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REVIEW

β_1- and β_2-Adrenoceptor polymorphisms and cardiovascular diseases

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β_1- and β_2-Adrenoceptors (AR) play a pivotal role in the regulation of cardiovascular function. Both β-AR subtypes are polymorphic: two single nucleotide polymorphisms (SNPs) have been described for the β_1- (Ser49Gly, Arg389Gly) and four for the β_2-AR (Arg-19Cys, Arg16Gly, Gln27Glu, Thr164Ile), and they are possibly of functional relevance. In recombinant cell systems, Gly49-β_1-AR are more susceptible to agonist-promoted down-regulation than Ser49-β_1-AR, whereas Arg389-β_1-AR are three to four times more responsive to agonist-evoked stimulation than Gly389-β_1-AR. With respect to β_2-AR, the Cys-19 variant is associated with greater β_2-AR expression than the Arg-19 variant; Gly16-β_2-AR are more susceptible, whereas Glu27-β_2-AR are almost resistant to agonist-promoted down-regulation; Thr164-β_2-AR are three to four times more responsive to agonist-evoked stimulation than Ile164-β_2-AR. Several studies addressed potential phenotypic consequences of these SNPs *in vivo* by influencing and/or contributing to the pathophysiology of cardiovascular/pulmonary diseases such as hypertension, congestive heart failure, arrhythmias or asthma. At present, it appears that these β-AR SNPs are very likely not disease-causing genes but possibly predictive for the responsiveness to agonists and antagonists. Patients carrying one or two alleles of the Gly389-β_1-AR are poor or non-responders to agonists and antagonists, whereas patients homozygous for the Arg389-β_1-AR are good responders. Subjects carrying the Ile164-β_2-AR exhibit blunted responses to β_2-AR stimulation. Asthma patients carrying the Arg16-Gln27-Thr164-β_2-AR haplotype who receive regularly short- or long-acting β_2-AR agonists are rather susceptible to agonist-induced desensitization and in consequence exhibit reduced bronchodilating and -protective effects and/or increased asthma exacerbations. The clinical relevance of these findings is still under debate.
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Keywords: β_1-Adrenoceptor; β_2-Adrenoceptor; cardiovascular diseases; genotype; haplotype; phenotype; single nucleotide polymorphism

Abbreviations: AC, adenylyl cyclase; ADR, adrenaline; AR, adrenoceptor; DOBU, dobutamine; GRK, G protein-coupled receptor kinase; ISO, isoprenaline; HF, heart failure; NA, noradrenaline; SALB, salbutamol; SNP, single nucleotide polymorphism; TER, terbutaline; TMD, transmembrane spanning domain; ZIN, zinterol

β-Adrenoceptors (AR) in the cardiovascular system

β-AR are characterized by a seven transmembrane spanning domain (TMD) structure, an extracellular amino terminus, three intra- and extracellular loops, and an intracellular carboxyl terminus. Agonist binding to β-AR induces the activation of an associated heterotrimeric G protein (containing the three subunits G_α, G_β and G_γ). The activated G_α-subunit dissociates from the G protein complex and stimulates ($G_{\alpha s}$) or inhibits ($G_{\alpha i}$) adenylyl cyclase (AC), and therefore modulates the intracellular amount of cyclic AMP (for review, see Brodde *et al.*, 2006; Leineweber *et al.*, 2006a).

At present, three β-AR subtypes have been identified in mammals, β_1-, β_2- and β_3-AR. In the human heart, both β_1- and β_2-AR coexist, and the β_1-AR subtype predominates in atrial (60–70%:40–30%) as well as in ventricular tissue

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(70–80%:30–20%) (for review, see Brodde and Michel, 1999). β_1-AR exclusively couple to the stimulatory $G_{\alpha s}$. In rat and mouse heart, however, β_2-AR couple to not only $G_{\alpha s}$, but also $G_{\alpha i}$, probably in an agonist-specific manner: fenoterol stimulates predominantly $G_{\alpha s}$ while terbutaline (TER), salbutamol (SALB) and zinterol (ZIN) stimulate both, $G_{\alpha s}$ and $G_{\alpha i}$ (Xiao *et al.*, 2003). Whether or not β_2-AR also couple to $G_{\alpha i}$ in human hearts, is still a matter of debate. However, in isolated human right atrial membranes pertussis toxin pretreatment (irreversible inhibition of $G_{\alpha i}$) enhances AC activation in response to ZIN (for review, see Steinberg, 1999).

Despite the fact that β_1-AR predominate in the human heart, β_2-AR are more effectively coupled to AC than β_1-AR and the extent of functional responsiveness of β_1- and β_2-AR is tissue- and/or agonist-specific (for review, see Brodde and Michel, 1999). In isolated human right atria, isoprenaline (ISO) and adrenaline (ADR) cause nearly identical increases in force of contraction via β_1- and β_2-AR, however, in isolated ventricular preparations increases in force of contraction are maximal via β_1-AR stimulation and only submaximal via β_2-AR stimulation. *In vivo*, ISO and ADR infusion-induced increases in heart rate are mediated by β_1- and β_2-AR stimulation to about the same degree. On the other hand, noradrenaline (NA) increases contractility almost exclusively via β_1-AR in isolated human right atria and ventricular preparations, and it exerts its positive inotropic and chronotropic effects in vivo also almost exclusively via β_1-AR.

In addition to their cardiac effects, β_1-AR also mediate lipolysis and regulate the release of renin (and by this activation of the renin-angiotensin-aldosterone system), while β_2-AR mediate vasodilation, bronchodilation, relaxation of uterine muscles and glycogenolysis (for review, see Brodde and Michel, 1999).

Physiologically, in white and brown adipocytes, β_3-AR are coupled to stimulatory $G_{\alpha s}$ and modulate energy metabolism and thermogenesis. Several reports have also discussed a potential role of β_3-AR in vasodilation and relaxation of airway smooth muscles (for review, see Leineweber *et al.*, 2004). Whether or not β_3-AR exist in the human heart, is still unclear. Several groups found neither on the transcriptional nor on the functional level any evidence for β_3-AR mRNA or β_3-AR mediated effects (for review, see Brodde and Michel, 1999). On the other hand, in endomyocardial biopsy samples from the right intraventricular septum of cardiac transplant patients, Gauthier *et al.*, (1996; 1998) found β_3-AR coupled to the $G_{\alpha i}$/nitric oxide (NO) pathway mediating negative inotropic effects.

β-AR in heart failure (HF)

In human HF, the β_1-AR density is decreased (down-regulation), β_2-AR are uncoupled from the $G_{\alpha s}$–AC-pathway (desensitization), amount and activity of the inhibitory $G_{\alpha i}$ are increased (while $G_{\alpha s}$ is unchanged) as is the expression and enzymatic activity of G protein-coupled receptor kinases (GRKs), which phosphorylate the agonist-occupied β-AR receptors and thus facilitate their endocytosis (predominately β_1-AR) or uncoupling from $G_{\alpha s}$ (predominately β_2-AR) (for review, see Brodde, 1991; Brodde and Leineweber, 2004). In ventricular cardiomyocytes from patients with HF (increased inhibitory $G_{\alpha i}$ activity, see above), but not in cardiomyocytes from non-failing human hearts (normal $G_{\alpha i}$ activity), β_2-AR couple to $G_{\alpha i}$, thereby directly mediating negative inotropic effects (Gong *et al.*, 2002).

The consequence of these changes is a reduction in cardiac β-AR functional responsiveness; the extent of β_1-AR down-regulation and β_2-AR desensitization is directly related to the severity of HF and an attribute to the compensatory and chronically elevated activity of the sympathetic nervous system, as reflected by increased plasma NA levels (for review, see Brodde and Leineweber, 2004). Consequently, antagonism of the deleterious effects of catecholamines on the heart in HF by β-AR blockers (predominantly β_1-AR blockade) has beneficial effects, reflected by the up-regulation of the down-regulated β_1-AR, the re-sensitization of the uncoupled β_2-AR to the $G_{\alpha s}$–AC pathway, normalization of $G_{\alpha i}$ activity, and the decrease in GRK amount and enzymatic activity (for review, see Brodde, 2007).

Thus, β-AR blockers improve left ventricular function, relieve HF symptoms and increase survival in patients with HF (Bouzamondo *et al.*, 2001). However, despite their success as therapeutic agents, clinical studies have also shown that the responses among patients with HF to β-AR blocker are variable (Shin and Johnson, 2007). Genetic variations may account – at least in part – not only for the development and progression of HF but also for the variable responses to β-AR blockers in patients with HF.

β-AR single nucleotide polymorphisms (SNPs)

β_1-AR

For the β_1-AR, 12 nucleotide polymorphisms (SNPs) exist, but only two have functional relevance (see Figure 1): in the amino terminus of the receptor at position 145 (Ser49Gly, allele frequency, see Table 1) and in the proximal part of the carboxyl terminus (within the $G_{\alpha s}$-binding domain of the β_1-AR) at position 1165 (Arg389Gly, allele frequency, see Table 1). A strong linkage disequilibrium (LD; a measurement how often alleles are inherited together) exists between both SNPs, thus creating common haplotypes (defined as a set of closely associated alleles inherited together on one chromosome). Gly49 is always associated with Arg389, while Gly389 is always associated with Ser49, so that the haplotype Gly49Gly–Gly389Gly occurs very rarely, if at all. Accordingly, the wild-type (WT)-β_1-AR consists of Ser49Ser–Arg389Arg (for review, see Leineweber *et al.*, 2004).

Consequently, because of such strong LD, conclusions from a single locus investigated *in vitro* (site-directed mutagenesis of the WT-β_1AR) are difficult to relate to *ex vivo* or *in vivo* findings. Investigations of single loci *in vitro* revealed that Gly49-β_1-AR had no effect on agonist binding and on basal and maximal ISO-stimulated AC-activity but enhanced agonist-induced down-regulation. Gly389-β_1-AR, on the other hand, exhibited slightly lower basal and three- to four-fold lower maximal ISO-stimulated AC-activity, probably due to reduced coupling to the $G_{\alpha s}$–AC-pathway. In addition, Gly389-β_1-AR had less short-term agonist-promoted desensitization than Arg389 β_1-AR (for review, see Leineweber *et al.*,

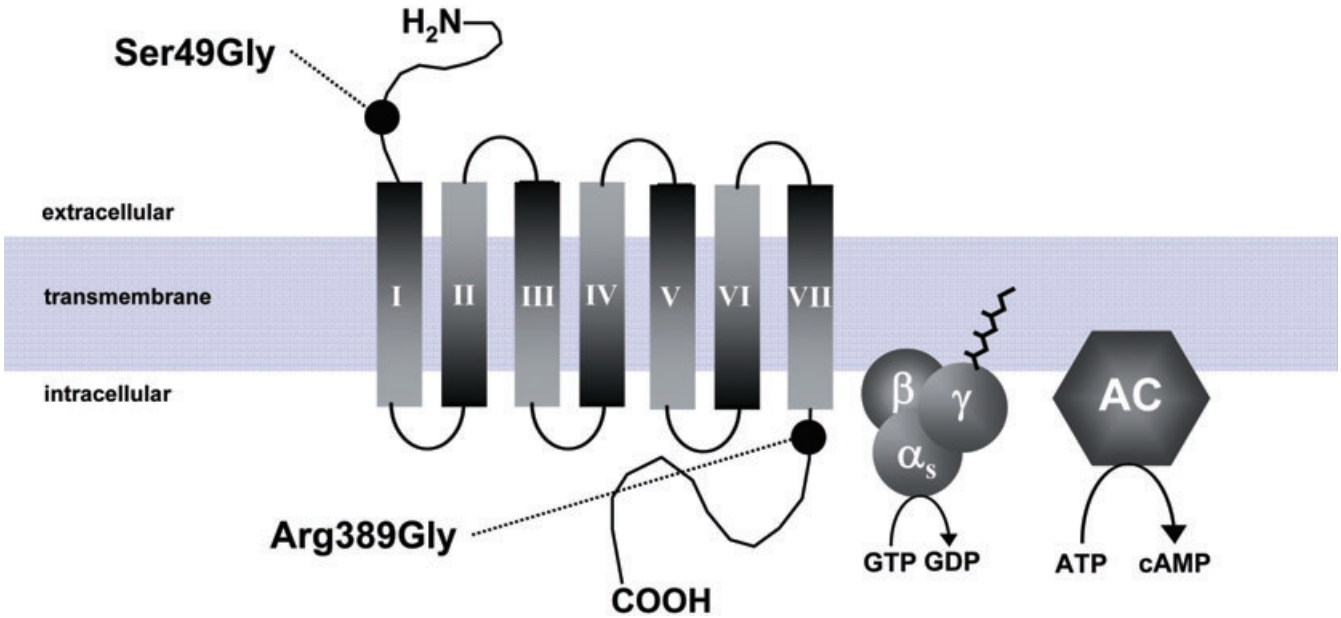

Figure 1 β_1-Adrenoceptor single nucleotide polymorphisms. AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate.

Table 1 Position, frequency and phenotype consequences of the functional β_1-Adrenoceptor single nucleotide polymorphisms

β_1-Adrenoceptor gene			*Published frequencies of the minor allele*			
SNP	Amino acid position	Common→minor allele	Caucasians	African-Americans	Asians	Latino-Hispanics
A145G	49	Ser→Gly	0.12–0.16	0.13–0.15 0.23–0.28	0.15	0.20–0.21
C1165G	389	Arg→Gly	0.24–0.34	0.39–0.46	0.20–0.30	0.31–0.33
In vitro						
Gly49	Enhanced agonist-induced down-regulation					
Gly389	Lesser coupling to $G_{\alpha s}$-protein Lower basal and maximal agonist-stimulated AC-activity Less short-term agonist-promoted desensitization					
Ex vivo						
Gly49	Right atria	No difference in potency or maximum of agonist-promoted increases in contractility				
Gly389	Right atria	No difference in maximum of agonist-promoted increases in contractility				
Gly389	Right ventricular trabeculae	Lower potency and maximum of agonist-promoted increases in contractility				
In vivo						
Gly389	Dynamic exercise; with endogenous increases in NA	Lower peak VO_2, but no differences in increases in heart rate, contractility, blood pressure and plasma renin activity				
Gly389	DOBU-infusion	Lower increases in heart rate, contractility and plasma renin activity				
Gly389	ADR-infusion after CABG with CPB	More and longer inotropic support				
Gly389	β_1-Adrenoceptor antagonists	Less reduction in heart rate, contractility, blood pressure and plasma renin activity				

AC, adenylyl cyclase; ADR, adrenaline; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; DOBU, dobutamine; NA, noradrenaline; SNP, single nucleotide polymorphism.

2004). Investigations of the four possible haplotypes *in vitro* revealed a rank order for the ISO-stimulated increase in cyclic AMP production and ISO-induced desensitization: Gly49Gly–Arg389Arg > Ser49Ser–Arg389Arg > Gly49Gly–Gly389Gly ≥ Ser49Ser–Gly389Gly (Sandilands *et al.*, 2004). Thus, position 389 obviously determines the functional responsiveness of the β_1-AR.

Although there is good *in vitro* evidence for such functional differences there is still controversy about their functional relevance *ex vivo* (in human tissue natively expressing the different β_1-AR SNPs) and *in vivo*. In isolated right atria obtained from patients undergoing coronary artery bypass grafting and chronically treated with atenolol, the inotropic potency but not the maximal effects of NA and ISO were lower when homozygous for the Gly389-β_1-AR than when carrying the Arg389-β_1-AR (Sandilands *et al.*, 2003). In isolated atria from patients chronically treated with metoprolol or atenolol or not treated with β_1-AR selective blockers at all, the inotropic potency or maximal effects of NA were not different when carrying the various Arg389Gly β_1-AR polymorphisms (Molenaar *et al.*, 2002). On the other hand, in isolated right ventricular trabeculae from non-failing and failing human

hearts, inotropic potency and maximal effects of ISO were lower when from patients homozygous for the Gly389-β_1-AR than from those carrying the Arg389-β_1-AR (Liggett *et al.*, 2006). In isolated human fat cells, however, the lipolytic response to dobutamine (DOBU, β_1-AR selective agonist) was not different with regard to the various Arg389Gly β_1-AR polymorphisms (Rydén *et al.*, 2001).

Whether or not these discrepancies are caused by genotype-dependent differences between the various β_1-AR SNPs in concert with a tissue and/or agonist-specific β_1-AR responsiveness (see above) is not known at present. Of note, in HF patients treated with β_1-AR selective blockers β_1-AR are up-regulated while β_2-AR are re-sensitized, and by using ISO (a non-selective β_1- and β_2-AR agonist) additional β_2-AR responses probably modulate putative β_1-AR genotype-dependent differences.

In young and middle-aged healthy subjects dynamic exercise (associated with increases in endogenous NA) revealed no genotype-dependent differences between subjects carrying the Gly389- or the Arg389-β_1-AR variant with regard to increases in heart rate, contractility, blood pressure and plasma renin activity (extracardiac β_1-AR mediated effect) (for review, see Leineweber *et al.*, 2004). On the other hand, exercise-evoked maximal aerobic power (peak VO_2) was least in subjects carrying the Gly389-β_1-AR (Defoor *et al.*, 2006). In contrast to the endogenously increased NA during exercise, increasing doses of exogenous DOBU caused significantly smaller increases in heart rate, contractility and plasma renin activity in healthy young subjects carrying one or two alleles of the Gly389-β_1-AR than in subjects carrying the Arg389-β_1-AR (La Rosee *et al.*, 2004; Bruck *et al.*, 2005a). Consistently, patients undergoing coronary artery bypass grafting under cardiopulmonary bypass and carrying one or two alleles of the Gly389-β_1-AR required more and longer inotropic support by ADR than those homozygous for the Arg389-β_1-AR (Leineweber *et al.*, 2007).

Thus, *in vivo* position 389 does not determine functional responsiveness to a physiologic stimulus (exercise-induced increase in endogenous NA), but modulates the β_1-AR response in a genotype-dependent manner to a pharmacological stimulus (exogenous infusion of DOBU and ADR). *Vice versa*, subjects carrying one or two alleles of the Gly389-β_1AR exhibit less responsiveness to β_1-AR selective blockers (i.e. reduction in heart rate, contractility, blood pressure and plasma renin activity), however, independent of the underlying β_1-AR stimulus: none (Sofowora *et al.*, 2003), exercise (Liu Liu *et al.*, 2003) or DOBU infusion (Bruck *et al.*, 2005a).

Thus, genetic variation in the β_1-AR – predominately at position 389 – not only modulates functional responsiveness to agonists but also affects the impact of β-AR blockers *in vivo*.

β_2-AR

For the β_2-AR, 19 SNPs have been identified, and at least four are of functional consequence (see Figure 2 and Table 2): (i) at position –47 (Cys-19Arg) within the short open reading frame of the *B*eta *U*pstream *P*eptide (BUP; a 1.5 kb region upstream to the start codon containing the main transcriptional regulatory activity for β_2-AR gene expression); (ii) and (iii) within the coding region within the extracellular amino terminus at

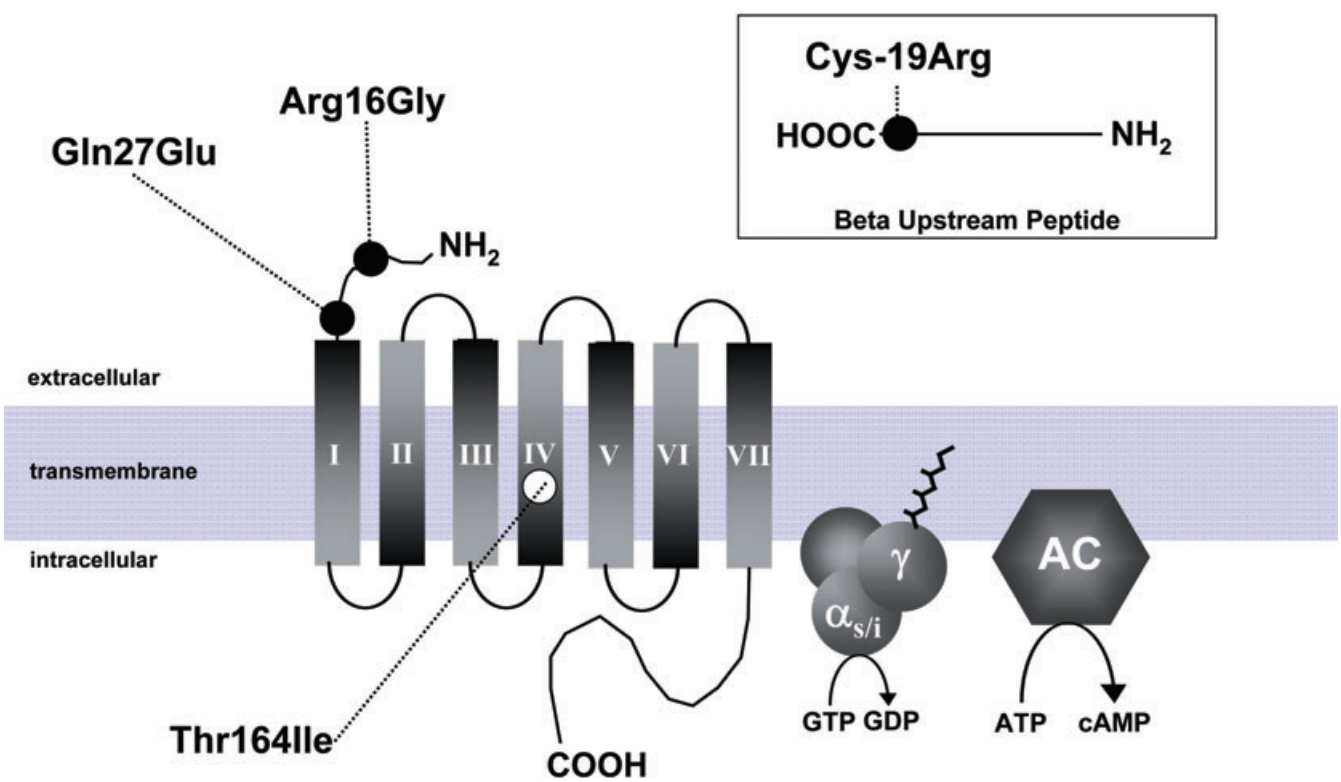

Figure 2 β_2-Adrenoceptor single nucleotide polymorphisms. AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate.

Table 2 Position, frequency and phenotype consequences of the functional β_2-Adrenoceptor single nucleotide polymorphisms

β_2-Adrenoceptor gene			*Published frequencies of the minor allele*			
SNP	Amino acid position	Common→minor allele	Caucasians	African-Americans	Asians	Latino-Hispanics
C-47T	–19	Cys→Arg	0.35	0.21	0.08–0.12	n.d.
A46G	16	Gly→Arg	0.38–0.46	0.49–0.51	0.54–0.59	n.d.
C79G	27	Gln→Glu	0.35–0.46	0.20–0.27	0.07–0.20	n.d.
C491T	164	Thr→Ile	0.02–0.04	0.02–0.04	0–0.01	0.03
In vitro						
Arg-19	Lower receptor expression					
Gly16	Enhanced agonist-induced down-regulation, no effects on agonist binding affinities and/or basal/maximal agonist-stimulated AC-activity					
Glu27	Reduced agonist-induced down-regulation, no effects on agonist binding affinities and/or basal/maximal agonist-stimulated AC-activity					
Ile164*	Lower agonist binding affinities, reduced basal/maximal agonist-stimulated AC-activity, lower maximal agonist-induced sequestration					
In vivo						
Gly16/Glu27	Heart rate and contractility	No differences				
Ile164*	Heart rate and contractility	Blunted responsiveness				
Gly16	Systemic infusion of SALB, TER and ADR	Less vasodilation				
Gly16	Local infusion of ISO into a brachial artery or hand vein	Larger vasodilator response				
Ile164*	Local infusion of ISO or TER into a brachial artery or hand vein	Blunted vasodilation				
Gly16	Receptor desensitization upon 2 h continuous ISO-infusion or 2 weeks oral treatment with TER	Less pronounced loss in venodilator response				
Gly16	Short-acting β_2-AR agonist treatment in asthma patients	Less pronounced loss in bronchodilating/protective effects and lower frequency of exacerbations				

*Only in the heterozygous state.
AC, adenylyl cyclase; ADR, adrenaline; n.d., not determined; ISO, isoprenaline; SALB, salbutamol; SNP, single nucleotide polymorphism; TER, terbutaline.

position 46 (Arg16Gly) and position 79 (Gln27Glu); and (iv) within the fourth TMD at position 491 (Thr164Ile) of the β_2-AR (for review, see Leineweber and Brodde, 2004; Leineweber *et al.*, 2004; Brodde and Leineweber, 2005). Strong LDs exist between these SNPs, resulting in common haplotypes: Arg-19 is always associated with Gly16, while Cys-19 is associated with either Arg16 or Gly16. Glu27 is almost always associated with Gly16, whereas Gln27 is associated with either Arg16 or Gly16. Finally, Ile164 is closely associated with Gly16 and Gln27. Accordingly, the WT-β_2-AR consists of Cys-19Cys–Arg16Arg–Gln27Gln–Thr164Thr (for review, see Leineweber and Brodde, 2004; Leineweber *et al.*, 2004; Brodde and Leineweber, 2005).

Investigations of single loci *in vitro* revealed: Cys-19Arg affects receptor expression on the transcriptional level (Arg-19 decreases β_2-AR expression); Gly16-β_2-AR enhance agonist-induced down-regulation but Glu27-β_2-AR reduce it (both without affecting agonist binding affinities and/or basal/maximal agonist-stimulated AC-activity); Ile164-β_2-AR have lower agonist binding affinities, reduced basal/maximal agonist-stimulated AC-activity and exhibit less maximal agonist-induced internalization. Studies investigating different combinations at position 16 and 27 *in vitro* revealed: *Arg-19Arg–Gly16Gly*–Gln27Gln–Thr164Thr-β_2-AR and *Arg-19Arg–Gly16Gly–Glu27Glu*–Thr164Thr-β_2-AR have a similar down-regulation profile in comparison with the WT-β_2-AR (suggesting that Gly16 dominates the phenotype), while Cys-19Cys–Arg16Arg–*Glu27Glu*–Thr164Thr-β_2-AR have no such agonist-promoted down-regulation (for review, see Leineweber and Brodde, 2004; Brodde and Leineweber, 2005).

With regard to the strong LD between position 16 and 27, the Cys-19Cys–Arg16Arg–*Glu27Glu*–Thr164Thr-β_2-AR does almost not exist in nature (<1% of the population). Also, the Ile164-β_2-AR is not only rare in nature, but exists only in the heterozygous state; that is, the chance of forming a gamete homozygous for Ile164-β_2-AR is therefore very low and possibly lethal due to a total loss of β_2-AR responsiveness.

As mentioned above, β_2-AR play a pivotal role in the regulation of heart rate and contractility, vasodilation and bronchodilation. *In vivo*, independent whether single loci or haplotypes were investigated, several studies found β_2-AR-mediated increases in heart rate and contractility to be genotype-independent for the β_2-AR variants Arg16Gly and Gln27Glu, but blunted in subjects carrying one allele of the Ile164-β_2-AR (for review, see Leineweber *et al.*, 2004). So far, studies investigating the impact of the β_2-AR variants Arg16Gly and Gln27Glu on vasodilation are inconclusive. Systemic infusion of SALB, TER and ADR resulted in less vasodilation, but local infusion of ISO into a brachial artery or hand vein evoked larger vasodilator responses in subjects carrying one or two alleles of the Gly16-β_2-AR than in those homozygous for the Arg16-β_2-AR (for review, see Leineweber and Brodde, 2004; Brodde and Leineweber, 2005). ISO-induced venodilation or increases in forearm blood flow were most pronounced in subjects carrying the Gly16Gly–Glu27Glu-β_2-AR and smallest in subjects carrying the Arg16Arg–Gln27Gln-β2-AR (Dishy *et al.*, 2001; Garovic *et al.*, 2003; Trombetta *et al.*, 2005); only one study found no differences between these haplotypes in the TER-stimulated vasodilator response in the dorsal hand vein (Bruck *et al.*, 2005b).

Similar to cardiac responses, vasodilator responses to β_2-AR stimulation are blunted in subjects carrying one allele of the Ile164-β_2-AR when compared with subjects homozygous for the Thr164-β_2-AR (Dishy *et al.*, 2004; Bruck *et al.*, 2005b).

β_2-AR agonists are widely used to treat acute bronchospasm (short-acting agonists) and as an adjunct to anti-inflammatory therapy (long-acting agonists) in asthma. Similar to β-AR blockers in the treatment of HF, responses to β_2-AR agonists are variable with regard to loss in bronchodilating and bronchoprotective effects and/or increased frequency of exacerbations due to β_2-AR desensitization (for review, see Taylor, 2007). Such adverse effects are less pronounced in asthma patients carrying the Gly16Gly–Gln27Gln- or Gly16Gly–Glu27Glu-β_2-AR than in patients carrying the Arg16Arg–Gln27Gln-β_2-AR when treated with regular short-acting β_2-AR agonists (for review, see Hawkins *et al.*, 2008). Consistent with these findings, also the venous vasodilator response in healthy subjects is desensitized upon 2 h continuous ISO-infusion or 2 weeks oral treatment with TER with the haplotype order Arg16Arg–Gln27Gln >> Gly16Gly–Gln27Gln ≥ Gly16Gly–Glu27Glu (Dishy *et al.*, 2001; Bruck *et al.*, 2005b).

Thus, while position 16 and 27 have no functional impact on heart rate and contractility, the Arg16Arg–Gln27Gln-β_2AR in vascular and bronchial smooth muscle is rather susceptible to agonist-induced desensitization. With regard to the agonist- and tissue-dependent responsiveness of cardiac β_2-AR (see above), an impact of the different β_2-AR variants Arg16Gly and Gln27Glu can not be excluded. The functional responsiveness of the Ile164-β_2-AR appears generally blunted, in an agonist- and tissue-independent manner.

Clinical implications of β-AR polymorphisms

In HF, both β_1- and β_2-AR and the accompanying signalling cascade ($G_{\alpha s}$, $G_{\alpha i}$, cyclic adenosine monophosphate (cAMP), GRKs) are altered, probably modulated also by the genetic variants of the β_1- and β_2-AR. Assuming that β_1- and β_2-AR SNPs are involved in the pathogenesis of HF, differences in the allele frequencies of these SNPs between cohorts with and without HF and in their prognosis are expected. However, neither for the Ser49- or Gly49- nor for the Arg389- or Gly389-β_1-AR differences in allele frequencies were found. Furthermore, studies investigating the outcome of HF (worsening of clinical conditions, hospitalization, transplantation or death, mortality) are rather inconsistent (for review, see Brodde, 2008). While in three studies the outcome was better in HF patients carrying one or two alleles of the Gly49-β_1AR, two studies did not find an association between the different Ser49Gly β_1-AR variants and the outcome of HF (for review, see Brodde, 2008). However, with regard to the strong LD between position 49 and 389, that is, that Gly49 is always associated with Arg389, inconsistent results are actually expected. Consistently, however, the Arg389Gly β_1-AR polymorphism seems not to affect the outcome of HF at all (for review, see Muthumala *et al.*, 2008).

β_2-AR mediate vasodilation; in hypertension vascular responses to β_2-AR stimulation are impaired (for review, see Brodde and Michel, 1999). Since β_2-AR SNPs modulate the desensitization of β_2-AR and by this vasodilator responsiveness (see above) the different β_2-AR variants might play a role in the development and/or maintenance of hypertension. However, in many studies the β_2-AR variants Arg16Gly or Gln27Glu had no influence on the development of hypertension (for review, see Brodde, 2008). However, a potential association between the Ile164-β_2-AR variant and hypertension was found in women (Sethi *et al.*, 2005) but not in men (Iaccarino *et al.*, 2004; Sethi *et al.*, 2005). No differences in the allele frequencies of the Arg16- or Gly16- and Gln27- or Glu27- and Thr164- or Ile164-β_2-AR were observed between cohorts with and without HF (for review, see Leineweber and Brodde, 2004; Brodde and Leineweber, 2005). While HF patients with the Arg16Arg–Gln27Gln-β_2-AR seem to have a more pronounced adverse outcome (heart transplantation) and increased risk for sudden cardiac death (for review, see Brodde, 2008) data on the role of the Ile164-β_2-AR in HF are rather conflicting. Chronic HF patients carrying the Thr164Ile-β_2-AR exhibit lower exercise capacity (peak VO_2) than Thr164Thr-β_2-AR patients (Wagoner *et al.*, 2000) and a rapid progression to either death or heart transplantation during a 3 year follow-up (Liggett *et al.*, 1998). However, because of the low prevalence of the Thr164Ile-β_2AR variant in the general population (see Table 2), the latter finding was obtained in only 10 out of 259 chronic HF patients (Liggett *et al.*, 1998). On the other hand, several recent studies could not confirm this finding of a rapid progression of HF in patients heterozygous for the Thr164Ile-β_2-AR. Forleo *et al.* (2004) in 171 consecutive patients with dilated cardiomyopathy did not find an association between the Thr164Ile-β_2-AR polymorphism and the risk of HF, and De Groote *et al.* (2005) in 444 consecutive patients with chronic HF and Barbato *et al.* (2007) in 31 chronic HF-patients and 24 controls did not find an association between the Thr164Ile-β2-AR polymorphism and 3.5 or 2 year, respectively, survival. We (Leineweber *et al.*, 2006b) genotyped 309 heart-transplanted patients, 520 patients with stable chronic HF and 328 controls for the Thr164Ile-β_2-AR polymorphism under the assumption that – if Thr164Ile-β_2-AR patients indeed undergo rapid progression to death or heart transplantation, as suggested by Liggett *et al.* (1998) – the prevalence of the Thr164Ile β_2-AR variant and the frequency of the Ile164 allele should be much higher in heart-transplanted patients than in patients with stable chronic HF or healthy controls. In contrast to this assumption, we found the prevalence of the Thr164Ile-β_2-AR variant in the heart-transplanted patients not different from that in the patients with stable chronic HF or the controls (Leineweber *et al.*, 2006b).

As mentioned, β-AR blockers up-regulate the down-regulated β_1-AR, re-sensitize the uncoupled β_2-AR, and improve left ventricular function, HF symptoms and survival in patient with HF, probably in a β_1- and/or β_2-AR genotype-dependent manner. However, investigations on the impact of the different β_1- and β_2-AR SNPs on left ventricular remodelling and the improvement in left ventricular function and survival in HF patients are limited and have to be interpreted carefully with regard to the study design [i.e. cohort registries vs. placebo-controlled or -uncontrolled clinical trials and β-AR blocker treatment: β_1-AR selective vs. β_1- and β_2-AR non-selective blockers as well as time of intake

and dose (for review, see Muthumala *et al.*, 2008)]. Studies investigating the effect of β-AR blockers on left ventricular remodelling and function found that the beneficial effects were more pronounced in HF patients homozygous for the Arg389-β_1-AR (Mialet-Perez *et al.*, 2003; Terra *et al.*, 2005) or Glu27-β_2-AR (Kaye *et al.*, 2003). Studies investigating the effect of β-AR blockers on survival are inconclusive: a substudy of the MERIT HF (Metoprolol CR/XL Randomized Intervention Trial in Chronic Heart Failure) found no association between the β_1-AR variants Ser49Gly and Arg389Gly and all-cause death and/or hospitalization (White *et al.*, 2003); in another study, five-year survival was significantly better in HF patients carrying one or two alleles of the Gly49-β_1-AR (and, due to the strong LD between position 49 and 389, carrying one or two alleles of the Arg389 β_1-AR) than in HF patients homozygous for the Ser49 β_1-AR (Magnusson *et al.*, 2005). On the contrary, in the BEST trial (Beta-blocker Evaluation of Survival Trial) survival and reduction in hospitalization were reduced more in HF patients homozygous for the Arg389-β_1-AR and treated with bucindolol (β_1- and β_2-AR non-selective β-AR blocker with sympatholytic effects) than in HF patients homozygous for the Arg389 β_1-AR on placebo, whereas there was no such difference in outcome between HF patients receiving bucindolol or placebo when homozygous for the Gly389-β_1-AR (Liggett *et al.*, 2006). Finally, a placebo-uncontrolled study investigating a cohort of clinically treated HF patients receiving metoprolol or carvedilol found no association between transplant-free survival and the different β_1- and β_2-AR SNPs (Sehnert *et al.*, 2008); unfortunately, information regarding duration and dose of the respective β-AR blocker before registration in this study is missing.

Recently, Rochais *et al.* (2007) investigated in HEK-293 cells expressing either the Gly389- or the Arg389-β_1-AR dynamic conformational changes of both receptor variants during activation (by ISO and NA) or inhibiton (by bisoprolol, metoprolol and carvedilol) via fluorescence resonance energy transfer microscopy. In this setting position 389 did neither affect the kinetics of agonist-induced receptor activation nor did it influence basal or maximal cAMP levels or even reveal differences in $G_{\alpha s}$ activation. On the other hand, while position 389 again did not affect the response to bisoprolol and metoprolol (decrease in basal cAMP levels), the Gly389-β_1-AR variant exhibited a more than two-fold lesser basal cAMP reduction in response to carvedilol than the Arg389-β_1-AR variant. Consistently, in neonatal rat cardiomyocytes expressing either the Gly389- or the Arg389-β_1-AR, the Gly389-β_1-AR did not respond to carvedilol while the Arg389-β_1-AR responded with a 25% reduction of the beating frequency. Of note, while Rochais *et al.* (2007) did not find genotype-dependent differences in basal and maximal cAMP formation or activation of $G_{\alpha s}$ in HEK-293 cells, the expression of the Arg389-β_1-AR variant in neonatal rat cardiomyocytes led already under basal conditions to a higher beating frequency than the Gly389-β_1-AR variant. In a clinical setting, Chen *et al.* (2007) investigated Caucasian patients with nonischemic cardiomyopathy treated ≥1 year with maximal tolerable carvedilol doses. They found that only 45% of patients carrying the Gly389-β_1-AR variant responded at all to carvedilol (left ventricular ejection fraction: basal 24 ± 7% vs. follow-up 30 ± 14%) whereas 81% of patients carrying the Arg389-β_1-AR variant responded with a significant increase in left ventricular ejection fraction (basal 22 ± 7% vs. follow-up 41 ± 12%).

Thus, at present, β-AR SNPs are very likely not disease-causing genes but possibly predictive for the responsiveness to agonists and antagonists. Patients carrying one or two alleles of the Gly389-β_1-AR are poor or non-responders whereas patients homozygous for the Arg389 β_1-AR are good responders to antagonists. Such SNP differences are not of consequence for survival in HF patients.

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Conflict of interest

None.

References

Barbato E, Penicka, M, Delrue L, Van Durme F, De Bruyne B, Goethals M *et al.* (2007). Thr164Ile polymorphism of β2-adrenergic receptor negatively modulates cardiac contractility: implications for prognosis in patients with idiopathic dilated cardiomyopathy. *Heart* **93**: 856–861.

Bouzamondo A, Hulot JS, Sanchez P, Cucherat M, Lechat P (2001). Beta-blocker treatment in heart failure. *Fundam Clin Pharmacol* **15**: 95–109.

Brodde O.-E. (1991). β_1- and β_2-adrenoceptors in the human heart: properties, function, and alterations in heart failure. *Pharmacol Rev* **43**: 203–242.

Brodde O.-E. (2007). Beta-adrenoceptor blocker treatment and the cardiac beta-adrenoceptor-G-protein(s)-adenylyl cyclase system in chronic heart failure. Naunyn-Schmiedeberg's. *Arch Pharmacol* **374**: 361–372.

Brodde O.-E. (2008). Beta-1 and beta-2 adrenoceptor polymorphisms: functional importance, impact on cardiovascular diseases and drug responses. *Pharmacol Ther* **117**: 1–29.

Brodde O.-E., Michel MC (1999). Adrenergic and muscarinic receptors in the human heart. *Pharmacol Rev* **51**: 651–690.

Brodde O.-E., Leineweber K (2004). Autonomic receptor systems in the failing and aging human heart: similarities and differences. *Eur J Pharmacol* **500**: 167–176.

Brodde O.-E., Leineweber K (2005). β2-Adrenoceptor gene polymorphisms. *Pharmacogenet Genomics* **15**: 267–275.

Brodde O.-E., Bruck H, Leineweber K (2006). Cardiac adrenoceptors: physiological and pathophysiological relevance. *J Pharmacol Sci* **100**: 323–337.

Bruck H, Leineweber K, Temme T, Weber M, Heusch G, Philipp T *et al.* (2005a). The Arg389Gly β_1-adrenoceptor polymorphism and catecholamine effects on plasma-renin activity. *J Am Coll Cardiol* **46**: 2111–2115.

Bruck H, Leineweber K, Park J, Weber M, Heusch G, Philipp T, Brodde O.-E. (2005b). Human β2-adrenergic receptor gene haplotypes and venodilation in vivo. *Clin Pharmacol Ther* **78**: 232–238.

Chen L, Meyers D, Javorsky G, Burstow D, Lolekha P, Lucas M *et al.*

(2007). Arg389Gly-β_1-adrenergic receptors determine improvement in left ventricular systolic function in nonischemic cardiomyopathy patients with heart failure after chronic treatment with carvedilol. *Pharmacogenet Genomics* **17**: 941–949.

Defoor J, Martens K, Zielinska D, Matthijs G, Van Nerum H, Schepers D *et al*. (2006). The CAREGENE study: polymorphisms of the β_1-adrenoceptor gene and aerobic power in coronary artery disease. *Eur Heart J* **27**: 808–816.

Groote D, Lamblin P, Helbecque N, Mouquet N, McFadden F, Hermant E *et al*. (2005). The impact of beta-adrenoreceptor gene polymorphisms on survival in patients with congestive heart failure. *Eur J Heart Fail* **7**: 966–973.

Dishy V, Sofowora GG, Xie HG, Kim RB, Byrne DW, Stein CM *et al*. (2001). The effect of common polymorphisms of the β2-adrenergic receptor on agonist-mediated vascular desensitization. *N Engl J Med* **345**: 1030–1035.

Dishy V, Landau R, Sofowora GG, Xie HG, Smiley RM, Kim RB *et al*. (2004). β2-Adrenoceptor Thr164Ile polymorphism is associated with markedly decreased vasodilator and increased vasoconstrictor sensitivity in vivo. *Pharmacogenetics* **14**: 517–522.

Forleo C, Resta N, Sorrentino S, Guida P, Manghisi A, De Luca V *et al*. (2004). Association of β-adrenergic receptor polymorphisms and progression of heart failure in patients with idiopathic dilated cardiomyopathy. *Am J Med* **117**: 451–458.

Garovic VD, Joyner MJ, Dietz NM, Boerwinkle E, Turner ST (2003). β2-Adrenergic receptor polymorphism and nitric oxide-dependent forearm blood flow responses to isoproterenol in humans. *J Physiol* **546**: 583–589.

Gauthier C, Tavernier G, Charpentier F, Langin D, Le Marec H (1996). Functional β_3-adrenoceptor in the human heart. *J Clin Invest* **98**: 556–562.

Gauthier C, Leblais V, Kobzik L, Trochu JN, Khandoudi N, Bril A *et al*. (1998). The negative inotropic effect of β_3-adrenoceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle. *J Clin Invest* **102**: 1377–1384.

Gong H, Sun H, Koch WJ, Rau T, Eschenhagen T, Ravens U *et al*. (2002). Specific beta(2)AR blocker ICI 118,551 actively decreases contraction through a G(i)-coupled form of the beta(2)AR in myocytes from failing human heart. *Circulation* **105**: 2497–2503.

Hawkins GA, Weiss ST, Bleecker ER (2008). Clinical consequences of ADRβ2 polymorphisms. *Pharmacogenomics* **9**: 349–358.

Iaccarino G, Lanni F, Cipolletta E, Trimarco V, Izzo R, Iovino GL *et al*. (2004). The Glu27 allele of the β2 adrenergic receptor increases the risk of cardiac hypertrophy in hypertension. *J Hypertens* **22**: 2117–2122.

Kaye DM, Smirk B, Williams C, Jennings G, Esler M, Holst D (2003). Beta-adrenoceptor genotype influences the response to carvedilol in patients with congestive heart failure. *Pharmacogenetics* **13**: 379–382.

La Rosee L, Huntgeburth K, Rosenkranz M, Böhm S, M, Schnabel P (2004). The Arg389Gly β_1-adrenoceptor gene polymorphism determines contractile response to catecholamines. *Pharmacogenetics* **14**: 711–716.

Leineweber K, Brodde O.-E. (2004). β2-Adrenoceptor polymorphisms: relation between in vitro and in vivo phenotypes.. *Life Sci* **74**: 2803–2814.

Leineweber K, Büscher R, Bruck H, Brodde O.-E. (2004). β-Adrenoceptor polymorphisms. Naunyn-Schmiedeberg's. *Arch Pharmacol* **369**: 1–22.

Leineweber K, Böhm M, Heusch G (2006a). Cyclic adenosine monophosphate in acute myocardial infarction with heart failure: slayer or savior? *Circulation* **114**: 365–367.

Leineweber K, Tenderich G, Wolf C, Wagner S, Zittermann A, Elter-Schulz M *et al*. (2006b). Is there a role of the Thr164Ile β2-adrenoceptor polymorphism for the outcome of chronic heart failure? *Basic Res Cardiol* **101**: 479–484.

Leineweber K, Bogedain P, Wolf C, Wagner S, Weber M, Jakob HG *et al*. (2007). In patients chronically treated with metoprolol, the demand of inotropic catecholamine support after coronary artery bypass grafting is determined by the Arg389Gly-β_1-adrenoceptor polymorphism. Naunyn-Schmiedeberg's. *Arch Pharmacol* **375**: 303–309.

Liggett SB, Wagoner LE, Craft LL, Hornung RW, Hoit BD, McIntosh TC *et al*. (1998). The Ile164 β_2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. *J Clin Invest* **102**: 1534–1539.

Liggett SB, Mialet-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D *et al*. (2006). A polymorphism within a conserved β_1-adrenergic receptor motif alters cardiac function and β-blocker response in human heart failure. *Proc Natl Acad Sci USA* **103**: 11288–11293.

Liu J, Liu Z.-Q, Tan Z.-R, Chen X.-P, Wang L.-S, Zhou G *et al*. (2003). Gly389Arg polymorphism of β_1-adrenoceptor is associated with the cardiovascular response to metoprolol. *Clin Pharmacol Ther* **74**: 372–379.

Magnusson Y, Levin MC, Eggertsen R, Nyström E, Mobini R, Schaufelberger M *et al*. (2005). Ser49Gly of β_1-adrenergic receptor is associated with effective beta-blocker dose in dilated cardiomyopathy. *Clin Pharmacol Ther* **78**: 221–231.

Mialet-Perez J, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A *et al*. (2003). β_1-Adrenergic receptor polymorphsims confer differential function and predisposition to heart failure. *Nat Med* **9**: 1300–1305.

Molenaar P, Rabnott G, Yang I, Fong KM, Savarimuthu SM, Li L *et al*. (2002). Conservation of the cardiostimulant effects of (-)-norepinephrine accross Ser49Gly and Gly389Arg beta$_1$-adrenergic receptor polymorphisms in human right atrium in vitro. *J Am Coll Cardiol* **40**: 1275–1282.

Muthumala A, Drenos F, Elliott PM, Humphries SE (2008). Role of beta adrenergic receptor polymorphisms in heart failure: systematic review and meta-analysis. *Eur J Heart Fail* **10**: 3–13.

Rochais F, Vilardaga J.-P, Nikolaev VO, Bünemann M, Lohse MJ, Engelhardt S (2007). Real-time optical recording of β_1-adrenergic receptor activation reveals supersensitivity of the Arg389 variant to carvedilol. *J Clin Invest* **117**: 229–235.

Rydén M, Hoffstedt J, Eriksson P, Bringman S, Arner P (2001). The Arg389Gly β_1-adrenergic receptor gene polymorphism and human fat cell lipolysis. *Int J Obes Relat Metab Disord* **25**: 1599–1603.

Sandilands AJ, O'Shaughnessy KM, Brown MJ (2003). Greater inotropic and cyclic AMP responses evoked by noradrenaline through Arg389 β_1-adrenoceptors versus Gly389 β1-adrenoceptors in isolated human atrial myocardium. *Br J Pharmacol* **138**: 386–392.

Sandilands A, Yeo G, Brown MJ, O'Shaughnessy KM (2004). Functional responses of human β_1 adrenoceptors with defined haplotypes for the common 389R > G and 49S > G polymorphisms. *Pharmacogenetics* **14**: 343–349.

Sehnert AJ, Daniels SE, Elashoff M, Wingrove JA, Burrow CR, Horne B *et al*. (2008). Lack of association between adrenergic receptor genotypes and survival in heart failure patients treated with carvedilol or metoprolol. *J Am Coll Cardiol* **52**: 644–651.

Sethi AA, Tybjaerg-Hansen A, Jensen GB, Nordestgaard BG (2005). 164Ile allele in the β2-Adrenergic receptor gene is associated with risk of elevated blood pressure in women. The Copenhagen City Heart Study. *Pharmacogenet Genomics* **15**: 633–645.

Shin J, Johnson JA (2007). Pharmacogenetics of β-blockers. *Pharmacotherapy* **27**: 874–887.

Sofowora GG, Dishy V, Muszkat M, Xie HG, Kim RB, Harris PA *et al*. (2003). A common β_1-adrenergic receptor polymorphism (Arg389Gly) affects blood pressure response to β-blockade. *Clin Pharmacol Ther* **73**: 366–371.

Steinberg SF (1999). The molecular basis for distinct β-adrenergic receptor subtype actions in cardiomyocytes. *Circ Res* **85**: 1101–1111.

Taylor DR (2007). β-Adrenergic receptor polymorphisms: relationship

to the β-agonist controversy and clinical implications. *Expert Opin Pharmacother* **8**: 3195–3203.

Terra SG, Hamilton KK, Pauly DF, Lee CR, Patterson JH, Adams KF *et al*. (2005). β_1-Adrenergic receptor polymorphisms and left ventricular remodelling changes in response to β-blocker therapy. *Pharmacogenet Genomics* **15**: 227–234.

Trombetta IC, Batalha LT, Rondon MU, Laterza MC, Frazzatto E, Alves MJ *et al*. (2005). Gly16 + Glu27 β2-adrenoceptor polymorphisms cause increased forearm blood flow responses to mental stress and handgrip in humans. *J Appl Physiol* **98**: 787–794.

Wagoner LE, Craft LL, Singh B, Suresh DP, Zengel PW, McGuire N *et al*. (2000). Polymorphisms of the β2-adrenergic receptor determine exercise capacity in patients with heart failure. *Circ Res* **86**: 834–840.

White HL, de Boer RA, Maqbool A, Greenwood D, van Veldhuisen DJ, Cuthbert R *et al*. (2003). An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals with heart failure: a MERIT-HF sub-study. *Eur J Heart Fail* **5**: 463–468.

Xiao RP, Zhang SJ, Chakir K, Avdonin P, Zhu W, Bond RA *et al*. (2003). Enhanced G(i) signaling selectively negates β2-adrenergic receptor (AR)- but not beta1-AR-mediated positive inotropic effect in myocytes from failing rat hearts. *Circulation* **108**: 1633–1639.