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REVIEW

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β-Adrenoceptor polymorphisms

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Abstract There can be no doubt that β_1-, β_2- and β_3-adrenoceptor genes have genetic polymorphisms. Two single nucleotide polymorphisms have been described for the β_1- (Ser49Gly; Gly389Arg), three for the β_2- (Arg16Gly; Gln27Glu; Thr164Ile) and one for the β_3-adrenoceptor subtype (Trp64Arg) that might be of functional importance. The possibility that changes in expression or properties of the β-adrenoceptors due to single nucleotide polymorphisms might have phenotypic consequences influencing their cardiovascular or metabolic function or may contribute to the pathophysiology of several disorders like hypertension, congestive heart failure, asthma or obesity is an idea that has attracted much interest during the last 10 years. At present, it appears that these β-adrenoceptor polymorphisms are very likely not disease-causing genes, but might be risk factors, might modify disease and/or might influence progression of disease. The aim of this review is to provide an overview of the functional consequences of such β-adrenoceptor polymorphisms in vitro, ex vivo and in vivo.

Keywords β-Adrenoceptors · Single nucleotide polymorphism · Linkage disequilibrium · Genotype · Haplotype · Phenotype

Introduction

β-Adrenoceptors are the targets for the endogenous catecholamines noradrenaline and adrenaline. They are expressed in many cell types throughout the body and play a pivotal role in regulation of cardiac, pulmonary, vascular, endocrine and central nervous system. Three different β-adrenoceptor subtypes have been cloned so far and identified pharmacologically: β_1-, β_2- and β_3-adrenoceptors (Bylund et al. 1994). Recent studies have discovered that for all these three β-adrenoceptor subtypes genetic polymorphisms exist (summarized in Table 1). The aim of this review is to provide an overview of the functional importance of such β-adrenoceptor polymorphisms and their (possible) association with certain cardiovascular diseases.

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β_1-Adrenoceptor polymorphisms

The β_1-adrenoceptor is encoded by an intron-less gene located on chromosome 10q24-26, consisting of a short 5′-untranslated region (UTR) of 86 bp, an open reading frame that encodes a protein of 477 amino acid residues and 3′-untranslated region of about 900 bp (Frielle et al. 1987). Two major single nucleotide polymorphisms (SNPs) have been detected in the human β_1-adrenoceptor coding region: at position 49 in the amino-terminus of the receptor a serine is substituted by a glycine (Maqbool et al. 1999; Börjesson et al. 2000); the minor allele is Gly, the allele frequency of this polymorphism is about 15% and shows no differences between Caucasians and Asian people (Moore et al. 1999; Johnson and Terra 2002). Divergent results have been reported, however, for the allele frequency of the Gly49 in African Americans with one group reporting 13% (Moore et al. 1999) and another group reporting 29% (Johnson and Terra 2002). At position 389 in the proximal part of the carboxy-terminus a glycine is substituted by an arginine (Maqbool et al. 1999; Mason et al. 1999; Tesson et al. 1999); the minor allele is Gly, the allele frequency of this polymorphism is in Caucasians and Asian people about 27% (Moore et al. 1999; Johnson and Terra 2002) but 42% of the African Americans carry the Gly389 allele (Moore et al. 1999; Johnson and Terra 2002). Codon 49 and codon 389 polymorphisms are in linkage disequilibrium (LD) so that the haplotype

(particular combination of SNPs) Gly49Gly/Gly389Gly occurs very rarely, if at all (Johnson et al. 2003). Interestingly the Gly389 allele was present in the first clone of the β_1-adrenoceptor and is therefore – although being the minor allele – often considered as the "wild-type" β_1-adrenoceptor (Frielle et al. 1987). In addition, in a preliminary report Podlowski et al. (2000) found further five polymorphisms within the coding region of the β_1-adrenoceptor (Ala59Ser, Arg399Cys, His402Arg, Thr404Ala, Pro418Ala); however, these polymorphisms appear to be very rare (allele frequencies approximately 1%) and have not been studied in details.

To study the functional importance of such polymorphisms heterologous expression of the genetically engineered mutant and the "wild-type" receptor is a commonly used method (summarized in Table 2). Two groups have recently studied by this approach the Ser49Gly β_1-adrenoceptor. Rathz et al. (2002) expressed Ser49 and Gly49 β_1-adrenoceptors in Chinese hamster fibroblasts (CHW) cells (at a density of about 220 fmol/mg protein) and in HEK 293 cells (density about 500 fmol/mg protein) and found nearly identical agonist and antagonist binding affinities for both mutants; in addition both mutants did also not differ in basal and agonist-stimulated adenylyl cyclase activity. However, the Gly49 mutant was down-regulated by long-term agonist activation to a significantly greater extent than was the Ser49 mutant. Levin et al. (2002) expressed both receptor variants in HEK 293 cells to a density up to tenfold higher than in the Rathz et al. (2002) study (see above). Under these conditions the Gly49 variant demonstrated characteristic features of a constitutively active receptor: in cells expressing the Gly49 β_1-adrenoceptor basal and agonist-stimulated adenylyl cyclase activity was higher than in cells expressing the Ser49 β_1-adrenoceptor; the Gly49 β_1-adrenoceptor was more sensitive to the inhibitory effects of inverse agonists such as metoprolol and displayed increased affinities for agonists. On the other hand, also in this study, it was found, that the Gly49 mutant was down-regulated by long-term agonist activation to a significantly greater extent than was the Ser49 mutant.

Thus, both groups found that the Gly49 variant is more susceptible to agonist-induced down-regulation than the Ser49 variant; the differences between both studies regarding the functional properties (agonist- and antagonist-binding, adenylyl cyclase activation) of the Ser49Gly polymorphism are most likely explained by the differences in receptor densities.

A similar approach has been used to study in vitro the functional importance of the Gly389Arg polymorphism of the β_1-adrenoceptor (Mason et al. 1999); both variants were expressed in CHW cells (at densities between 150 and 250 fmol/mg protein). In functional studies with matched expression the Arg389 variant exhibited a slightly higher basal and a three- to fourfold higher maximal isoprenaline-induced activation of adenylyl cyclase than did the Gly389 variant. These differences were found to be due to a greater coupling of the Arg389 receptor to G_s-protein than the Gly389 receptor as assessed by GTPγS-binding and agonist competition curves in radioligand binding assays the presence and absence of guanine nucleotides (Mason et al. 1999). Thus, the Gly389Arg polymorphism of the β_1-adrenoceptor results in alterations in receptor-G_s-coupling with the functional consequence of differential activation of the effector mechanisms.

Conflicting data have been published during recent years regarding the functional ex vivo importance of the Gly389Arg "gain-of-function" β_1-adrenoceptor polymorphism (summarized in Table 3). Two recent studies have investigated the impact of the Gly389Arg polymorphism ex vivo on isolated human right atrial preparations obtained from patients undergoing coronary artery bypass grafting on the positive inotropic effects of noradrenaline. However, results were divergent: while Sandilands et al. (2003) demonstrated greater inotropic and cyclic AMP responses to noradrenaline at the Arg389 variant of the β_1-adrenoceptor versus the Gly389 variant, Molenaar et al. (2002) did not find any influence of the Gly389Arg (or the Ser49Gly) polymorphism of the β_1-adrenoceptor on the positive inotropic effect of noradrenaline. Similarly, in human right atrial preparations Sarsero et al. (2003) did not observe any differences in potency or maxima for the positive inotropic effects of CGP 12177 (via the propranolol-resistant state of the β_1-adrenoceptor, see Kaumann et al. 2001) at Ser49Gly or Gly389Arg β_1-adrenoceptor polymorphisms; furthermore, the lipolytic response to β_1-adrenoceptor stimulation in human adipocytes did not differ between the natively expressed Gly389 and Arg389 variants of the β_1-adrenoceptor (Ryden et al. 2001).

In vivo the effects of dynamic exercise on heart rate (the standard model to test in vivo the responsiveness of β_1-adrenoceptors in the human heart, for reviews see McDevitt 1989; Brodde 1991) in volunteers homozygous for the Gly389 or Arg389 β_1-adrenoceptors polymorphism have been investigated; no differences in heart rate response to bicycle exercise between the two genotypes have been found (Xie et al. 2001; Büscher et al. 2001; Sofowora et al. 2003; Liu et al. 2003). Moreover, exercise induced increase in contractility and plasma renin activity (Büscher et al. 2001) – two other well known β_1-adrenoceptor responses in humans – were also not different between Gly389 and Arg389 β_1-adrenoceptor volunteers. More detailed studies are hampered by the fact that a selective β_1-adrenoceptor agonist for human use in vivo is not available. The only "β_1-adrenoceptor agonist" available is dobutamine that is a preferentially β_1-adrenoceptor agonist but has also β_2-adrenoceptor agonistic and α-adrenergic activities (Ruffolo et al. 1981). Thus, only a very few preliminary studies with dobutamine have been published, Dishy et al. (2001a) studied, in healthy volunteers homozygous for the Arg389 (n=9) or Gly389 (n=8) polymorphism of the β_1-adrenoceptor, the effects of dobutamine-infusion (0.05–9 µg/kg/min) on heart rate and plasma renin activity, and found no differences in dobutamine-induced increases in heart rate and plasma renin activity between the two genotype groups. LaRosee et al. (2003) studied dobutamine stress echocardiography in male healthy volunteers either homozygous for Arg389 (n=18) or homozygous (n=1) or heterozygous for the Gly389 allele (n=7). Dobutamine

was infused in doses from 10–40 μg/kg/min and heart rate and contractility (assessed as fractional shortening) were determined. They found that the increase in heart rate was identical in the two genotype groups while increases in contractility was at each dose of dobutamine higher in the volunteers homozygous for Arg389 than in the volunteers with one or two Gly389 alleles. No studies are published so far on the functional role of the Ser49Gly polymorphism of the β_1-adrenoceptor in vivo.

An alternative approach to test the impact of the Gly389Arg polymorphism on β_1-adrenoceptor sensitivity could be to determine the response of heart rate and blood pressure to selective β_1-adrenoceptor antagonists. Initially in a retrospective study, O'Shaughnessy et al. (2000) failed to find, in hypertensive patients, any significant differences between the Gly389 and Arg389 allele in blood pressure and heart rate responses to chronic β_1-adrenoceptor blockade with either 50 mg atenolol or 5 mg bisoprolol. However, very recently several papers have been published that show that there might be indeed differences between the Gly389 and Arg389 variant of the β_1-adrenoceptor in heart rate and blood pressure responses to β_1-adrenoceptor selective antagonists. Sofowora et al. (2003) studied in 21 subjects homozygous for the Arg389 and 13 subjects homozygous for the Gly389 β_1-adrenoceptor resting and exercise haemodynamic responses before and 3 h after 25 mg atenolol. They found no differences in baseline haemodynamics (heart rate, blood pressure) but atenolol caused a significantly larger decrease in resting systolic and mean arterial blood pressure in volunteers homozygous for Arg389 than it did in volunteers homozygous for Gly389. On the other hand, exercise induced increase in heart rate and systolic blood pressure as well as their attenuation by the atenolol treatment did not differ between the two genotype groups of volunteers.

Similarly, Liu et al. (2003) studied exercise-induced increase in heart rate and systolic blood pressure in healthy Chinese males homozygous for Arg389 (n=8) and Gly389 (n=8) before and after three doses of metoprolol. Metoprolol caused a dose-dependent decrease in resting heart rate and systolic blood pressure that was significantly higher in volunteers homozygous for the Arg389 allele than in volunteers homozygous for the Gly389 allele. Exercise-induced maximal increase in heart rate was not different between the two genotype groups; however, in contrast to the Sofowora et al. (2003) data, this group also found significant differences in the effects of metoprolol on exercise heart rate with Arg389 showing greater metoprolol-induced inhibition than the Gly389. Metoprolol is metabolized by the genetically polymorphic cytochrome P450 2D6 (CYP2D6) enzyme that might contribute to the observed differences. However, plasma metoprolol concentrations did not differ between the two genotype groups; furthermore, at each metoprolol concentration reduction in resting heart rate and blood pressure as well as in exercise heart rate was larger in volunteers homozygous for Arg389 than in volunteers homozygous for Gly389. This strongly indicates that differences in the observed effects are not due to differences in drug metabolism.

Finally in a prospective study Johnson et al. (2003) studied in 40 hypertensive patients the impact of the Gly389Arg and Ser49Gly polymorphisms of the β_1-adrenoceptors on the antihypertensive effect of metoprolol. They found that patients homozygous for Arg389 had a significant greater reduction in 24-h and day-time diastolic blood pressure than patients with the Gly389 allele. Moreover, they also found that the Ser49Gly polymorphism might contribute to different blood pressure responses to metoprolol. Thus, patients with the haplotype Ser49Arg389/Ser49Arg389 showed a significant reduction in diastolic blood pressure whereas patients with the haplotype Ser49Gly389/Gly49Arg389 showed nearly no reduction in diastolic blood pressure. A similar tendency was also found for reduction in systolic blood pressure although this failed to reach statistical significance. On the other hand, interestingly in this study, reduction in heart rate was not at all associated with genotypes at codon 49 or 389. Plasma concentrations of metoprolol were not different between Arg389 homozygotes and Gly389 carriers; thus, the differences in the observed response between the two groups were not due to differences in drug metabolism or plasma concentrations. The data of Johnson et al. (2003) are in contrast to the data published by O'Shaughnessy et al. (2000, see above). The major difference between the two studies is drug treatment: O'Shaughnessy et al. (2000) used a fixed dose of either atenolol (50 mg) or bisoprolol (5 mg) while Johnson et al. (2003) used dose-titration of metoprolol to response (diastolic blood pressure <90 mmHg). Thus it might well be that in carriers of Gly389 allele (who are according to the data of Johnson et al. (2003) poor responders) increases in drug concentration will not change responsiveness. On the other hand, Johnson et al. (2003) also found that patients with Arg389Arg (who are good responders) did often need dose-titration to relative high doses to achieve responsiveness, which would be missed using a fixed dose. Finally, in the preliminary study of LaRosee et al. (2003) dobutamine-induced increase in contractility, that was higher in volunteers homozygous Arg389Arg than volunteers carrying one or two Gly389 alleles (see above), was significantly attenuated by metoprolol only in the volunteers homozygous for Arg389 polymorphism of the β_1-adrenoceptor, whereas metoprolol had nearly no effect on the dobutamine-induced increase in contractility in the volunteers with one or two Gly389 alleles (summarized in Table 4).

Taken together these consistent findings of a greater response to β_1-blockers in volunteers or patients homozygous for the Arg389 variant of the β_1-adrenoceptor might have important clinical implications for the treatment of patients with β_1-blockers. It is well known that blood pressure responses to treatment with a β-blocker vary widely among individuals. Some of this variability results from differences in renin levels, race, and pharmacokinetic; now differences in β_1-adrenoceptor genotype might be an additional factor. For example, it is recognized that African Americans tend to have poorer responses to β-blockers than Caucasians. Since the Arg389 allele ("the responsive allele") occurs in about 71–78% of Caucasians and Chi-

nese subjects but only in 58% of African Americans this difference in β_1-adrenoceptor genotype might be one explanation for the observed clinical different responses in these two ethnic groups.

Several studies have investigated possible associations between resting haemodynamics and the β_1-adrenoceptor polymorphisms. Humma et al. (2001) determined in 142 patients undergoing dobutamine stress echocardiography resting heart rate and blood pressure after 30 min of rest in supine position and found that patients homozygous for the Arg389 variant of the β_1-adrenoceptor had significantly higher heart rate and diastolic blood pressure than patients carrying one or two Gly389 alleles; when Caucasians alone were evaluated also systolic blood pressure was significantly higher in patients homozygous for the Arg 389 β_1-adrenoceptor polymorphism. On the other hand, polymorphisms at codon 49 of the β_1-adrenoceptor had no influence on resting haemodynamics in this study population. In contrast, Ranade et al. (2002) studied about 1400 individuals of Chinese and Japanese descent and found that subjects homozygous for the Ser49 β_1-adrenoceptor polymorphism had a significantly higher resting heart rate than subjects carrying one or two Gly49 alleles. The Gly389Arg polymorphism had no influence on resting heart rate and neither variants were associated with resting blood pressure in this study population. Bengtsson et al. (2001a) performed a case-control association study and a sibling study to determine the association of hypertension with the Gly389Arg and/or Ser49Gly polymorphisms of the β_1-adrenoceptor. In the case-control study the age- and body mass index adjusted odds ratio for hypertension was in subjects homozygous for the Arg389 allele 1.9 when compared with subjects carrying one or two Gly389 alleles. In the sibling study they found that siblings homozygous for the Arg389 variant of the β_1-adrenoceptor had significantly higher resting diastolic blood pressure and heart rate than siblings with one or two Gly389 alleles; the Ser49Gly polymorphism was not associated with heart rate or blood pressure. From these results it was concluded that individuals with Arg389Arg have an increased risk to develop hypertension (Bengtsson et al. 2001a). It should be mentioned, however, that also several studies have been published that did not find any significant influence of the Gly389Arg polymorphism of the β_1-adrenoceptor on resting haemodynamics in volunteers (Büscher et al. 2001; Xie et al. 2001; Sofowora et al. 2003; Liu et al. 2003) or hypertensives (O'Shaughnessy et al. 2000; Johnson et al. 2003); in most of these studies, however, study population was rather small (n=16–40).

Because of the great importance of β_1-adrenoceptors in the human heart to regulate heart rate and contractility (Brodde 1991; Brodde and Michel 1999) attempts have been made to find possible associations between the Ser49Gly and/or Gly389Arg polymorphisms of the β_1-adrenoceptor and heart failure. However, the studies published so far failed to find any association between the Ser49 or Gly49 alleles and heart failure (Börjesson et al. 2000) and between the Arg389 or Gly389 allele and heart failure (Tesson et al. 1999; Iwai et al. 2002; Wagoner et al. 2002; Small et al. 2002). Börjesson et al. (2000), however, found, that patients homozygous for the Gly49 variant of the β_1-adrenoceptor had a decreased 5-year mortality risk when compared with patients carrying one or two Ser49 alleles. Wagoner et al. (2002) observed that in heart failure patients homozygous for Gly389 peak $\dot{V}O_2$ during exercise (i.e. a clinically relevant measure of the capacity of the heart to increase cardiac output) was significantly lower than in patients homozygous for Arg389; patients heterozygous Gly389Arg had an intermediate level of performance. In addition, the Ser49Gly polymorphism of the β_1-adrenoceptor displayed a small contribution to exercise performance in these patients. Thus, patients with the haplotype Ser49/Gly389 exhibited the lowest peak $\dot{V}O_2$, patients with the haplotype Gly49/Arg389 the highest peak $\dot{V}O_2$. Interestingly, in this study heart failure patients homozygous for the Arg389 had a higher resting systolic blood pressure than patients heterozygous Arg389Gly or homozygous Gly389. Very recently, Small et al. (2002) assessed a possible association between the combination of the Arg389Gly polymorphisms of the β_1-adrenoceptor and deletion polymorphism of the α_{2c}-adrenoceptor (Del322-325) and heart failure. The α_{2c}-adrenoceptor is involved in the presynaptic release of catecholamines from sympathetic nerve terminals and the deletion mutant exhibited a markedly reduced agonist-response (i.e. a blunted agonist-induced inhibition of noradrenaline release; Small et al. 2000). Thus, it might be possible that subjects homozygous for the α_{2c}Del322-325 polymorphism might have an enhanced basal release of noradrenaline, and this might well contribute to the development of heart failure. In fact, Small et al. (2002) found that, in African Americans, the combination of the deletion mutant of the α_{2C}-adrenoceptor together with the Arg389 allele of the β_1-adrenoceptor, significantly increases the risk to have heart failure; the Arg389 polymorphism of the β_1-adrenoceptor alone, on the other hand, had no predictive value for heart failure (in agreement with several other studies, see above).

Moreover, in a study on more than 1500 subjects in the West of Scotland Coronary Prevention Study, White et al. (2002) did not find any association between the Ser49Gly or the Gly389Arg polymorphisms of the β_1-adrenoceptor and coronary artery disease. In a study with 66 patients Kanki et al. (2002) failed to find any association between the Ser49Gly or the Gly389Arg variants of the β_1-adrenoceptor. Finally, as mentioned above Iwai et al. (2002), studying 163 patients with dilated cardiomyopathy and 157 sex- and age-matched controls, did not find any association between the Arg389 or Gly389 allele and heart failure; however, they noted that the frequency of the occurrence of ventricular tachycardias was lower in patients carrying one or two Gly389 alleles than in patients homozygous for the Arg389 polymorphism of the β_1-adrenoceptor.

Taken together, it appears that β_1-adrenoceptor genotype dependent differences in vivo and ex vivo for agonist-induced effects are small or inconsistent. Only in patients with heart failure exercise evoked a clear-cut smaller response (peak $\dot{V}O_2$) in subjects with the Gly389 variant

than in subjects with the Arg389 variant (Wagoner et al. 2002) which might indicate that the β_1-adrenoceptor polymorphisms play a more important role in chronic heart failure where they are desensitized (Brodde 1991; Brodde and Michel 1999). On the other hand, effects for β_1-adrenoceptor antagonists quite consistently are found to be larger in Arg389Arg subjects than in subjects carrying the Gly389 allele. The reason for this discrepancy in responses of the β_1-adrenoceptor polymorphisms to agonists and antagonists is not clear at present. Furthermore, β_1-adrenoceptor polymorphisms appear not to be cause of heart failure but they may be risk factors, might modify the disease or the response to drug treatment.

β_2-Adrenoceptor polymorphisms

The human β_2-adrenoceptor, encoded by an intron-less gene, is located on chromosome 5q31-32 (Kobilka et al. 1987). In the β_2-adrenoceptor coding region, nine single base substitutions (occurring at position 46 (N-terminus; Arg16Gly), 79 (N-terminus; Gln27Glu), 100 (first transmembrane spanning region; Val34Met), 252, 491 (fourth transmembrane spanning region; Thr164Ile), 523, 1053, 1098 and 1239) have to date been identified; five of these are degenerate and are unlikely to be functionally significant. Still, three (position 46, 79 and 491) of the other four non-degenerate substitutions have demonstrable functional effects in vitro and in vivo (for review, see Liggett 1995),whereas the extremely rare Val34Met polymorphism seems not to alter receptor function (Green et al. 1995a).

Recent studies have demonstrated the presence of eight additional SNPs (–20T→C, –47T→C, –367T→C, –468C→G, –654G→A, –1343A→G, –1429T→A) within the 1.5-kb 5′-untranslated region (UTR) upstream from the ATG start codon (Scott et al. 1999). This region is believed to contain the main transcriptional regulatory activity for the β_2-adrenoceptor gene. It consists of a number of putative controlling elements including a short open reading frame for a 19 amino acid leader peptide, called the Beta Upstream Peptide (BUP) or the 5′-leader cistron (LC), that affects the β_2-adrenoceptor gene expression at a translational level, a cyclic AMP response element (CRE), two NF-IL6 sites, four AP-2 sites and a steroid binding hexamer (Parola and Kobilka 1994; Scott et al. 1999). Two of the eight 5′-UTR SNPs create and ablate restriction enzyme sites (Mspa 1 and BSu36 I, respectively; Scott et al. 1999) and were therefore intensively investigated. The first of these SNPs results from a base change –47 bp from the start codon within the BUP region which substitutes an Arg for a Cys and possibly represents a genetic basis for variable β_2-adrenoceptor expression. The second 5′-UTR SNP results from a base change (T/C) at –367 bp from the start codon within a consensus AP-2 site.

Sequence analysis of several animals (e.g. guinea pig, bovine, rhesus monkey, dog, rat, mouse, hamster) revealed five additional SNPs occurring in the β_2-adrenoceptor open reading frame in mammalians. However, these polymorphisms are degenerate and dissimilar to polymorphisms found in the human β_2-adrenoceptor (Oostendorp et al. 2002).

In vitro, phenotypic consequences of a given SNP at position 16, 27 and 164 were assessed by expressing β_2-adrenoceptor constructs in specialized cell lines, like COS-7 cells and CHW cells; in addition primary cultures of human airway smooth muscle (HASM) cells natively expressing these SNPs were studied (summarized in Table 5). By using those cell lines, it could be demonstrated that neither ligand binding nor adenylyl cyclase activity is altered either by the presence of codon 16 (Arg→Gly) or codon 27 (Gln→Glu) polymorphism in the extracellular amino terminus of the β_2-adrenoceptor (Green et al. 1994). However, transfected CHW cells as well as HASM cells expressing the Gly16 receptor genotype have shown that this adrenergic receptor variant undergoes significantly enhanced agonist promoted down-regulation compared with the wild type (Green et al. 1994, 1995b); the Arg16Gly genotype has similar patterns of agonist-induced down-regulation, implying that Arg16 seems to be a recessive allele (Liggett 1997). On the other hand the Gln27Glu polymorphism of the β_2-adrenoceptor gene seems to confer on the receptor a strong resistance towards agonist-promoted down-regulation. In the HASM cell system a 60-fold greater concentration of isoprenaline was required to down-regulate the homozygous Glu27 variant to the same extent as the homozygous Gln27 form (Green et al. 1995b). Additionally performed Western blot analysis of the β_2-adrenoceptor with the Glu27 allele revealed an altered electrophoretic mobile conformational isoform, which might affect the susceptibility of the Glu27 genotype to receptor protein degradation (Green et al. 1994). By using site-directed mutagenesis to generate and express Gly16/Glu27 double mutant receptors it could be demonstrated, however, that the Gly16 effects dominate the phenotype over Glu27 since these receptors underwent even greater agonist-promoted down-regulation than did the wild type Gln27 β_2-adrenoceptor (Green et al. 1994). On the other hand, the Arg16/Glu27 double mutant β-adrenoceptor variant was found to be completely resistant to down-regulation (Green et al. 1994).

At position 164 the amino acid Thr or Ile can be found (C491T). Studies in transfected CHW cells have shown, that the Ile164 β_2-adrenoceptor binds isoprenaline, adrenaline and noradrenaline with fourfold lower affinity than the wild type β_2-adrenoceptor variant (Green et al. 1993, 2001). Likewise, the Ile164 variant exhibits reduced basal and agonist-induced activation of the adenylyl cyclase (rightward shift in the agonist concentration-response curve), implying a diminished β_2-adrenoceptor-G protein interaction (Green et al. 1993, 2001). Consistently, in agonist-competition binding studies carried out in the absence of guanidine nucleotides the Ile164 β_2-adrenoceptor failed to display high-affinity agonist binding (Green et al. 1993).

At last, an amino acid change to Cys19 at position –47 (5′-LC-Arg19Cys) leads in transfected COS-7 cells to a consistently greater β_2-adrenoceptor expression as com-

pared with the Arg19 variant (McGraw et al. 1998). Thus, the Arg19Cys polymorphism could possibly represents a genetic basis for variable β_2-adrenoceptor expression, responsiveness or by this a predictive for phenotype variations. The second 5′-UTR SNP, which appears potentially to be important, results from a base change (T/C) at –367 bp from the start codon. It interrupts a consensus AP-2 site 7 bp downstream of an overlapping Sp-1/AP-2 site, a region also containing strong positive promoter activity. By this it possibly alters gene expression through differences in transcription factor transactivation (Parola and Kobilka 1994; Scott et al. 1999).

To assess the functional ex vivo importance of the Arg16Gly, Gln27Glu and Thr164Ile polymorphisms of the β_2-adrenoceptor isolated human lung mast (HLM) cells, HASM cells, human adipocytes and human lymphocytes natively expressing the SNPs have been studied (summarized in Table 6). For the Arg16Gly and Gln27Glu several ex vivo studies have confirmed the in vitro findings that these genotypes do not affect functional β_2-adrenoceptor properties. Thus in HASM cells Moore et al. (2000) did not find any influence of the β_2-adrenoceptor genotypes or haplotypes on basal or on isoprenaline-induced increase in cyclic AMP levels or decreases in cell stiffness. Similarly, three studies investigating isolated human circulating lymphocytes failed to find any influence of the Arg16Gly or Gln27Glu genotypes as well as haplotypes on basal or isoprenaline-induced increases in cyclic AMP levels (Lipworth et al. 1999, 2002; Bruck et al. 2003c). Slightly divergent results, on the other hand, were reported by Large et al. (1997) who found that, in isolated human adipocytes, terbutaline evoked a lipolytic response with a potency (pD_2 value) that was in carriers with one or two Gly alleles fivefold higher than in Arg16 homozygotes; maximum response to terbutaline, however, was not affected by the genotype at position 16. On the other hand, there was no influence of the Gln27Glu polymorphism of the β_2-adrenoceptor on this terbutaline response.

In contrast to the ex vivo data on functional properties of the Arg16Gly and/or Gln27Glu variants of the β_2-adrenoceptor rather conflicting data have been published on the ex vivo agonist-induced desensitization of these β_2-adrenoceptor isoforms. Whereas Chong et al. (2000) found in HLM cells that both mutant (Gly16 and Glu27) forms of the β_2-adrenoceptor were resistant to isoprenaline-induced desensitization compared to the wild type (Arg16 and Gln27), Moore et al. (2000) reported that in HASM cells β_2-adrenoceptors homozygous or heterozygous for Glu27 showed greater short- and long-term desensitization than those homozygous for Gln27, whereby in this population sample the presence of Glu27 was always associated with the presence of Gly16. Moreover, whereas Chong et al. (2000) speculated that position 27 is more important in influencing the degree of functional desensitization, Aziz et al. (1998) reported that the degree of agonist-induced β_2-adrenoceptor down-regulation in isolated human lymphocytes is rather more associated with codon-16 polymorphism, particular Gly16, than with the polymorphic allele at position 27.

The inconsistency which of these SNPs (at codon 16 or codon 27) enhances or depresses agonist promoted desensitization of the β_2-adrenoceptor ex vivo could result from the fact that those studies underestimated the degree of LD in the β_2-adrenoceptor gene where e.g. Glu27 essentially always occurs with Gly16 (Dewar et al 1997; Xie et al. 2000; Drysdale et al. 2000; Bruck et al. 2003a) and, with regard to agonist-induced desensitization, that Gly 16 dominate the phenotype over Glu27 (see above). Thus, in vitro studies using site-directed mutagenesis techniques as well as ex vivo studies assessing the phenotypic consequence of only one isolated SNP did not take into account the combined effect or potential interaction of all polymorphic loci within a gene, or even that the receptor function can be highly dependent on cell type. This holds especially true for the paradigm that the natively expressed Glu27 β_2-adrenoceptor variant is resistant to agonist-promoted down-regulation per se. However, in this context it should be mentioned that this "resistance" could exclusively be observed only for the Arg16/Glu27 β_2-adrenoceptor variant that is particularly rare in nature (present in less than 1% of the population; Martinez et al. 1997; Large et al. 1997; Hancox et al. 1998; Weir et al. 1998; D'Amato et al. 1998).

Another example, is the tight LD between the Gln27 allele within the coding region and the Cys19 allele of the 5′-LC-Arg19Cys polymorphism (Bengtsson et al. 2001b), that is, as mentioned above, thought to affect β_2-adrenoceptor gene expression.

Therefore, despite a considerable amount of research on these β_2-adrenoceptor coding SNPs ex vivo it is not surprising that some results are divergent, particularly when these are compared with isolated in vitro phenotypes.

The Thr164Ile polymorphism is rare (≈2–4% in the heterozygous state; to date no homozygous Ile164 individuals have been identified, Liggett et al. 1998) and therefore studies in non-transfected cell systems carrying the mutation have proven to be difficult. The impact of the Thr164Ile mutation on β_2-adrenoceptor function in vivo was first studied in transgenic mice, expressing the Ile164 β_2-adrenergic receptor specifically only in cardiomyocytes. This study confirmed a lower myocardial basal and isoprenaline-stimulated adenylyl cyclase activity as described above, resulting in those animals in lower resting heart rates and inotropic and lusitropic indices (Turki et al. 1996). However, it should be mentioned that this expressed Ile164 β_2-adrenoceptor construct was generated by oligonucleotide-directed site-specific mutagenesis of a wild type template. Therefore those mice possessed an artificial haplotype (Arg16Arg/Gln27Gln/Thr164Ile) not occurring in nature. The existence of the Ile164-β_2-adrenoceptor variant in humans is in fact closely associated with the existence of the Gly16 variant in combination with the Gln27 variant (Drysdale et al. 2000; Bruck et al. 2003a).

To the best of our knowledge only three ex vivo studies on the functional impact of the natively expressed Thr164Ile variant of the β_2-adrenoceptor have been published. Büscher et al. (2002) found that in lymphocytes of 3 cystic fibrosis patients carrying the Thr164Ile variant

isoprenaline-induced increase in cyclic AMP was significantly reduced when compared with lymphocytes of 4 cystic fibrosis patients carrying the wild type (Thr164Thr) β_2-adrenoceptor. A similar decreased cyclic AMP response was, however, also found by the authors in 4 cystic fibrosis patients being heterozygous Arg16Gly and Gln27Glu. Hoffstedt et al. (2001) investigated adipocytes from 236 patients, 12 of them being heterozygous Thr164Ile. They found that the potency (pD_2-value) of terbutaline induced lipolytic response – but not that of dobutamine – was about sixfold lower in Thr164Ile patients than in Thr164Thr carriers; basal as well as maximal lipolytic response, however, was not affected by β_2-adrenoceptor genotypes. And finally, very similar results were obtained by Kay et al. (2003) in isolated human lung mast cells: pD_2-value for isoprenaline – or salbutamol – evoked inhibition of IgE-mediated histamine-release was in cells from four subjects heterozygous Thr164Ile markedly lower than in cells from 120 subjects homozygous Thr164Thr; again, basal as well as maximum response to isoprenaline or salbutamol was not genotype-dependent.

The occurrence of the Gly16 form of the receptor in Caucasians (≈50% in the homozygous state) is higher than the Arg16 which has traditionally been considered as the wild type (Green et al. 1995a; Liggett 1997). Several in vivo studies have shown that basal heart rate and blood pressure are not significantly affected by the Arg16 or Gly16 β_2-adrenoceptor genotype in healthy subjects (Cockroft et al. 2000; Hoit et al. 2000; Dishy et al. 2001b; Bruck et al. 2003a; Garovic et al. 2003); only Gratze et al. (1999) found, in normotensive Austrian Caucasians, that basal mean blood pressure was higher in volunteers homozygous for Gly16Gly than in volunteers homozygous for Arg16Arg. As mentioned earlier, in vitro and ex vivo studies had shown that the functional properties of the Arg16Gly and Gln27Glu variants of the β_2-adrenoceptor did not differ from those of the wild type β_2-adrenoceptor; the existing in vivo data regarding β_2-adrenoceptor mediated changes in heart rate, contractility and blood pressure in general confirm these findings. Thus, salbutamol-infusion induced increases in heart rate were only at lower doses (0.07 and 0.14 µg salbutamol/kg/min) in volunteers homozygous Arg16Arg significantly higher than in volunteers carrying one or two Gly16 alleles; however, at the highest dose used in this study (0.21 µg salbutamol/kg/min) genotype-dependent differences had disappeared (Gratze et al. 1999). Similar, Hoit et al. (2000) and Bruck et al. (2003a) did not observe any genotype-dependent difference in the terbutaline-induced increase in heart rate (and contractility) in volunteers homozygous for Gly16Gly or Arg16Arg. However, while Bruck et al. (2003a) did not observe any influence of genotypes on terbutaline-induced increases in systolic and decreases in diastolic blood pressure, Hoit et al. (2000) observed that the terbutaline-infusion-induced increase in systolic blood pressure was higher in volunteers homozygous for the Gly16 while decreases in diastolic blood pressure were larger in volunteers homozygous for the Arg16 β_2-adrenoceptor. Gratze et al. (1999) unfortunately measured only mean arterial blood pressure and since during salbutamol-infusion heart rate and stroke index increased and total peripheral resistance fell, the mean arterial pressure remained unchanged.

One major function of the β_2-adrenoceptor is vasodilation. Thus, various studies have investigated the impact of the Arg16Gly and/or Gln27Glu polymorphisms of the β_2-adrenoceptor on vascular responsiveness. Gratze et al. (1999), studying the salbutamol-infusion-induced decrease in total peripheral resistance found that volunteers homozygous Arg16 showed a larger decrease in peripheral resistance than volunteers homozygous Gly16. Similar results were obtained by Hoit et al. (2000) who found that the terbutaline-induced increase in lower limb flow was in volunteers homozygous Arg16 significantly higher than in volunteers homozygous Gly16. On the other hand, three studies investigated isoprenaline-induced increases in forearm blood flow or dilation of hand vein and found that volunteers homozygous Gly16 exhibited larger vasodilatory responses than did volunteers homozygous Arg16 (Cockroft et al. 2000; Dishy et al. 2001b; Garovic et al. 2003). The difference between these three studies – using local infusion techniques – and the former two studies – using systemic infusion – might be that systemic infusions of salbutamol or terbutaline are associated with significant changes in heart rate and blood pressure that may induce compensatory cardiovascular reflexes that might be involved in the vasodilatory action of the β_2-adrenoceptor agonists. In contrast, local infusion does not exert any haemodynamic changes. It is worthwhile to note that Garovic et al. (2003) observed that isoprenaline-induced increase in forearm blood flow in volunteers homozygous Gly16 was strongly dependent on the generation of endothelial NO while volunteers homozygous Arg16 were much less dependent on NO-release.

The occurrence of Glu at codon 27 in the β_2-adrenoceptor gene accounts in Caucasians about 25–35% in the homozygous state (Liggett 1995; Xie et al. 2000). According to in vitro studies the Glu27 variant of the β_2-adrenoceptor exhibits a strong resistance towards agonist-promoted down-regulation by which the responsiveness in individuals with the Glu27 polymorphism should be greater than in those with the Gln27 variant (see above). Correspondingly, Cockroft et al. (2000) found that isoprenaline-induced increase in forearm blood flow and dilation of hand vein was larger in volunteers homozygous Glu27 than in volunteers homozygous Gln27. This was confirmed in the study of Dishy et al. (2001b) who observed, that isoprenaline-induced venodilation was larger in volunteers homozygous Glu27 than in volunteers homozygous Gln27. Among those studies only Dishy et al. (2001b) looked for dependence of venodilation on haplotypes. Because of the strong LD between codon 16 and codon 27 all volunteers homozygous Glu27 are homozygous Gly16, while volunteers homozygous Gln27 can be homozygous Arg16 or Gly16 or heterozygous Arg16Gly. Accordingly, Dishy et al. (2001b) found that the haplotype Gly16Gly/Glu27Glu showed the largest venodilatory response to isoprenaline, while the haplotypes Gly16Gly/Gln27Gln and Arg16Arg/Gln27Gln were similar. On the other hand, Garovic et al.

(2003) found in their study that the haplotype Gly16Gly/Gln27Gln exhibited the largest increase in forearm blood flow induced by isoprenaline, when compared with the haplotypes Gly16Gly/Glu27Glu and Arg16Arg/Gln27Gln. Thus, it remains to elucidate whether the haplotypes Gly16Gly/Glu27Glu or Gly16Gly/Gln27Gln are associated with enhanced vasodilatory responsiveness.

The impact of the β_2-adrenoceptor variants at codons 16 and 27 on agonist-induced desensitization have been investigated only in a very few studies in vivo, and data were quite controversial. Dishy et al. (2001b) studied the effects of a 2-h continuous infusion of isoprenaline into the hand vein in volunteers either homozygous wild type β_2-adrenoceptor, Gly16Gly/Gln27Gln or Gly16Gly/Glu27Glu β_2-adrenoceptor. They found that subjects homozygous Arg16Arg/Gln27Gln exhibited strong desensitization of hand vein dilation, while volunteers homozygous Gly16, irrespective of the amino acid at position 27 (Gln or Glu), were rather resistant against desensitization. In contrast, Bruck et al. (2003a) studied the effects of a two-weeks treatment of volunteers with the haplotypes Arg16Arg/Gln27Gln, Gly16Gly/Gln27Gln and Gly16Gly/Glu27Glu with 3×5 mg/day oral terbutaline on terbutaline-infusion induced increase in heart rate and contractility, a protocol that had previously been shown to desensitize cardiac β_2-adrenoceptor responses (Poller et al. 1998). In this study after the two-weeks oral treatment with terbutaline the extent in desensitization of cardiac responses to terbutaline-infusion was not different between the codon 16 or 27 genotypes. Very similar results were obtained by the same authors in human lymphocytes natively expressing the different β_2-adrenoceptor genotypes (Bruck et al. 2003c): again, the extent in β_2-adrenoceptor down-regulation following a two-weeks oral treatment with 3×5 mg/day terbutaline was not different between the codon 16 and codon 27 genotypes. These results uniformly show that the resistance against down-regulation of the Glu27 observed in the initial in vitro studies (Green et al. 1994, 1995b) does not hold true in the in vivo situation. This discrepancy between in vitro and in vivo studies is most likely explained by the fact that the original in vitro studies did not recognize the strong LD between codon 16 and 27 and created a haplotype (Arg16Arg/Glu27Glu) that was resistant against agonist-induced down-regulation but is – as we know today – nearly not natively expressed in humans (see above). Thus, neither in the study of Dishy et al. (2001b) nor in the two studies of Bruck et al. (2003a, 2003c) volunteers with the haplotype Arg16Arg/Glu27Glu existed; in contrast, nearly all volunteers homozygous Glu27 were also homozygous Gly16. The haplotype Gly16Gly/Glu27Glu, however, was also in the in vitro studies of Green et al. (1994) found to be as susceptible to agonist-induced down-regulation as was the wild type β_2-adrenoceptor (Arg16Arg/Gln27Gln). The differences between the data of Dishy et al. (Gly16 resistant against isoprenaline-induced desensitization of hand vein β_2-adrenoceptors) vs. Bruck et al. (no genotype-dependent effect of desensitization of cardiac β_2-adrenoceptors or down-regulation of lymphocyte β_2-adrenoceptors) might be due to different duration of agonist-challenge (2 h intravenously into the hand vein vs. two-weeks oral treatment), concentration of agonist (quite high concentrations of isoprenaline vs. therapeutic doses of terbutaline) or tissue (vascular tissue vs. cardiac tissue or circulating lymphocytes).

Interestingly, however, Bruck et al. (2003a, 2003c) found that volunteers homozygous for Glu27 β_2-adrenoceptors exhibited a slowed onset in desensitization of cardiac responses or in down-regulation of lymphocyte β_2-adrenoceptor density, and this occurred although volunteers carried two or one allele Gly16. Two weeks after oral terbutaline-treatment, however, both haplotype variants (Arg16Arg/Gln27Gln vs. Gly16Gly/Glu27Glu) showed equivalent desensitization in cardiac β_2-adrenoceptor responsiveness, leading to the assumption that Gly16 might dominate the phenotype with ongoing agonist-treatment. The mechanism underlying this initial "resistance" of the Glu27 β_2-adrenoceptor variant against agonist-induced desensitization remains to be elucidated.

The Thr164Ile variant of the β_2-adrenoceptor occurs only rarely and is found only in the heterozygous form; moreover, the majority of volunteers carrying the Thr164Ile polymorphisms carry at position 16 a Gly and at codon 27 a Gln (see above). Brodde et al. (2001) studied in 6 volunteers heterozygous for the Thr164Ile variant the effects of terbutaline-infusion on heart rate and contractility in comparison with 12 volunteers with the wild type β_2-adrenoceptor. Increases in heart rate and contractility were in the Thr164Ile volunteers significantly blunted vs. those in wild type β_2-adrenoceptor volunteers. Preliminary data from Barbato et al. (2002) confirmed these results: they also found in subjects heterozygous for the Thr164Ile β_2-adrenoceptor a blunted increase in heart rate and contractility vs. wild type β_2-adrenoceptor subjects. Bruck et al. (2003b) very recently studied the effects of two-weeks oral treatment with 3×5 mg/day terbutaline on terbutaline-infusion induced increases in heart rate and contractility in 6 volunteers heterozygous Thr164Ile and in 10 wild type β_2-adrenoceptor volunteers. As to be expected, wild type as well as Thr164Ile β_2-adrenoceptors were desensitized after the two-weeks oral terbutaline-treatment; interestingly however, after the two-weeks treatment cardiac β_2-adrenoceptor responses did not differ anymore between wild type and Thr164Ile volunteers indicating that either maximum in terbutaline desensitization was obtained or the Thr164Ile (that is already somewhat desensitized, see above) appears to be somewhat protected agonist agonist-induced desensitization. In this context it is interesting to note, that Barbato et al. (2002) in their preliminary study observed that in CHF-patients where β_2-adrenoceptors are somewhat desensitized (uncoupled from the adenylyl cyclase system, for references see Brodde and Michel 1999) terbutaline-induced increases in heart rate, but *not* in contractility, were not different in patients with the Thr164Thr or the Thr164Ile variant of the β_2-adrenoceptor. On the other hand, Wagoner et al. (2000) assessed in CHF-patients either heterozygous Thr164Ile or homozygous Thr164Thr exercise capacity and found that patients with the Thr164Ile variant of the β_2-adrenoceptor

had a lower peak $\dot{V}O_2$ than patients homozygous Thr164Thr. Moreover, Liggett et al. (1998) genotyped 259 patients with CHF due to ischemic or dilated cardiomyopathy and found that the allele frequencies for the Arg16Gly, Gln27Glu and Thr164Ile polymorphisms of the β_2-adrenoceptor did not differ with those assessed in 212 healthy controls. However, those patients carrying the Thr164Ile polymorphism had much more rapid progression to transplantation or death. Unfortunately because of the rare existence of the Thr164Ile β_2-adrenoceptor variant in the human population, these findings were based only on 10 patients with this genotype, and it would be highly desirable to confirm these findings in a larger population of CHF-patients. Over-expression of the Ile164 β_2-adrenoceptor variant in mice, on the other hand, had no deleterious effects per se (Turki et al. 1996), suggesting that this β_2-adrenoceptor polymorphism is unlikely to be a major causative factor in the development of heart failure but perhaps modify disease progression (summarized in Table 7).

Again, one reason for the inconclusive in vivo findings mentioned above might be the fact that also those studies were not designed to investigate volunteers either homozygous for all SNPs or heterozygous at only one single position to maximize the chance of observing effects. However, such an analysis is inevitably complicated by the strong LD between SNPs which results in the occurrence of several common haplotypes resulting in multilocus effects. Furthermore, Drysdale et al. (2000) noted a deep divergence in the distribution of some haplotypes in Caucasian, African-American, Asian and Hispanic-Latino ethnic groups. With regard to those limitations mentioned above it is most unlikely that genetic-epidemiological data alone give details in relevant functional alterations of polymorphic β_2-adrenoceptors. It is therefore not surprising that investigations of single genotypes in different ethnic groups with regard to disease susceptibility resulted, for example, either in an association of the Gly16 variant with hypertension in African Caribbeans (Kotanko et al. 1997) *or* in an association of the alternate allele Arg16 with increased blood pressure values in the Bergen Blood Pressure Study (Timmermann et al. 1998) *or* in no association either for Arg16 or Gly16 with hypertension in a Japanese population (Kato et al. 2001) or black and white Americans (Hermann et al. 2000; Xie et al. 2000). Similar interethnic differences were obtained for the Gln27Glu β_2-adrenoceptor polymorphism. Whereas in Caucasians, the allelic frequency of Gln27 and Glu27 are both close to 50% (Dewar et al. 1998), the prevalence of the Glu27 allele is significantly lower in Turks (about 32%; Aynacioglu et al. 1999), Hispanics (about 27%; Martinez et al. 1997), African Americans (about 14%; Xie et al. 2000) and even substantially lower (about 3–7%) in Black South Africans (Candy et al. 2000). Consequently, it should be considered that differences in population frequencies of a given gene variant may produce inconsistent claims for an association, probably by ethnic variability of the variant.

A combined linkage-association analysis of the β_2-adrenoceptor gene in German twins, investigating the Arg16Gly, Gln27Glu and Thr164Ile variants in the coding and the Arg-19Cys in the promoter region, however, indeed conferred that the Arg16Gly polymorphism seems to be responsible for the effects on blood pressure and heart size rather than the Gln27Glu polymorphism, whereby the Arg16 variant seems to be associated with increased blood pressure values and a higher risk to develop hypertension in white subjects (Busjahn et al. 2000). Correspondingly, in a similar designed study investigating the association between the β_2-adrenoceptor polymorphisms with hypertension in patients with or without Type II diabetes and normotensive controls, Bengtsson et al. (2001b) showed that subjects carrying at least one copy of the Arg16 allele had a higher systolic blood pressure whereas homozygous carriers of the Arg16 allele had already an increased odds ratio for hypertension.

In contrast, Bray et al. (2000), who studied sib-pairs from 55 pedigrees and about 2500 additional subjects from 589 families, found that the risk for hypertension was greater for those subjects carrying the Gly16 and Glu27 alleles. However, β_2-adrenoceptor polymorphisms were responsible for only 2% of total variation in blood pressure. On the other hand, Candy et al. (2000) did not find any association between β_2-adrenoceptor genotypes at either codon 16 or codon 27 and hypertension in Black South Africans.

Recently, Heckbert et al. (2003) studied in more than 5000 elder patients a possible relationship between the Arg16Gly and Gln27Glu polymorphisms of the β_2-adrenoceptor and the risk of cardiovascular events. They found that carriers of the Glu27 allele had a lower risk of coronary events than Gln27 homozygotes; a similar tendency was also observed for carriers of the Gly16 allele – as to be expected from the LD between codon 16 and codon 27. On the other hand, there was no association of the β_2-adrenoceptor genotypes with ischemic stroke or combined cardiovascular events.

β_2-Adrenoceptor agonists are widely used as bronchodilator drugs in the treatment of asthma. Hence, numerous studies have investigated possible associations between β_2-adrenoceptor polymorphisms and the diagnosis of asthma, disease severity and response to β_2-adrenoceptor agonists. Most studies have shown no or weak association between the β_2-adrenoceptor polymorphisms and the presence of asthma (for recent review, see Fenech and Hall 2001; Joos and Sandford 2002); however, they might modify the progress and severity of disease. Thus, a number of studies suggested that the Gly16 allele might be associated with a greater-than-expected frequency of steroid-dependent (Reishaus et al. 1993) and nocturnal asthma (Turki et al. 1995), severity of asthma (Weir et al. 1998) and, in children, with a decreased bronchodilator response to inhaled β_2-adrenoceptor agonists (Martinez et al. 1997). In contrast, Summerhill et al. (2000) found in a large study in asthma and related phenotypes that no phenotype was associated with the Arg16Gly or Gln27Glu variants of the β_2-adrenoceptor, however, patients homozygous Arg16 exhibited a small, but significant reduction in lung function. In addition, Hancox et al. (1998) did not find any association between Gly16 and any increased deterioration of asthma when compared with patients carrying the Arg16Arg allele.

The Glu27 variant of the β_2-adrenoceptor has been reported to be associated with less airway reactivity (Hall et al. 1995; Hopes et al. 1998) but seems not to influence nocturnal asthma (Turki et al. 1995) or bronchodilator responsiveness (Martinez et al. 1997). Consistent with a somewhat protective role of the Glu27 SNP are the findings of D'Amato et al. (1998) who showed that patients with the haplotype Gly16Gly/Gln27Gln exhibited increased bronchial hyperresponsiveness. In contrast to these reports, Ulbrecht et al. (2000) recently reported that the Gly16Gly/Gln27Gln/Thr164/Thr was protective against bronchial hyperresponsiveness. No clinical data regarding asthma are available for the (very rare occurring) Thr164Ile polymorphism of the β_2-adrenoceptor.

Taking together clinical data on the impact of β_2-adrenoceptor polymorphisms on bronchodilator therapy on asthma are still rather controversial. Thus studies with a large number of patients are needed and haplotypes should taken into consideration. In fact, a recent study by Drysdale et al. (2000) has shown that at least responsiveness to β_2-adrenoceptor bronchodilators might be associated with haplotypes.

Additionally, current studies lead to the suggestion that the Glu27 variant might be associated with higher indices of obesity, higher body fat, larger fat cell volume and higher fasting insulin levels when compared with the Gln27 allele (Large et al. 1997). However, although the Glu27 variant has been associated with obesity (Large et al. 1997; Hellstrom et al. 1999; Mori et al. 1999) and Type II diabetes (Ishiyama-Shigemoto et al. 1999; Yamada et al. 1999), the findings have not been replicated in all studies (Kortner et al. 1999; Echwald et al. 1998). In fact Carlsson et al. (2001) observed a more frequent prevalence of diabetes in subjects with the Gln27 variant. Moreover, the vulnerable Gln27 allele has been associated with elevated IgE levels in the serum of asthmatics (Dewar et al. 1997). Deichmann et al. (1999), on the other hand, were not able to find any association between the polymorphisms at codon 16 and codon 27 of the β_2-adrenoceptor gene with elevated total IgE levels or enhanced IgE responsiveness in an atopic population.

Taken together, the possibility that changes in expression or properties of the β_2-adrenoceptor due to SNPs influence cardiovascular function or may contribute to the pathophysiology of several disorders including hypertension, obesity and Type II diabetes, congestive heart failure or asthma, is an idea that has attracted much interest during the last 10 years. Nevertheless, whether the β_2-adrenoceptor polymorphisms play a role for disease susceptibility (as disease-causing gene), progression (as disease-modifying gene) or variations in drug response (as treatment response gene) still remains to be elucidated.

β_3-Adrenoceptor polymorphism

The human β_3-adrenoceptor gene, located on chromosome 8p11.1-8p12, consists, in contrast to the β_1- and β_2-adrenoceptor, of a large exon of 1.4 kb encoding the first 402 amino acid residues, an intron and a second exon of 0.7 kb containing the coding sequence for the six carboxy-terminal residues of the receptor and the entire mRNA 3′-UTR (Emorine et al. 1989). The β_3-adrenoceptor, is primarily expressed in white and brown adipose tissue, where it mainly regulates noradrenaline-induced changes in lipolysis and thermogenesis (Arch et al. 1984; Wilson et al. 1984; Emorine et al. 1989; Hollenga and Zaagsma 1989). Further attempts to detect β_3-adrenoceptor mRNA or protein in additional tissues by using Northern and Western blot analysis, reverse-transcription PCR and RNase protection assays have proven to be difficult and resulted in conflicting data. While Krief et al. (1993) detected β_3-adrenoceptor mRNA in subcutaneous, femoral and mesenteric adipose tissue as well as in smooth muscle cells form the gall bladder, small intestine, stomach, colon and prostate, Thomas and Liggett (1993) failed to detect a β_3-adrenoceptor signal in such samples obtained from adult humans. In the case of right atrium or the left ventricle Berkowitz et al. (1995), Krief et al. (1993) as well as Evans et al. (1996) speculated that the weak and variable mRNA signals seen in their samples seemed be more likely originated in attached brown adipocytes leading to false-positive signals. On the other hand, Gauthier et al. (1996) described pharmacologically and on the mRNA level the existence of a β_3-adrenoceptor coupled to the inhibitory G protein (G_i) in the human ventricle, whereas Chamberlain et al. (1999) were not able to immunologically detect the β_3-adrenoceptor protein in isolated myocardial cells obtained from ventricle at all, but they detected the protein in isolated myocardial cells obtained from right atria and skeletal muscle.

Evidence for the physiological role of the β_3-adrenoceptor in modulating noradrenaline-induced changes in energy metabolism and thermogenesis in adipose tissue was first established in rodents, but in the meantime it is also well documented in humans (Lönnqvist et al. 1993; Guillaume et al. 1994; Portillo et al. 1995; Zilberfarb et al. 1997; Chamberlain et al. 1999). From those studies it is known that the β_3-adrenoceptor in white and brown adipocytes is coupled to the stimulatory G protein (G_s) and adenylyl cyclase. The β-adrenergic stimulation by noradrenaline raises the intracellular levels of cyclic AMP and leads to the activation of the hormone-sensitive lipoprotein lipase that is responsible for lipolysis of triglycerides (for review see Strosberg 1997). A decrease in the expression of the β_3-adrenoceptor in adipose tissue could therefore contribute to the obese insulin-resistant phenotype observed in (ob/ob) mice (Collins et al. 1994) and (fa/fa) Zucker rats (Charon et al. 1995a), whereas, on the other hand, treatment with β_3-adrenoceptor agonists may reduce diet-induced and genetically determined obesity (Susulic et al. 1995; Charon et al. 1995b) and may also have anti-diabetic effects (Kiso et al.1999). Additionally, several reports have discussed a potential role of the β_3-adrenoceptor in regulating intestinal motility by causing vasorelaxation (Anthony et al. 1998), peripheral vasodilation primarily in skin and adipose tissue (Shen et al. 1994) and the relaxation of airway smooth muscle (Martin and Advenier 1995).

In 1995 simultaneously three papers appeared describing one major SNP in the β_3-adrenoceptor gene: at codon 64 at the beginning of the first intracellular loop, a tryptophan is substituted by an arginine (Trp64Arg β_3-adrenoceptor). These papers described that homozygotes for the Arg64 mutant exhibited abdominal obesity and resistance to insulin, increased capacity to gain weight and a lower age of onset of non-insulin-dependent diabetes mellitus (NIDDM) and a lower resting metabolic rate (Clément et al. 1995; Walston et al. 1995; Widén et al. 1995). Thereafter, it rapidly became apparent that the Arg64 genotype is present in nearly all populations of the world; allele frequency, however, varied from as high as 30% in Pima Indians, 19% in Japanese to as low as 4.7% in French (for review see Strosberg 1997). It should be emphasized that these studies have been mainly focused on obese subjects; since there might be a link between the Arg64 genotype and obesity, allele frequencies of this genotype in a large, not obese population might be in fact lower. Furthermore, Carlsson et al. (2001) found a strong linkage between the occurrence of the Gln27Glu β_2-adrenoceptor variant and the Trp64Arg β_3-adrenoceptor variant. Genotyping of 1054 non-diabetic and diabetic Caucasians revealed that subjects who were homozygous Glu27Glu (β_2-adrenoceptor) were either heterozygous or homozygous Trp at position 64 in the β_3-adrenoceptor gene but *not* homozygous for Arg64Arg.

To date, five studies have directly assessed the functional properties of the Trp64Arg β_3-adrenoceptor variants in vitro (summarized in Table 8). Whereas Candelore et al. (1996) found no significant genotype-dependent differences in agonist binding properties or adenylyl cyclase activation between CHO cells expressing the Trp64 or the Arg64 β_3-adrenoceptor, Piétri-Rouxel et al. (1997) demonstrated that maximal cyclic AMP accumulation in response to various β-adrenoceptor agonists of the Arg64 β_3-adrenoceptor variant was lowered in two different cell lines. Similarly, Kimura et al. (2000) found in mouse 3T3-L1 preadipocytes stably expressing the Arg64 β_3-adrenoceptor variant significantly lower intrinsic activities for isoprenaline, adrenaline and the specific and human-selective β_3-adrenoceptor agonist L-755,507. Moreover, Perfetti et al. (2001) demonstrated that cultured rat β-cells expressing the Arg64 β_3-adrenoceptor variant secreted less insulin, basal and after exposure to the human-selective β_3-adrenoceptor agonist CL316 243-5 and to the rat-selective β_3-adrenoceptor agonist BRL37 344. Recently, rather conflicting results have been published by Isogaya et al. (2002), who compared the biochemical and pharmacological characteristics of the mutant Arg64 β_3-adrenoceptor variant to those of the Trp64 β_3-adrenoceptor variant in COS-7 cells co-expressing adenylyl cyclase III, that adenylyl cyclase isoform that is selectively increased in brown adipocytes due to stimulation of the sympathetic nervous system (Granneman 1995). Under these condition, when adenylyl cyclase III was co-expressed with the Arg64 β_3-adrenoceptor variant, CGP12177A- and isoprenaline-stimulated cyclic AMP response were, on the contrary to the mentioned observations above, significantly enhanced as compared to cyclic AMP response in the cells expressing the Trp64 β_3-adrenoceptor variant and adenylyl cyclase III. Interestingly, in COS-7 cells lacking adenylyl cyclase III, basal and CGP12177A-stimulated cyclic AMP response of the Arg64 β_3-adrenoceptor variant were again almost the same as those of the Trp64 β_3-adrenoceptor variant. Therefore, according to these conflicting results, it still remains to be determined whether the Arg64 β_3-adrenoceptor variant has a decreased, increased or unchanged ability to induce the cAMP response.

Ex vivo, in adipocytes obtained from subjects heterozygous for the Trp64Arg mutant or homozygous Trp64Trp no genotype-dependent changes in the lipolytic response to noradrenaline or CGP 12177 could be found (Li et al. 1996). On the other hand, Hoffstedt et al. (1999) found in visceral adipocytes of subjects homozygous for the Arg64Arg polymorphism of the β_3-adrenoceptor a tenfold lower sensitivity for the lipolytic response to the β_3-adrenoceptor agonist CGP 12177 compared with adipocytes from subjects homozygous Trp64Trp. Umekawa et al. (1999), on the other hand, found in omental adipocytes obtained from subjects who were homozygous for the Arg64Arg polymorphism no differences in the potency and responsiveness of isoprenaline or CGP12177 to induce lipolysis, but they found that the potency of the specific and human-selective β_3-adrenoceptor agonist L755,507 was significantly lowered. Therefore, according to these conflicting results, certainly more studies have to be done to clarify whether or not the Trp64Arg polymorphism of the β_3-adrenoceptor has any functional importance (summarized in Table 9).

To investigate the functional impact of the Trp64Arg β_3-adrenoceptor variants in vivo several groups investigated whether they affect serum lipids and lipoproteins, insulin sensitivity and energy metabolism. Whereas Snitker et al. (1997) found in Pima Indians after local isoprenaline-infusion into the subcutaneous adipose tissue of the abdomen no genotype-dependent differences in basal and agonist-induced increase in lipolysis, Melis et al. (2002) reported that normotensive and normolipaemic non-diabetic subjects heterozygous for the Trp64Arg polymorphism showed a significant increase in plasma triacylglycerol and non-esterified fatty acids levels as well as a higher increase in diastolic blood pressure after low-dose noradrenaline-infusion than subjects homozygous for Trp64Trp.

Additionally, several groups studied the influence of the Trp64Arg polymorphism of the β_3-adrenoceptor on glucose tolerance and insulin-sensitivity. However, the results are rather conflicting. Whereas Christiansen et al. (1999) found higher fasting glucose and lower fasting insulin levels in elderly (aged 65–74 years) healthy dizygotic twins heterozygous for the Trp64Arg β_3-adrenoceptor polymorphism, neither Urhammer et al. (1996) by investigating young (aged 18–32 years) healthy individuals nor Janssen et al. (1998) by investigating elderly (aged 55–75 years) patients with type II diabetes mellitus, found a genotype-dependent difference in the fasting plasma glucose and insulin level. On the other hand, Urhammer et al. (1996) found higher fasting serum levels of total cho-

lesterol, LDL-cholesterol and triglycerides in carriers of the Trp64Arg polymorphism. Furthermore, whereas two groups found no genotype-dependent differences in plasma glucose and serum insulin levels after ingestion of glucose (Janssen et al. 1998; Pihlajamäki et al. 1998), Widén et al. (1995), by investigating patients with NIDDM, and Urhammer et al. (1996) found higher blood glucose and insulin levels in subjects heterozygous for the Trp64Arg β_3-adrenoceptor polymorphism. Finally, by investigating the activity of the autonomic nervous system (ANS) during supine rest and postural change to standing, Shihara et al. (1999, 2001) showed that young healthy subjects homozygous Arg64Arg exhibited a lower resting ANS activity but a higher response in para- and sympathetic nervous system activity than subjects carrying the Trp64Trp β_3-adrenoceptor (summarized in Table 10).

Since the initial observation that the Trp64Arg polymorphism of the β_3-adrenoceptor is associated with weight gain, insulin resistance and earlier time of onset of NIDDM (Clément et al. 1995; Walston et al. 1995; Widén et al. 1995), numerous studies have been performed on a possible association between the Trp64Arg polymorphism of the β_3-adrenoceptor and obesity and associated metabolic disorders; these studies were mostly performed in obese patients or in patients with NIDDM. Results were very divergent: about 50% of the studies found an association between the β_3-adrenoceptor mutant and obesity or NIDDM, while the other 50% of studies failed to do so (for reviews see Strosberg 1997; Arner and Hoffstedt 1999). Furthermore, two meta-analyses examining the effects of the Trp64Arg mutant of the β_3-adrenoceptor on body mass index (BMI) have been published: one analysis included a total of 9238 subjects and found significant BMI differences between carriers and non-carriers of the Arg64 allele (Fujisawa et al. 1998); the second analysis included 7399 subjects and found no association (Allison et al. 1998).

The presence and function of β_3-adrenoeptors in the human heart is still a matter of debate (for recent reviews see Kaumann and Molenaar 1997; Brodde and Michel 1999; Gauthier et al. 2000). Nevertheless in the past few years some data have been published about the association of the β_3-adrenoceptor polymorphism with cardiovascular diseases like hypertension and coronary artery disease – which are diseases that often accompany the metabolic syndrome. Besides one study investigating 494 Sardinian, non-diabetic subjects (43.2% with essential hypertension), which showed a significantly higher frequency of the Trp64Arg variant in hypertensives (13.6%) than in normotensives (6.8%) (Tonolo et al. 1999) and a Japanese study showing a higher frequency of the mutant in a small number of only 83 coronary artery disease patients versus 107 control subjects (Higashi et al. 1997) all other studies failed to find a higher allelic frequency of the Trp64Arg β_3-adrenoceptor variant in hypertensive patients (Ikegami et al. 1996; Fujisawa et al. 1997; Baba et al. 1998, Thomas et al. 2000) or patients with coronary heart disease (Pulkkinen et al. 1999; Sheu et al. 1999; Stangl et al. 2001; Tamaki et al. 1999). It should be mentioned that in some studies that showed an association between the β_3-adrenoceptor variant and arterial blood pressure, an association with other known independent risk factors for the development of hypertension has been described: higher serum lipid levels (cholesterol and triglycerides), obesity markers (BMI and body weight), serum uric acid or leptin concentrations (Tonolo et al. 1999; Strazzullo et al. 2001; Kurabayashi et al. 1996; Chen et al. 2001; Inukai et al. 2001). Thus, it cannot be differentiated in these patients whether the link between the Arg64 allele of the β_3-adrenoceptor and arterial blood pressure is secondarily to the presence of metabolic syndrome-related risk factors (which might be linked to the Arg64 allele, see above) or to the presence of the allele itself.

Taken together, it appears that the Trp64Arg polymorphism of the β_3-adrenoceptor might affect functional responsiveness in vitro, ex vivo and in vivo and might, by this, contribute to an accelerated onset of metabolic disorders.

Conclusion

There can be no doubt that β_1-, β_2- and β_3-adrenoceptor genes have genetic polymorphisms that are of functional importance. This has been studied so far mainly only with single SNPs for each β-adrenoceptor but evidence is emerging that studies of certain haplotypes (particular combinations of SNPs within one gene) or even "functional haplotypes" (particular combinations of SNPs within and between those three genes) might be a better tool to study the functional role and the interaction of the polymorphic β-adrenoceptors. Future studies will help to better understand the associations between these polymorphisms (haplotypes/"functional haplotypes") and certain diseases. At present, it appears that these β-adrenoceptor polymorphisms are very likely not disease-causing genes, but might be risk factors, might modify disease and/or might influence progression of disease. In addition, only very few studies have investigated the impact of the genetic profile of the β-adrenoceptor subtypes on drug responses although genetic variations in receptor genes might be one explanation for the well known interindividual variations in drug responses in the human population.

Appendix

Tables 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

Table 1 Single nucleotide polymorphisms in the three β-adrenoceptor (AR) subtypes. (*SNP* single nucleotide polymorphism, *AA* amino acid, *UTR* untranslated region, *BUP* beta-upstream peptide)

Position	SNP	AA-Position	AA-Substitution	Putative regulatory site
β_1-Adrenoceptor				
145	A/G	49	Ser→Gly	N-Terminus
175	G/T	59	Ala→Ser	N-Terminus
1165	G/C	389	Gly→Arg	C-Terminus
1195	C/T	399	Arg→Cys	C-Terminus
1205	A/G	402	His→Arg	C-Terminus
1210	A/G	404	Thr→Ala	C-Terminus
1252	C/G	418	Pro→Ala	C-Terminus
β_2-Adrenoceptor				
–20	T/C	–	–	5′-UTR
–47	T/C	–19	Arg→Cys	BUP in 5′-UTR
–367	T/C	–	–	7 bp 3′ of an AP2/SP1 site in 5′-UTR
–468	C/G	–	–	5′-UTR
–654	G/A	–	–	p53 site in 5′-UTR
–1023	G/A	–	–	2 bp 3′ of a NF-IL6 site in 5′-UTR
–1343	A/G	–	–	5′-UTR
–1429	T/A	–	–	3 bp 3′ of steroid binding hexamer
46	A/G	16	Arg→Gly	N-Terminus
79	C/G	27	Gln→Glu	N-Terminus
100	G/A	34	Val→Met	1. TMD
252	A/G	84	Leu	2. TMD
491	C/T	164	Thr→Ile	4. TMD
523	A/C	175	Arg	4. TMD
1053	C/G	351	Gly	C-Terminus
1098	C/T	366	Tyr	C-Terminus
1170	C/T	390	His	C-Terminus
1179	C/T	393	Thr	C-Terminus
1239	C/G/T	413	Leu	C-Terminus
β_3-Adrenoceptor				
827	T/G	64	Trp→Arg	1. Intracellular Loop
1856	G/T		–	Intron-Region
3139	G/C		–	3′-UTR

Table 2 In vitro β_1-AR phenotypes (*ISO* isoprenaline, *AC* adenylyl cyclase, G_s stimulatory G protein)

SNP	Cell	In vitro phenotype	Reference
Ser49Gly	CHW	No effects on agonist binding affinities No effect on basal/maximal ISO Enhanced agonist	Rathz et al. 2002
	HEK293	Increased agonist binding affinities Higher basal/maximal ISO-stimulated AC activity Enhanced agonist-induced down-regulation	Levin et al. 2002
Gly389Arg	CHW	Higher basal and three- to fourfold higher maximal ISO-stimulated AC activity Greater coupling to G_s protein	Mason et al. 1999

Table 3 Ex vivo β_1-AR phenotypes. (*NA* noradrenaline, *cAMP* cyclic AMP)

SNP	Cell/tissue	Ex vivo phenotype	Reference
Ser49Gly	Isolated right atria	No difference in NA-induced increase in contractility	Molenaar et al. 2002
	Isolated right atria	No difference in potency or maximum of CGP12177-induced increase in contractility	Sarsero et al. 2003
Gly389Arg	Isolated right atria	No difference in NA-induced increase in contractility	Molenaar et al. 2002
	Isolated right atria	Greater NA-induced increase in contractility and cAMP response	Sandilands et al. 2003
	Isolated right atria	No difference in potency or maximum of CGP12177-induced increase in contractility	Sarsero et al. 2003
	Adipocytes	No difference in agonist-stimulated lipolysis	Ryden et al. 2001

Table 4 In vivo β_1-AR phenotypes

SNP	Method	In vivo phenotype	Reference
Gly389Arg	Bicycle exercise	No differences in heart rate response	Xie et al. 2001
		No differences in heart rate response, contractility and plasma renin activity	Büscher et al. 2001
		No differences in heart rate response and systolic blood pressure	Sofowora et al. 2003
		No difference in maximal increase in heart rate	Liu et al. 2003
	Bicycle exercise/atenolol	Larger decrease in resting systolic and mean arterial blood pressure, no difference in atenolol-induced attenuation of exercise-induced increase in heart rate and systolic blood pressure	Sofowora et al. 2003
	Bicycle exercise/metoprolol	Higher decrease in resting heart rate and systolic blood pressure, greater metoprolol-induced inhibition of exercise-induced increase in heart rate	Liu et al. 2003
	Dobutamine-infusion	No differences in heart rate response and plasma renin activity	Dishy et al. 2001a
		No differences in heart rate response but higher increase in contractility	LaRosee et al. 2003

Table 5 In vitro β_2-AR phenotypes

SNP	Cell	In vitro phenotype	Reference
Arg-19Cys	COS-7	Increase in β_2-AR density	McGraw et al. 1998
Arg16Gly	CHW-1102	No effects on agonist binding affinities	Green et al. 1994
		No effect on basal/maximal ISO	
		Enhanced agonist	
Gln27Glu	CHW-1102	No effects on agonist binding affinities	
		No effect on basal/maximal ISO	
		Resistance to agonist	
Thr164Ile	CHW-1102	Lower agonist binding affinities	Green et al. 1993
		Reduced basal/maximal ISO-stimulated AC activity, lower maximal agonist-induced sequestration	
Arg16/Gln27		No effects on agonist binding affinities	Green et al. 1994
		No effect on basal/maximal ISO-stimulated AC activity	
Arg16/Glu27		No effects on agonist binding affinities	
		No effect on basal/maximal ISO	
		Resistance to agonist	
Gly16/Gln27		No effects on agonist binding affinities	
		No effect on basal/maximal ISO	
		Enhanced agonist	
Gly16/Glu27	CHW-1102	No effects on agonist binding affinities	
		No effects on basal/maximal ISO-stimulated AC activity	
		Enhanced agonist-induced down-regulation	

Table 6 Ex vivo β_2-AR phenotypes (*HASM* human airway smooth muscle *TER* terbutaline, *SALB* salbutamol, *HLM* human lung mast cells)

SNP	Cell/tissue	Ex vivo phenotype	Reference
Arg-19Cys	HASM	Increase in receptor density	McGraw et al. 1998
	Lymphocytes	No effect on receptor expression	Lipworth et al. 2002
T/C-367	Lymphocytes	No effect on receptor expression	
Arg16Gly	Lymphocytes	No effect on receptor expression No effect on basal/maximal AC activity	Lipworth et al. 1999
	Lymphocytes	No effect on basal/maximal AC activity	Bruck et al. 2003c
	HASM	No effect on basal/maximal AC activity	Moore et al. 2000
	Adipocytes	Higher potency of TER in inducing lipolysis No effect on basal/maximal lipolysis	Large et al. 1997
	HLM	Resistance to agonist-induced down-regulation	Chong et al. 2000
	Lymphocytes	Greater agonist-induced down-regulation	Aziz et al. 1998
Gln27Glu	Lymphocytes	No effect on receptor expression No effect on basal/maximal AC activity	Lipworth et al. 1999
	Lymphocytes	No effect on basal/maximal AC activity	Bruck et al. 2003c
	HASM	Greater short- and long-term desensitization	Moore et al. 2000
	HLM	Resistance to agonist-induced desensitization	Chong et al. 2000
Val34Met	HASM	No effect on receptor expression No effect on basal/maximal AC activity	Green et al. 1995b
Thr164Ile	Lymphocytes*	Decreased maximal cAMP formation	Büscher et al. 2002
	Adipocytes	Lower potency of TER in inducing lipolysis No effect on basal/maximal lipolysis	Hoffstedt et al. 2001
	HLM	Lower potency of SALB in inducing inhibition of IgE-mediated histamine release No effect on basal/maximal histamine release	Kay et al. 2003

*From patients with cystic fibrosis

Table 7 In vivo β_2-AR phenotype. (*CHF* chronic heart failure, $\dot{V}O_2$ maximum pulmonary O_2 uptake rate)

SNP	Method	In vivo phenotype	Reference
Cardiac response			
Arg16Gly	SALB-induced increase in heart rate	Lower responsiveness	Gratze et al. 1999*
	TER-induced increase in heart rate	No differences in responsiveness	Hoit et al. 2000**
	TER-induced increase in heart rate and contractility	No differences in responsiveness	Bruck et al. 2003c**
Gln27Glu	TER-induced increase in heart rate and contractility	No differences in responsiveness	
Thr164Ile	TER-induced increase in heart rate and contractility	Blunted responsiveness	Brodde et al. 2001
	TER-induced increase in contractility	Blunted responsiveness	Barbato et al. 2002
Vascular response			
Arg16Gly	SALB-induced decrease in total peripheral resistance	Lower responsiveness	Gratze et al. 1999*
	TER-Induced increase in lower limb blood flow	Lower responsiveness	Hoit et al. 2000**
	ISO-induced increase in forearm blood flow	Higher responsiveness	Cockroft et al. 2000**
	ISO-induced dilation of hand vein	Higher responsiveness	
	ISO-induced dilation of hand vein	No differences in responsiveness	Dishy et al. 2001b**
	ISO-induced increase in forearm blood flow	Higher responsiveness	Garovic et al. 2003**
Gln27Glu	ISO-induced increase in forearm blood flow	Higher responsiveness	Cockcroft et al. 2000
	ISO-induced dilation of hand vein	Higher responsiveness	
	ISO-induced dilation of hand vein	Higher responsiveness	Dishy et al. 2001b
Thr164Ile	Exercise capacity in CHF patients	Lower peak $\dot{V}O_2$	Wagoner et al. 2000
Agonist-promoted desensitization			
Arg16/Gln27	2-h continuous ISO infusion into hand vein	Strong desensitization of venodilation	Dishy et al. 2001b
Gly16/Gln27	Cardiac responses after 2 weeks oral TER treatment	No differences in the extent of desensitization	Bruck et al. 2003a
	Cardiac responses over 3 days oral TER treatment	No differences in the extent of desensitization 24 h after first TER intake	
	Lymphocyte β-AR density after 2 weeks oral TER treatment	No differences in the extent of down-regulation	Bruck et al. 2003c
	Lymphocyte β-AR density over 3 days oral TER treatment	No differences in the extent of down-regulation 24 h after first TER intake	
	Two-h continuous ISO infusion into hand vein	Resistant against desensitization	Dishy et al. 2001b

Table 7 (continued)

SNP	Method	In vivo phenotype	Reference
Gly16/Glu27	Cardiac responses after 2 weeks oral TER treatment	No differences in the extent of desensitization	Bruck et al. 2003a
	Cardiac responses over 3 days oral TER treatment	Slowed onset of desensitization	
	Lymphocyte β-AR density after 2 weeks oral TER treatment	No differences in the extent of down-regulation	Bruck et al. 2003c
	Lymphocyte β-AR density over 3 days oral TER treatment	Slowed onset of down-regulation	
	Two-h continuous ISO infusion into hand vein	Resistant against desensitization	Dishy et al. 2001b
Thr164Ile	Cardiac responses after 2 weeks oral TER treatment	Desensitization to the same level	Bruck et al. 2003b

*Higher basal mean blood pressure
**No differences in basal heart rate and blood pressure

Table 8 In vitro β_3-AR phenotype. (*A* adrenaline)

SNP	Cell	In vitro phenotype	Reference
Trp64Arg	CHO	No effects on agonist binding affinities No effect on basal/maximal agonist-stimulated AC activity	Candelore et al. 1996
	COS-7	Enhanced cAMP response	Isogaya et al. 2002*
	CHO-K1	No effects on agonist binding affinities Decreased maximal agonist-stimulated AC activity	Pietri-Rouxel et al. 1997
	HEK293	No effects on agonist binding affinities Decreased maximal agonist-stimulated AC activity	
	Rat insulinoma	Decreased basal, agonist- or glucose-stimulated secretion of insulin	Perfetti et al. 2001
	3T3-L1 preadipcytes	Lower intrinsic activities of ISO, A and L-755,507 Decreased K_{act} for cAMP accumulation for ISO, NA and L-755,507	Kimura et al. 2002

*Only when Arg64Arg-β_3-AR variant was co-expressed with AC-III

Table 9 Ex vivo β_3-AR phenotype

SNP	Cell/tissue	Ex vivo phenotype	Reference
Trp64Arg	Subcutaneous and visceral white adipocytes	No differences in the potency of CGP12177 and NA in inducing lipolysis	Li et al. 1996
	Omental adipose tissue biopsies	No difference in basal and ISO-induced maximal lipolysis No difference in potency of dobutamine or TER in inducing lipolysis Tenfold lower potency of CGP12177 in inducing lipolysis	Hoffstedt et al. 1999
	Omental adipocytes	No difference in the potency and responsiveness of ISO or CGP12177 in inducing lipolysis Lower potency of L755,507 in inducing lipolysis	Umekawa et al. 1999

Table 10 In vivo β_3-AR phenotype

SNP	Method	In vivo phenotype	Reference
Trp46Arg	Local ISO infusion-induced lipolysis in abdominal adipose tissue	No differences in basal and ISO-induced increase in lipolysis	Snitker et al. 1997
	Oral glucose tolerance test	No difference in fasting and post-load glucose-levels No difference in fasting and post-load insulin levels	Janssen et al. 1998
		Higher fasting glucose Lower fasting insulin levels and response	Christiansen et al. 1999

Table 10 (continued)

SNP	Method	In vivo phenotype	Reference
Trp46Arg	Hyperinsulinaemic-euglycaemic clamp	No difference in fasting glucose and insulin levels Lower fasting serum free fatty acid levels No effect on insulin-stimulated lipid-oxidation, whole-body glucose uptake and glucose non-oxidation Higher insulin-stimulated glucose oxidation	Pihlajamäki et al. 1998
		Reduced insulin-stimulated glucose disposal Higher blood glucose and serum insulin levels after ingestion of glucose	Widén et al. 1995
	Combined intravenous glucose and tolbutamide tolerance test	No effect on fasting plasma catecholamine levels Higher fasting serum levels of total cholesterol, LDL-cholesterol, triglycerides and C-peptide Lower insulin sensitivity*	Urhammer et al. 1996
		No difference in insulin sensitivity	Urhammer et al. 2000
	Postural change to standing	Lower resting autonomic nervous system activity Higher response in para- and sympathetic nervous system activity	Shihara et al. 1999, 2001
	Low-dose NA infusion	Increase in diastolic blood pressure Increase in triacylglycerol levels and non-esterified fatty acids	Melis et al. 2002

*Estimated in only three subjects carrying the homozygous Arg64Arg β_3-AR variant

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