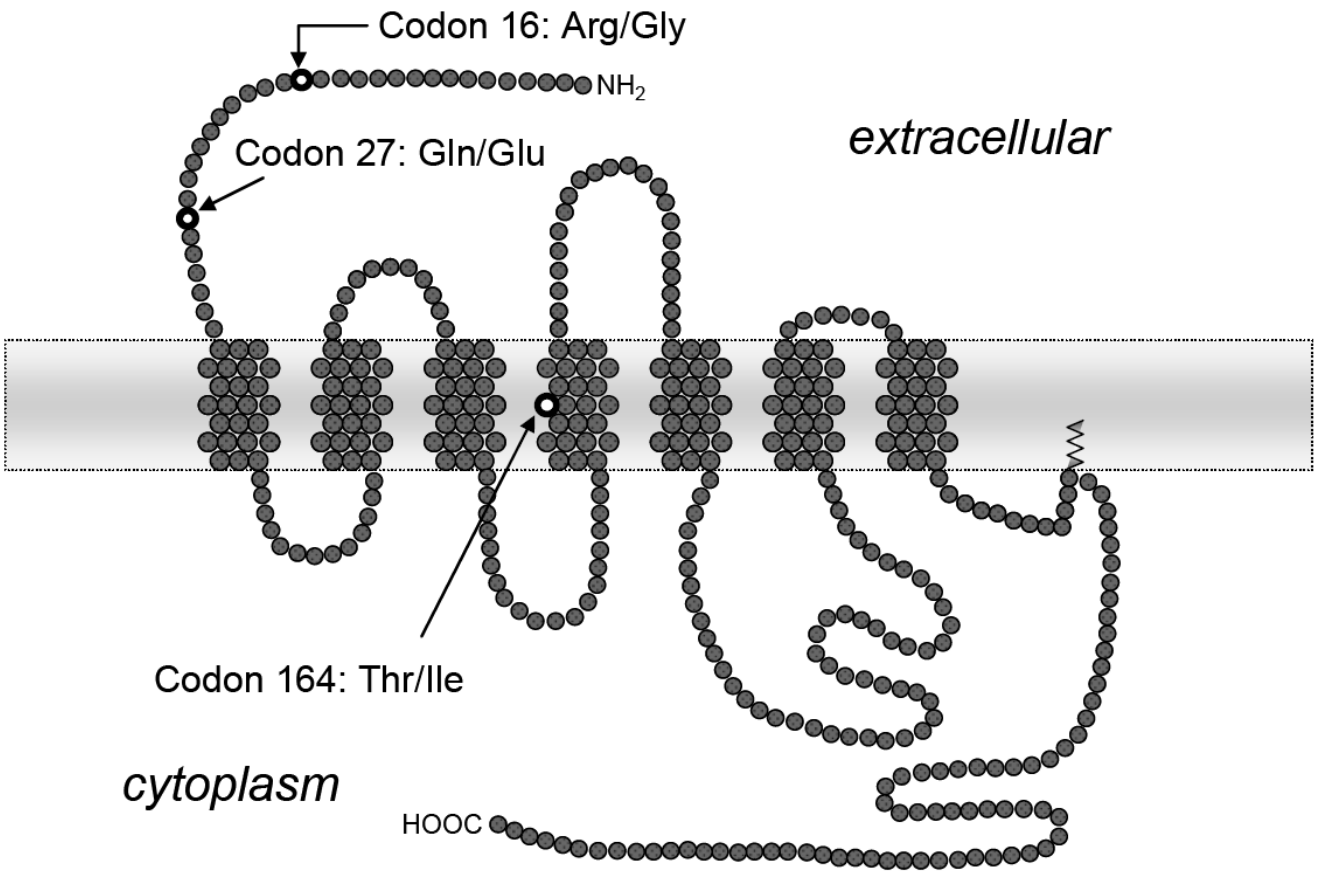

Figure 2.
Localization of polymorphisms in the human β_2-adrenergic receptor (β_2-AR). The β_2-AR is a 413 amino-acid long member of the "superfamily" of adrenergic receptors, with seven membrane-spanning regions. Common single nucleotide polymorphisms (SNPs) include a change at nucleotide position 46 which results results in the amino acid sequence of the β_2-AR containing a glycine (Gly) at position 16 instead of an arginine (Arg), and a change at nucleotide position 79 which results in glutamine (Gln) instead of glutamate (Glu) at amino acid position 27. Variation at amino acid position 164 has the most significant cardiovascular consequence; however, the isoleucine (Ile) variant is rare, and the homozygous form is not present in humans. From [McNamara, D. M. *et al.* 2002].

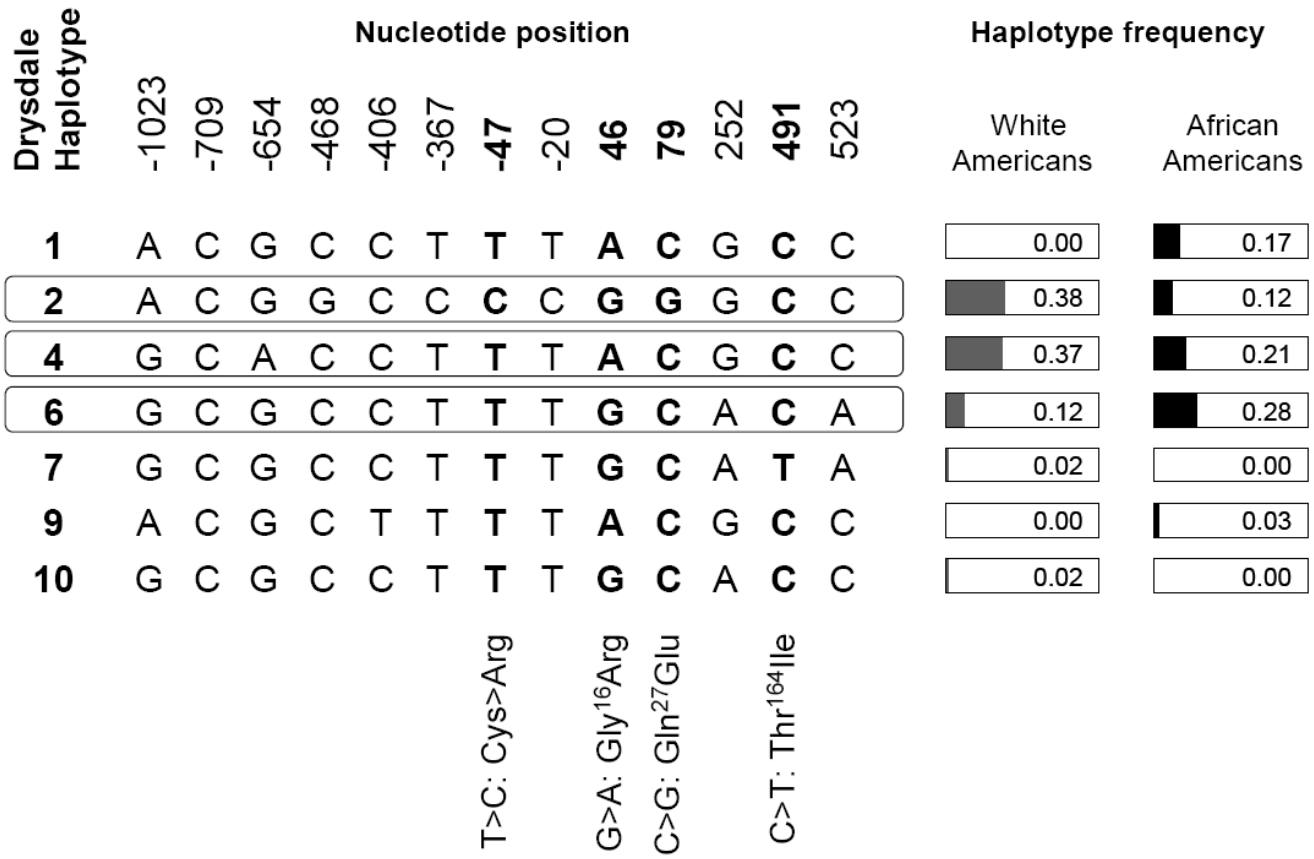

Figure 3.

Frequency of common haplotypes of the β_2-AR in Whites and African Americans. The figure shows the haplotypes defined by Drysdale et al. [Drysdale, C. M. *et al.* 2000] and their frequencies in Whites and African Americans as observed by Hawkins et al. [Hawkins, G. A. *et al.* 2006]. Only haplotypes with a frequency >0.01 are shown. Only 3 haplotypes (#2, #4, and #6) occur with frequencies >5% in the white population. Bold letters indicate tagging SNPs often used in association studies.

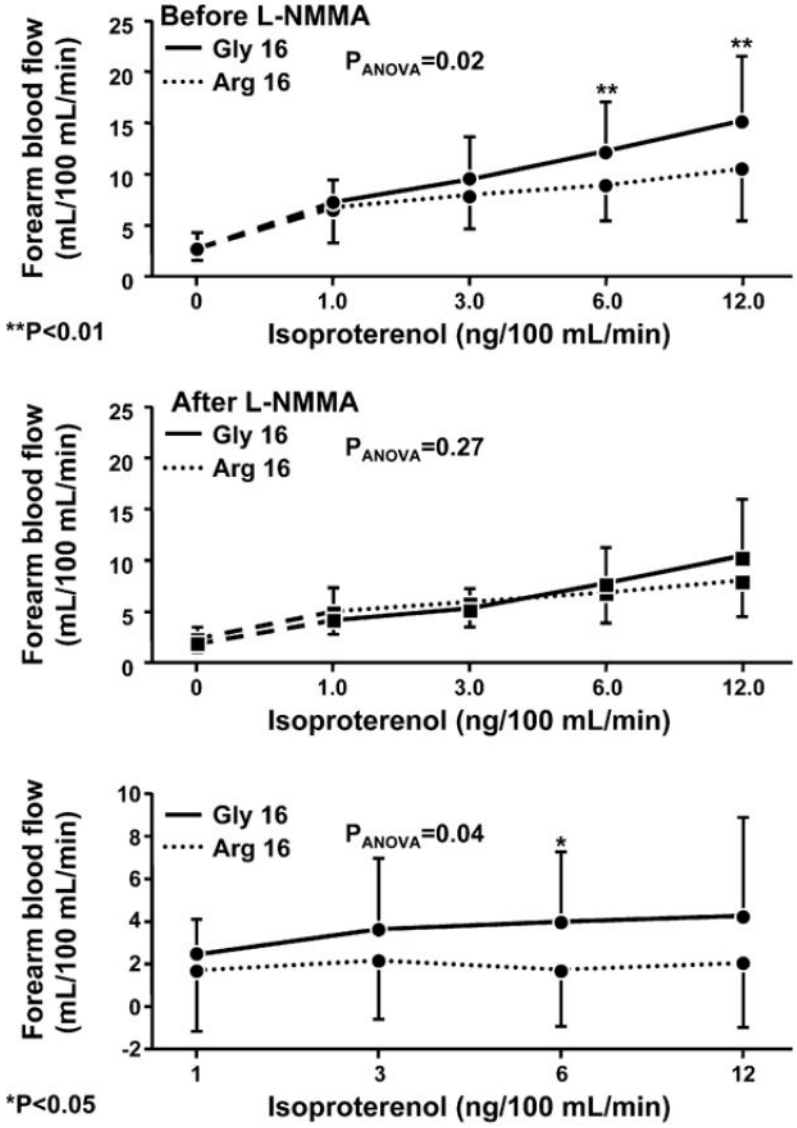

Figure 4.

Forearm blood flow responses to graded infusion of isoproterenol in subjects homozygous for the β_2-AR Arg^{16} variant (n=18) or the Gly^{16} variant (n=23). Gly^{16} homozygotes demonstrated significantly greater forearm blood flow responses to isoproterenol than Arg^{16} homozygotes (P=0.02; upper panel). After inhibition of endothelial nitric oxide synthase with L-NMMA, the forearm blood flow responses to isoproterenol were significantly reduced in both the Arg^{16} and Gly^{16} homozygotes, and they no longer differed significantly between the genotype groups (P=0.27; middle panel). Consequently, the nitric oxide-dependent component of the vasodilator response to isoproterenol, calculated by subtracting the post-L-NMMA responses from the pre-L-NMMA responses, was significantly greater in Gly^{16} than Arg^{16} homozygotes (P=0.04; bottom panel). P_{ANOVA} is the P value for the contrast between genotypes from the repeated measures ANOVA; at each dose of isoproterenol, statistically significant differences between genotype groups are denoted by: *P<0.05; **P<0.01. From [Garovic, V. D. *et al.* 2003].

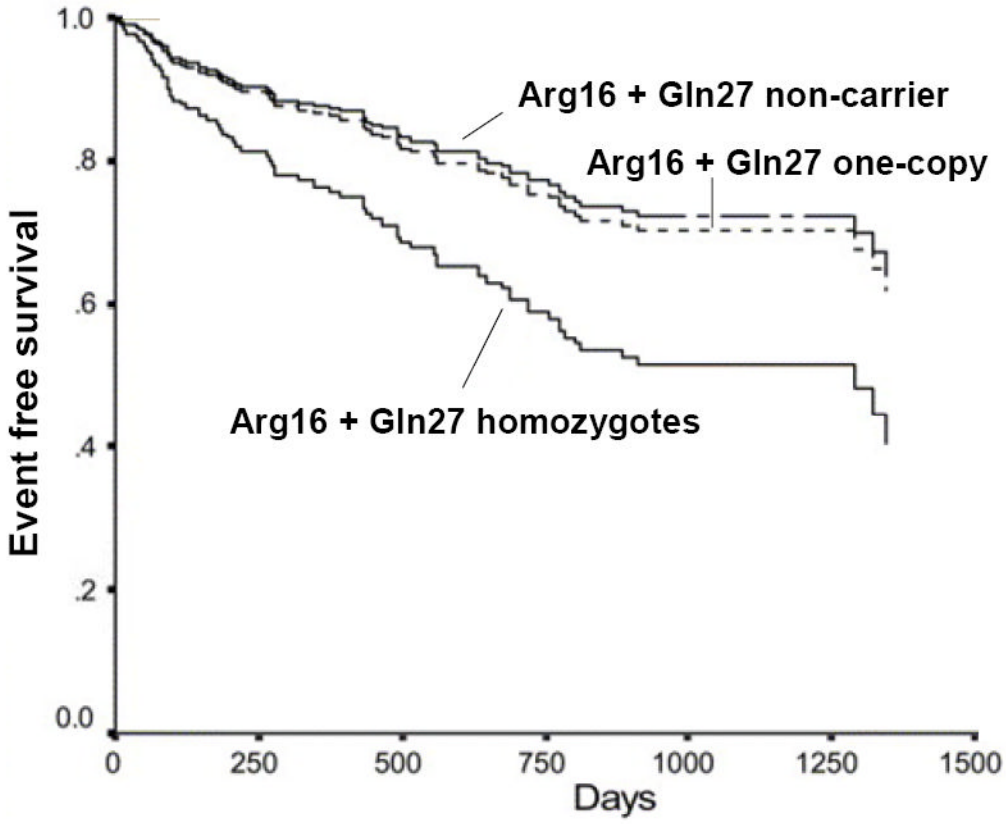

Figure 5.
Event-free survival (event defined as death or heart transplantation) in patients with heart failure. Possession of 2 copies of the Arg^{16} and Gln^{27} allele was associated with a 90% increase in the risk of death or heart transplantation, after adjustment of gender, etiology, NYHA class, ejection fraction, serum sodium levels, creatinine clearance, and medications. Modified from [Shin, J. *et al.* 2007].

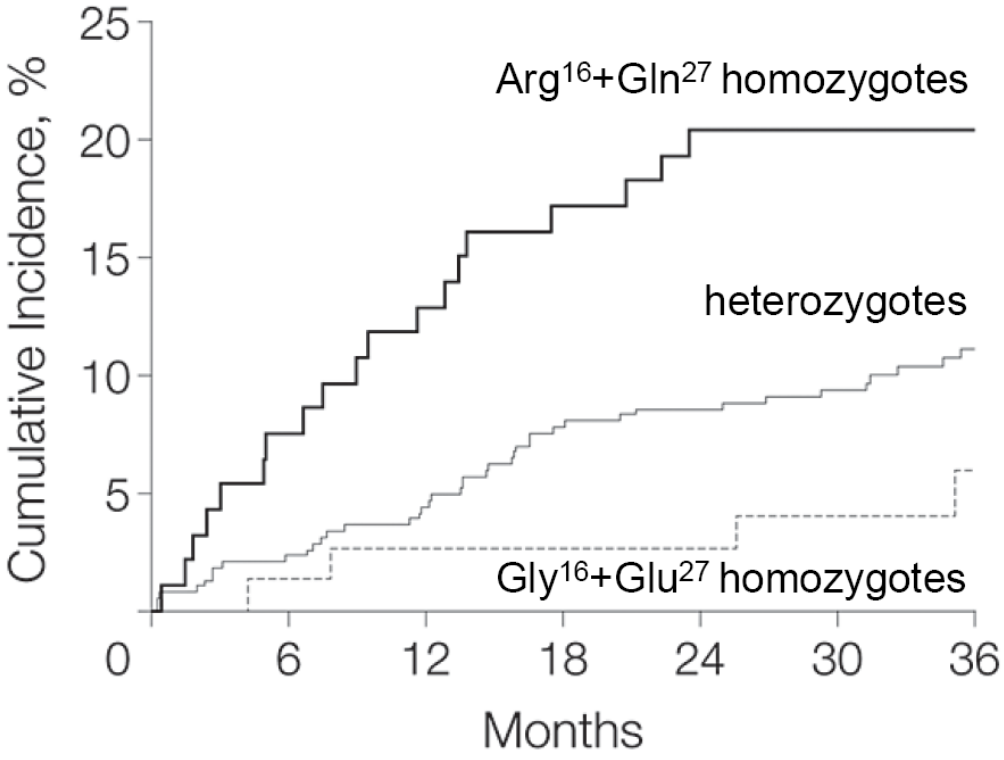

Figure 6.
Cumulative incidence of mortality in patients placed on β-blockers following an acute coronary syndrome. Individuals homozygous for both Arg^{16} and Gln^{27} were at high-risk with a 3-year Kaplan-Meier mortality rate of 20%, whereas individuals homozygous for both Gly^{16} and Glu^{27} were at low risk having a 3-year Kaplan-Meier mortality rate of only 6%. Modified from [Lanfear, D. E. *et al.* 2005].