Pharmacogenomics of G Protein-Coupled Receptor Ligands in Cardiovascular Medicine

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Abstract——Agonists and antagonists of G protein-coupled receptors are important drugs for the treatment of cardiovascular disease, but the therapeutic response of any given patient remains difficult to predict because of large interindividual variability. Among the factors potentially contributing to such variability, we have reviewed the evidence for a role of pharmacodynamic pharmacogenetics (i.e., polymorphisms in the cognate receptors for such drugs as well as other proteins potentially modifying their action). Based upon the availability of data and the prevalence of use, we have focused on ligands at adrenergic and angiotensin II receptors (including the indirectly acting angiotensin-converting enzyme inhibitors). The vast majority of gene polymorphisms reviewed here have shown only inconsistent effects on drug action, which does not make these polymorphisms useful genetic markers to predict treatment responses. We conclude that considerable additional research, partly involving other types of study than those available now, will be necessary to allow a definitive judgment whether pharmacodynamic pharmacogenetic markers are useful in an individualized approach to cardiovascular therapy. Moreover, we predict that even such additional research will result in only few cases where the promise of tailored treatment can be fulfilled; however, some of these few cases may be of major clinical relevance.

I. Introduction

G protein-coupled receptors (GPCRs)[1] are of prime importance for cardiovascular homeostasis under physiological and pathophysiological conditions (Insel et al., 2007; Hendriks-Balk et al., 2008). Although a considerable number of the more than a thousand different human GPCRs have been identified in cardiovascular tissues or have been implicated in the control of cardiovascular function, only a few GPCRs are actual direct targets of currently available drugs. In this regard, the sympathetic nervous system and the renin-angiotensin-

[1] Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AR, adrenergic receptor; AT, angiotensin II; BHT933, azepexole; EPI, epinephrine; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; I/D, insertion/deletion; kb, kilobase pair(s); NE, norepinephrine; NET, norepinephrine transporter; RAAS, renin-angiotensin-aldosterone system; SNP, single nucleotide polymorphism; UTR, untranslated region.

aldosterone system (RAAS) are of key importance, and directly and indirectly acting agonists, as well as antagonists, of these systems are the most frequently used classes of drugs in the treatment of cardiovascular diseases.

It has long been recognized that treatment responses to drugs acting on cardiovascular GPCRs vary markedly among individual subjects. Although part of this variability has been attributed to disease-associated alterations of corresponding receptors and their signal transduction mechanisms (Brodde, 2007), it has long been speculated that genetic heterogeneity also contributes to the observed variability in drug responses. Pharmacogenomics is the science that systematically studies the role of such genetic heterogeneity on beneficial and adverse drug responses. It is a promising field because it theoretically allows improving the risk-benefit ratio of treatment. This can occur by selecting patients more likely to be good therapeutic responders and/or by selecting against patients prone to experience adverse treatment effects. Such individualized medicine may help to improve health as well as cost-effectiveness of treatment.

Our current pharmacogenomic knowledge mirrors the historical developments in this rather young specialty. A key discovery was the observation that acetylation of isoniazid is genetically determined, a first example that the pharmacokinetics of a given drug is affected by inherited mechanisms (Goedde et al., 1964). Genetically determined profound toxicities of the antihypertensive sympatholytic debrisoquine and the antiarrhythmic sparteine, both no longer on the market, further fostered prime concepts of pharmacogenomics and led to the discovery of individual variances in the activities of P450 enzymes, CYP2D6 (Mahgoub et al., 1977; Eichelbaum et al., 1979; Gonzalez et al., 1988). In addition to variant drug metabolism, genetically determined differences in drug transporter activities have been identified as an additional source of the individual heterogeneity in drug disposition (for review, see Eichelbaum et al., 2006). Whereas the pharmacokinetic branch of pharmacogenomics accumulated considerable knowledge based on the availability of large amounts of data from drug monitoring, the investigation of genetic variances in pharmacodynamics started roughly a decade ago. The cloning of drug targets (e.g., GPCRs or components of the RAAS) sparked the search for genetic polymorphisms in their genes and the association of such polymorphisms with physiological or pharmacological phenotypes. Admittedly, the contribution of genetic polymorphisms in these genes to the observed variances remained modest in most cases. Integrative concepts extended research on signaling mechanisms involved in drug actions. In the field of GPCRs this includes not only variants in the receptors but also polymorphisms in genes contributing to the signal transduction of such receptors (e.g., G proteins and other genes linked physiologically to those

GPCRs). The disadvantage of such candidate gene approaches is that we basically do not comprehend the complex system of the biology of drug actions in vivo. An alternative approach for the investigation of complex genetic diseases or phenotypes, including drug responses, is genome-wide association studies that do not rely on prespecified hypotheses. At present, we experience exciting discoveries in the field of complex diseases (e.g., the identification of genes apparently involved in cardiometabolic diseases, including obesity, diabetes, myocardial infarction, or aneurysms) (Frayling et al., 2007; Sladek et al., 2007; Helgadottir et al., 2008). In the end, these discoveries will also affect pharmacogenomics and individualized therapy. Available data now support long proposed concepts that variants in different genes may lead to the same disease phenotype. Future personalized medicine will take such information into account for making therapeutic decisions. Genome-wide association studies have not only been used for the identification of disease genes, two examples underscore the power of this new method for pharmacogenomics research itself. Hence, one genome-wide association study confirmed the contribution of a gene (*VKORC1*) to the anticoagulant responses to warfarin and its derivatives (Cooper et al., 2008). Another study associated variants in the gene of a hepatic drug transporter (SLCO1B1) with statin-induced myopathy (The SEARCH Collaborative Group, 2008). Such genome-wide association studies will also contribute to the future identification of novel genes relevant to the pharmacogenomics of the drugs discussed here.

Despite these encouraging developments, the promise of current pharmacogenomics to provide tailored therapies remains to be fulfilled for most cardiovascular drugs because of various stumbling blocks. First, the observed contributions of single genes to explain therapeutic effects are of modest degree and do not allow for individual predictions of responder state, occurrence of adverse effects, or dosing regimens. Second, particularly for well tolerated drugs for which a therapeutic response can be assessed quickly, it remains open whether pharmacogenomic information is advantageous compared with trial-and-error treatment. The balance between the two approaches will depend on several factors, including the predictive value and the cost of the genetic test as well as the cost and risk of exploratory treatment. Third, much of the pharmacogenomic information available so far has remained equivocal, thereby questioning one of the key premises of pharmacogenomic-based treatment (i.e., whether a given genetic test is indeed predictive). Such controversial data may be due to many factors, including lack of solid knowledge on the biology of a given polymorphism and statistical under-powering of many studies. Finally, almost all pharmacodynamic pharmacogenomics studies in the cardiovascular system have focused on desired drug effects, whereas only little information is available on the potential flip side of the

coin (i.e., pharmacogenomic information on adverse effects).

Against this background, the present article reviews the current state of the evidence for a role of pharmacodynamic pharmacogenomics in response to GPCR ligands in the cardiovascular system. Reflecting both their prominent role in cardiovascular therapy and the available amount of data, ligands at adrenergic receptors (ARs) and at angiotensin II (AT) receptors will be the main focus. With regard to AT receptors, we not only look at direct ligands (i.e., AT receptor blockers) but also address indirectly acting inhibitors [i.e., the angiotensin-converting enzyme (ACE) inhibitors]. The much more limited information from other receptor systems will be summarized only briefly. Where applicable, we also include data on GPCR signaling mechanisms.

II. Adrenergic Receptor Ligands

The sympathoadrenal system plays a major role in the control of cardiovascular functions. It largely does so through the neurotransmitter norepinephrine (NE) and the adrenal hormone epinephrine (EPI). NE and EPI act via three subfamilies of ARs, with three subtypes each, termed $\alpha_{1A,B,D}$, $\alpha_{2A,B,C}$, and $\beta_{1,2,3}$ (Bylund et al., 1994). The most frequently used drug class in cardiovascular medicine acting on these receptors is the β-AR antagonists, such as atenolol, bisoprolol, and metoprolol. Typical indications of β-AR antagonists are long-term treatment of coronary heart disease and congestive heart failure, chronotropic control, and arterial hypertension, the latter with increasing controversy (Messerli et al., 2008). β-AR agonists, such as EPI, are sometimes used in the short-term treatment of heart failure, resuscitation, or shock. α_1-AR antagonists, such as doxazosin and terazosin, are indicated for the treatment of hypertension, although they are no longer considered a first-line option in this indication (ALLHAT Officers, 2000). α_1-AR agonists are sometimes used to elevate low blood pressure. α_2-AR agonists, such as clonidine, lower blood pressure, but there is continuing debate whether their effects may also involve nonadrenergic mechanisms often referred to as "imidazoline sites." α_1-AR signal mainly via G proteins of the $G_{q/11}$ family coupling to activation of a phospholipase C. α_2-AR are predominantly coupled to G proteins of the $G_{i/o}$ family, mediating the modulation of various ion channel activities and the inhibition of adenylyl cyclase isoforms, whereas β-AR mainly activate G proteins of the G_s family, thus stimulating adenylyl cyclase isoforms of some of the ion channels. Most studies on the pharmacogenomics of AR ligands have focused on polymorphisms in the corresponding AR. The role of genetic variants in the signaling cascades of ARs has only been addressed in some smaller studies.

A. α_1-Adrenergic Receptor Ligands

Among the α_1-AR subtypes, the α_{1A}-AR has received the most attention, with regard to genetic heterogeneity (Kirstein and Insel, 2004). Nine naturally occurring single nucleotide polymorphism (SNPs) have been identified in the coding region of the gene for this subtype (ADRA1A), of which seven result in amino acid changes. Upon heterologous transfection, some of them yield altered responses to agonists; however, the extent of alterations is small in each case (Lei et al., 2005). Polymorphisms in the promoter of the α_{1A}-AR gene have also been identified but similarly remain of uncertain biological relevance (Huang et al., 2008). With regard to cardiovascular drug responses, only information on the frequent R492C SNP (rs1048101; polymorphism is also referred to as R347C given the occurrence of various splice variants; Fig. 1) is available. The frequency of the Cys allele varies with the investigated ethnicity and ranges from 10% in Asians to 57% in Europeans (Table 1). This variant was apparently not associated with alterations of agonist-induced contraction of the dorsal hand vein in situ (Sofowora et al., 2004) and did not exhibit significantly altered blood pressure responses to EPI in one small study with healthy volunteers (Table 2) (Snapir et al., 2003). Likewise, a study in patients with voiding dysfunction attributed to benign prostatic hyperplasia did not detect a relationship between α_{1A}-AR polymorphisms and the clinical response to treatment with α_1-AR antagonists (Mochtar et al., 2006). Whereas a few polymorphisms have also been identified in the α_{1B}-AR gene (ADRA1B) (Kirstein and Insel, 2004), they were not associated with alterations of blood pressure responses to infusion of the agonist phenylephrine (Büscher et al., 1999). Although β_2-AR can counteract vascular effects of α_1-AR stimulation, an R16G polymorphism in the β_2-AR (see section II.D) was reported not to affect the pressor response to the α_1-AR agonist phenylephrine (Herrmann et al., 2000). Therefore, the presently available data do not support a role of identified genetic factors in the heterogeneity of cardiovascular responses to α_1-AR ligands.

With regard to the α_{1D}-receptor, two SNPs in the 3′-untranslated region (UTR) of its gene (ADRA1D), rs2236554 (A1848T) and rs709524 (A1905G), have been associated alone and in concert with a promoter polymorphism in the norepinephrine transporter (NET) gene, with differential improvement of ventricular shortening in patients with dilated cardiomyopathy upon treatment with β-blockers (Nonen et al., 2008). This result awaits independent verification and clarification of the involved mechanisms on how the α_{1D}-AR contributes to hemodynamic control under β-blockade.

In summary, a relevant contribution of variants in the α_1-AR genes to the effects of α_1-AR ligands has not been demonstrated so far. It is noteworthy that it remains
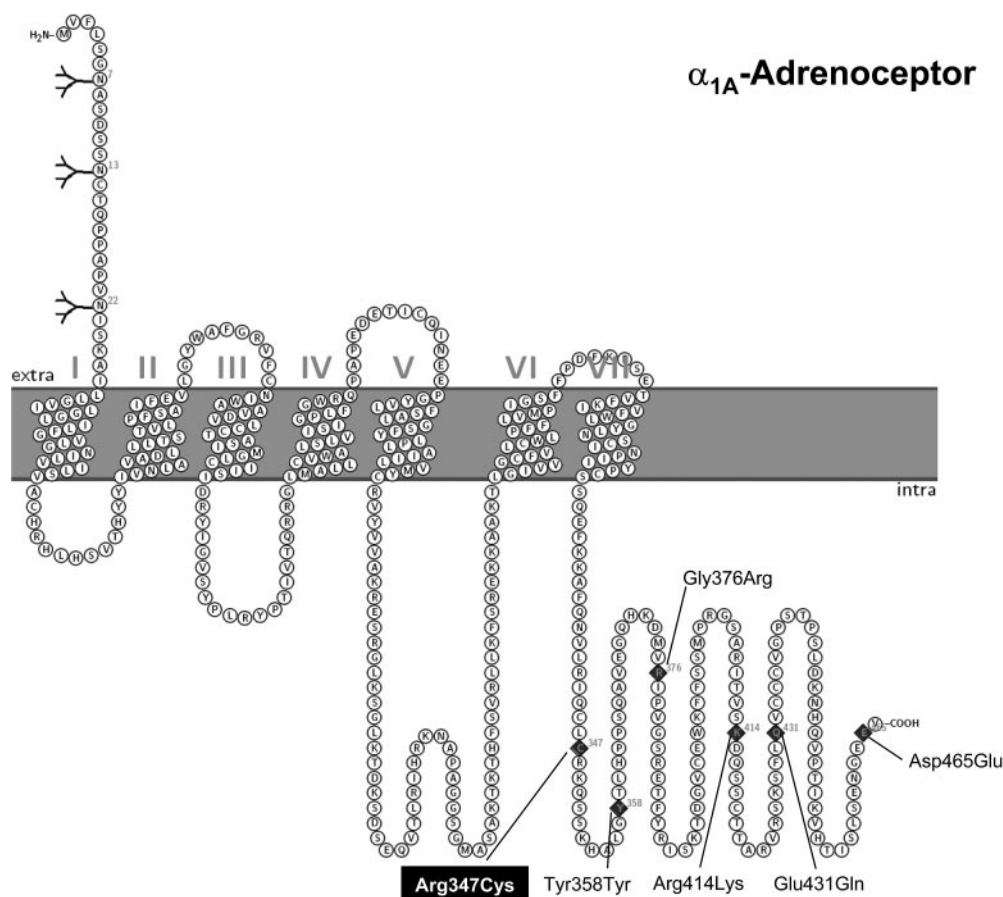
FIG. 1. Topological model of the α_{1A}-AR. The gene is located on chromosome 8p21-p11.2. Five exons have been identified that give rise to four different α_{1A}-AR splice variants with different C termini. Depicted is the most frequently occurring variant 1. Glycosylation sites are denoted by "y-like" structures at the external loop. Variants to the coding sequence are indicated by diamonds. They comprise the rare variants Y358Y (rs56318220), G376R (rs1496121), R414K (rs3730247), E431Q (rs1048102), and D465E (rs2229126), which have not been analyzed functionally. The SNP rs1048101 is a frequently occurring variant that causes an R347C exchange. It originally was identified in the cDNA for the longest splice variant of the α_{1A}-AR and, hence, termed R492C. The allele frequencies for the Arg and Cys variants are 0.45 and 0.55 in whites of European descent. In Asians and Africans, the Arg variant prevails with frequencies of 0.9 and 0.75, respectively. Controversy exists whether the Arg347 allele is associated with hypertension (Xie et al., 1999; Gu et al., 2006).

largely unknown whether genetic variants in α_1-AR genes modulate the actions of α_1-AR blockers.

B. α_2-Adrenergic Receptor Ligands

Apart from their involvement in the central regulation of blood pressure, α_2-AR can contribute to cardiovascular function in four main ways: prejunctional inhibition of transmitter release involving multiple subtypes but mostly α_{2A}-AR (Trendelenburg et al., 2003,), promotion of platelet aggregation exclusively via α_{2A}-AR (Bylund et al., 1988), endothelium-dependent vasodilatation, and endothelium-independent vasoconstriction, the last two potentially involving multiple subtypes (Guimarães and Moura, 2001). Sequence variants have been identified in each of the three α_2-AR genes (Kirstein and Insel, 2004). Early work on the α_{2A}-AR has used restriction fragment length polymorphisms to explore variants in its gene (ADRA2A), specifically using the restriction enzyme DraI (Michel et al., 1999). Although this polymorphism was associated with differences in catecholamine-induced platelet aggregation (Freeman et al., 1995), no

additional studies on its role in cardiovascular drug responses have been reported. Meanwhile, other polymorphisms in the ADRA2A have been identified at the nucleotide level, but their relationship to cardiovascular drug responses remains unclear (Kirstein and Insel, 2004).

Several polymorphisms have been described in the promoter of the α_{2B}-AR gene (ADRA2B); the most prevalent among them (−98G/C; rs3111873) had no effect on promoter activity but was in linkage disequilibrium, with a deletion polymorphism (del301-303; rs29000568; Table 1) in the coding region (Fig. 2) (Cayla et al., 2004). The latter is located in a region of the α_{2B}-AR, which is important for receptor phosphorylation and desensitization. Hence, the del301-303 variant is expected to enhance receptor function (Small and Liggett, 2001). Actually, the del301-303 variant was reported to be associated with several physiological alterations, but these did not necessarily reflect an enhanced receptor function (Kirstein and Insel, 2004). Carriers of the deletion variant exhibited an increased blood pressure ele-

TABLE 1
Allele frequencies of common variations in different ethnicities

Allele frequencies of the indicated variants refer, if not indicated otherwise, to the HapMap data phases II and III (accessed October 2008).

Gene	Variation (rs Nomenclature)	Common Designation	Reference Allele	African Americans	Sub-Saharan Africans	Europeans (North-West Europe)	Hispanics	Chinese	Japanese
ADRA1A	rs1048101	R492C = R347C	Cys	0.39	0.13–0.28	0.55–0.57	0.423	0.10–0.13	0.15–0.16
ADRA2B	rs29000568	del301-303	del	0.12–0.21[a]		0.31–0.37[a]			
ADRA2C	rs61767072	del322-325	del	0.41–0.43[b]		0.04–0.06[b]		0.13[b]	0
ADRB1	rs1801252	S49G	Gly	0.13–0.29[c]	0.10	0.10–0.15[c]		0	0–0.13[c]
ADRB1	rs1801253	R389G	Arg	0.59		0.68		0.75	0.85
ADRB2	rs1042713	R16G	Gly	0.43	0.46–0.50	0.63–0.68	0.55	0.41–0.44	0.56
ADRB2	rs1042714	Q27E	Glu		0.18	0.47		0.12	0.08
ADRB2	rs1800888	I164T	Ile	1.0		0.99		1.0	1.0
GNB3	rs5443	C825T	C		0.09	0.61		0.66	0.60
GNAS	rs7121	T393C	T		0.87	0.46		0.69	0.58
GNAS	rs6123837	G(−1211)A	A		0.08	0.11[d]		0.5	0.51
ACE	rs1799752	I/D	I	0.40[e]		0.46[e]	0.52[e]		0.66[e]
AGT	rs699	M235T	Thr	0.75	0.85–0.92	0.38–0.42	0.68	0.72–0.79	0.82–0.84
AGT	rs4762	T174M	Met	0.03	0.03–0.05	0.08–0.14	0.16	0.08–0.144	0.08–0.10
AGTR1	rs5186	A1166C	C	0.05	0.08	0.25		0.04	0.03

Frequencies for variants not represented in HapMap were taken from the following references:
[a] Small et al. (2001b); Muszkat et al. (2005).
[b] Small et al. (2002, 2004).
[c] Brodde (2008); Kirstein and Insel (2004).
[d] Frey et al. (2008).
[e] Haiman et al. (2003).

vation in response to EPI (administered in the presence of propranolol), whereas they had a smaller increase in coronary blood flow in the absence but not the presence of propranolol (Table 3) (Snapir et al., 2003). In another trial, intravenous administration of the α_2-agonist dexmedetomidine to 80 anesthetized patients resulted in an enhanced initial vasoconstriction in homozygous carriers of the deletion allele after 3 min, an effect that was not sustained after this period (Table 3) (Talke et al., 2005). On the other hand, studies using the α_2-agonist azepexole (BHT933) in the dorsal human hand vein in situ did not detect significant contributions of this polymorphism to the vasoconstriction response (King et al., 2005). Studies using the same technique but dexmedetomidine as the agonist also found no relationship between any of the nine common variants in the α_{2B}-AR gene and vasoconstriction; however, a haplotype occurring only in African Americans characterized by two SNPs in the promoter and one in the 3′-UTR of ADRA2B was associated with a smaller maximal vasoconstriction response (Muszkat et al., 2005). On the other hand, hemodynamic effects of the α_2-AR antagonist yohimbine were not associated with this and other genetic variants in ADRA2B (Etzel et al., 2005). Table 3 summarizes key findings relating to the ADRA2B del301-303 variants.

A deletion mutant involving 12 nucleotides and 4 encoded amino acids (Fig. 2, Del322-325; I/D variant) has been found in the gene of the α_{2C}-AR (ADRA2C) and apparently is more prevalent in African Americans than in white persons (Table 1). Whereas this variant exhibits a reduced affinity for and functional response to agonists in transfected cells (Table 4) (Small et al., 2000), little is known about possible implications of this polymorphism for cardiovascular drug responses. In theory, the reduced activity of the α_{2C}-AR, with the deletion of amino acids 322 to 325, should lead to a reduced inhibition of NE release from the presynaptic nerve terminals and ultimately result in increased adrenergic drive. However, in healthy persons, basal parameters of the sympathetic nervous system, including heart rate, heart rate variability, blood pressure, and concentrations of NE and EPI were not affected by this variant (Table 4) (Kurnik et al., 2007). Intravenous administration of the α_2-agonist dexmedetomidine to healthy subjects results in blood pressure lowering and a decrease in plasma catecholamine concentrations. These effects exhibit a considerable interindividual variability; however, the I/D polymorphism in the α_{2C}-AR gene did not contribute to this heterogeneity (Table 4) (Kurnik et al., 2008a). Another group reported enhanced sympathetic nervous and adrenomedullary activities in carriers of the deletion variant. In the presence of the α_2-AR blocker yohimbine, catecholamine release and heart rate were higher in persons with the deletion variant (Neumeister et al., 2005). Likewise, application of a cold pressure test resulted in increased heart rate responses in healthy vol-

TABLE 2
Association of the ADRA1A R492C variant with different cardiovascular and pharmacogenomic phenotypes

Parameter	Result	Reference
Association study with benign prostate hyperplasia in Japanese; expression studies with recombinant receptor variants	No association with prostate hyperplasia; no differences in ligand binding or signal transduction	Shibata et al., 1996
Hand vein constriction in response to phenylephrine in 74 healthy adults	No effects on investigated parameters	Sofowora et al., 2004
Hemodynamic epinephrine response in 16 young healthy men	CysCys Genotype, longer ECG PR interval; no effect on blood pressure response	Snapir et al., 2003
Association study with hypertension in 480 Chinese patients with hypertension and control subjects	Arg allele more frequent in patients with hypertension	Gu et al., 2006

unteers carrying the deletion variant (Table 4) (Kurnik et al., 2008b).

Apart from its effects on ligand activities, the *ADRA2C* deletion variant has been identified to increase the risk for congestive heart failure, an effect that is further augmented in presence of the β_1-AR Arg389 variant. Based on these data, it has been proposed to guide preventive treatment regimes by pharmacogenomic measures (i.e., genotyping of both variants in patients at risk) (Small et al., 2002). In a later trial, patients with heart failure who had α_{2C}-AR deletion variant actually exhibited a more pronounced increase in the ejection fraction upon treatment with β-blockers (Lobmeyer et al., 2007). However, the original observation of an increased risk for heart failure in carriers of the deletion allele has not been confirmed in other studies, particularly in non-African American populations (Nonen et al., 2005; Metra et al., 2006; Canham et al., 2007). Key findings relating to the *ADRA2C* del322-325 variant are summarized in Table 4.

Independent from polymorphisms in their cognate receptors, responses to α_2-AR ligands may be affected by genetic variability in elements of their signaling cascade. Several studies have addressed potential effects of a common genetic variant in the gene for the G protein $\beta3$ subunit (Table 5, *GNB3*). G proteins consist of a Gα subunit that carries the enzymatic activity and a stable G$\beta\gamma$ dimer. In its inactive GDP-bound state, Gα subunits form heterotrimers with free G$\beta\gamma$ subunits. Upon ligand binding to a GPCR, a conformational change of the receptor occurs that is transmitted to an interacting G protein. This favors the exchange of Gα-bound GDP for GTP, which is the active state of the Gα subunit. Subsequently, the GTP-bound Gα separates from the G$\beta\gamma$ dimer. In case of active GTP-bound Gα_S, stimulation of adenylyl cyclase isoforms is the next step of the signaling cascade. Free G$\beta\gamma$ subunits are themselves key regulators of numerous effector systems, including adenylyl cyclases, ion channels, and phospholipase C isoforms. The catalytic GTPase activity of the Gα subunit mediates the hydrolysis of GTP to GDP and induces the inactive GDP-bound Gα state in which it reassociates with a G$\beta\gamma$ dimer (for review, see Wettschureck and Offermanns, 2005). A common polymorphism (C825T, rs5443) in the *GNB3* has been associated with increased signal transduction (Fig. 3) (Siffert et al., 1998). Major

ethnic differences exist in the prevalence of this variant (Table 1). A consistent mechanistic explanation for this observation is still lacking. Available evidence suggests that the 825T allele favors alternative splicing, ultimately resulting in two protein variants termed Gβ3s and Gβ3s2 (Rosskopf et al., 2003; Fig. 4). Because G proteins are involved in the signaling cascades of more than a thousand different receptors, complex phenotypes in association with the Gβ3 rs5443 alleles have been described, although not without controversy (for review, see Siffert, 2005). Variants in *GNB3* have also been attributed to pharmacogenomic effects, particularly on adrenergic and serotonergic mechanisms (Hauner et al., 2003; Schürks et al., 2007; Wilkie et al., 2007). With regard to ARs, intracoronary administration of the α_2-AR agonist azepexole was associated with increased vasoconstriction in carriers of the 825T allele compared with persons with the wild-type C variant (Table 5) (Baumgart et al., 1999). It is interesting that coronary vasoconstriction induced by the α_1-AR agonist methoxamine was not affected by *GNB3* variants, potentially indicating a differential coupling of Gβ3 to α_1- and α_2-ARs (Baumgart et al., 1999). In an independent study of 131 patients, an increased coronary vasoconstrictor response was observed in association with the 825T allele upon injection of methylergonovine, a semisynthethic ergot alkaloid that acts as an agonist on α_1- and α_2-ARs and on serotonin receptors (Table 5) (Meirhaeghe et al., 2001). Additional evidence for pharmacogenomic modulation of α_2-AR responses by *GNB3* variants has been provided by a clinical trial in 30 young healthy subjects who were treated with clonidine. Carriers of the 825T allele exhibited an increased lowering of systolic blood pressure, a greater reduction in peripheral resistance, and a more pronounced slowing of pulse wave velocity (Table 5) (Nürnberger et al., 2003). Likewise, NE-induced vasoconstriction in the microcirculation was increased in carriers of the 825T allele (Table 5) (Wenzel et al., 2002). In contrast, hand vein vasoconstriction upon administration of the α_2-AR agonist azepexole and changes in total peripheral resistance in the presence of α-methylnorepinephrine were similar in carriers of the C and T alleles of the C825T variant (Schäfers et al., 2001). A similar finding was observed with NE-induced venoconstriction, which was not different between carriers of the
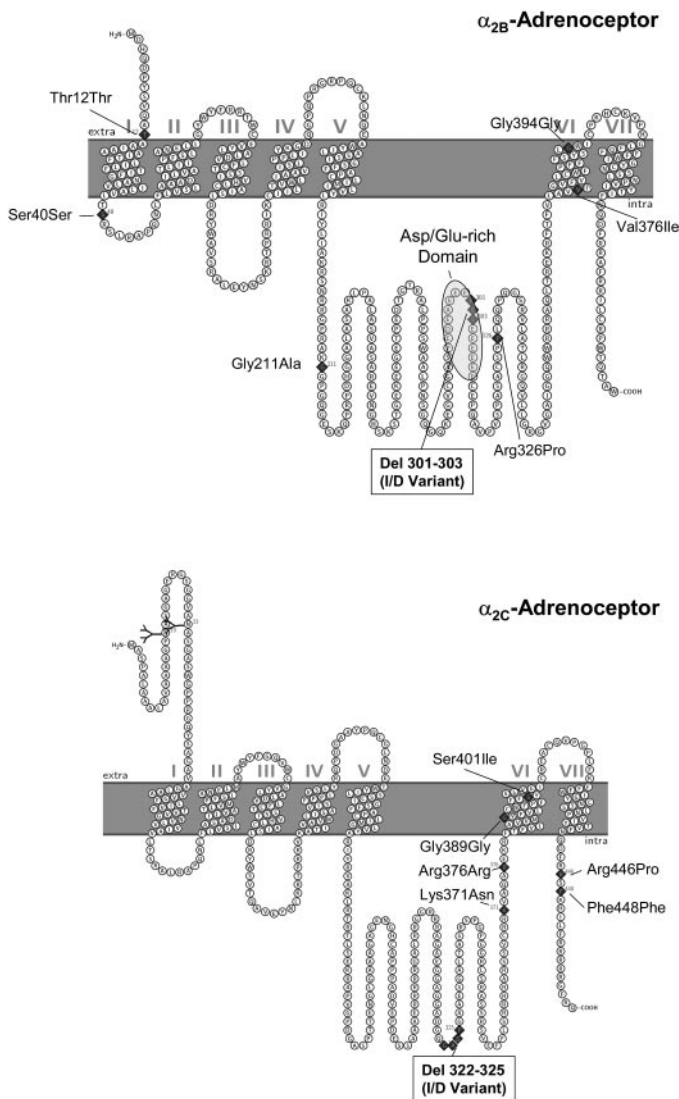
FIG. 2. Top, topological model of the α_{2B}-AR. The gene is located on chromosome 2p13-q13 and consists of only one exon. In the C terminus an Arg/Glu-rich acidic domain exists that has been implicated in receptor kinase-mediated phosphorylation and desensitization. The deletion allele of the frequent I/D variant (referred to in the dbSNP database as rs29000568) has been associated with enhanced receptor function. This polymorphism is also referred to as Glu9/Glu12 variant. Other variants in the coding sequence include the rare SNPs, T12T (rs9333567), S40S (rs35812638), A211G (rs9333568), and R326P (rs45494291), and the more frequently occurring SNPs, I376V (rs29000569) and G394G (rs2229169). Detailed functional characterizations only exist for the I/D variant. Bottom, topological model of the α_{2C}-AR. The gene is located on chromosome 4p16 and consists of only one exon. Glycosylation sites are denoted by "y-like" structures at the external loop. Variants to the coding sequence are indicated by diamonds. Functionally characterized has been the frequent insertion/deletion variant affecting amino acids 321 to 325 (rs61767072). Allele frequencies vary considerably between ethnicities; whereas the allele frequency for the deletion allele amounts up to 40% in African Americans, it occurs in less than 5% whites. Rare variants in the coding region comprise N371K (rs1800037), R376R (rs7435505), G389G (rs7434630), I401S (rs1133450), P446R (rs1133451), and F448F (rs1133453).

cies that are not easily explained (Mitchell et al., 2004). In contrast to the effects on vasoconstriction, clonidine-mediated antilipolytic responses were less potent in carriers of the 825T allele compared with persons with the wild-type allele (Rydén et al., 2002). Although the possibility of genetic heterogeneity in receptor signaling molecules, rather than in the receptors, is intriguing and deserves further investigation, the inconclusive data on the C825T polymorphism are far from being understood. The possibility of tissue-specific signaling mechanisms has to be taken into account. On the other hand, effects of the *GNB3* C825T polymorphism seem to be of only moderate degree as recently shown in a *meta*-analysis for hypertension, the most frequently studied phenotype in association with the *GNB3* variants (Bagos et al., 2007).

In summary, there is good evidence that the α_{2C}-AR deletion variant prevailing in African ethnicities is of functional relevance. Several independent studies underscore that the *GNB3* 825T allele further modulates effects of α_2-AR agonists (Table 5). ARs and G proteins are interacting partners in signal transduction. The observed effects of both, the *GNB3* C825T and the *ADRA2C* del322-325 variants, are of moderate degree (Tables 4 and 5). Hence, it is sensible to search for interactions of such variants, as recently demonstrated for these polymorphisms in *GNB3* and *ADRA2C* (Kurnik et al., 2008b). The major drawback of such approaches is of course the necessity for much larger cohorts.

C. β-Adrenergic Receptor Ligands

Several polymorphisms have been described in the human β_1-AR gene (*ADRB1*), among which S49G and G389R have been studied most intensively (Fig. 4) (Brodde, 2008). Other SNPs within the coding region of the β_1-AR, such as A59S, R399C, H402R, T404A, or P418A, have allele frequencies of only approximately 1%, and little information is available regarding their functional role, particularly with regard to drug effects (Leineweber et al., 2004). Besides these SNPs in the coding sequence, numerous variants in the 5′-flanking region of the β_1-AR gene have been identified and clustered in haplotypes. In vitro transfection experiments of whole-gene constructs revealed major differences in β_1-AR expression levels. At present, it remains open how this translates in altered function in vivo (Small et al., 2008).

The S49G and G389R SNPs are in tight linkage disequilibrium, and a haplotype with a homozygous Gly in both positions exists very rarely, if at all. The Gly49 and Gly389 variants are found with allele frequencies of 25 and 27%, respectively, in white populations (Brodde, 2008). Although both variants are cosmopolitan, allele frequencies vary to a minor extent in other ethnicities (Table 1) (Moore et al., 1999; Xie et al., 2001; Belfer et al., 2005; Nonen et al., 2005). The Ser49 and Gly49 variants apparently respond similarly to agonists at

T and C alleles (Table 5) (Mitchell et al., 2006). On the other hand, these investigators reported that, after systemic treatment with an endothelin receptor antagonist, NE-induced vasoconstriction of the dorsal hand vein was much more pronounced in T allele carriers, discrepan-

TABLE 3
Association of the ADRA2B del301-303 (DD) variant with different cardiovascular and pharmacogenomic phenotypes

Parameters Analyzed	Results	Reference
Expression of receptor variants in cell lines	No effect on ligand binding; slightly reduced coupling efficiency to $G\alpha_S$ in deletion variant. Markedly reduced receptor phosphorylation and loss of short term desensitization in deletion variant	Small et al., 2001
Hemodynamic epinephrine responses in 16 volunteers	DD associated with blunted epinephrine-induced coronary blood flow increases	Snapir et al., 2003
Vasoconstrictive response to dexmedetomidine in 80 (α_2-AR agonist) in 80 surgical patients	DD associated with short lasting vasoconstriction	Talke et al., 2005
Hand vein constriction after azepexole (α_2-AR agonist) in 50 healthy volunteers	No effects on investigated parameters	King et al., 2005

TABLE 4
Association of the ADRA2C del322-325 (D) variant with different cardiovascular and pharmacogenomic phenotypes

Parameters Analyzed	Results	Reference
Expression of receptor variants in cell lines	D variant: decreased ligand binding affinity; decreased coupling to $G\alpha_i$ & adenylyl cyclase	Small et al., 2000
Association study of this and the *ADRB1* G389R variant in 159 heart failure patients	Odds ratio of 5 for homozygous black carriers with DD genotype for heart failure; interaction with *ADRB1* Arg389.	Small et al., 2002
Plasma catecholamine levels at rest and during yohimbine in 29 African-Americans	DD higher sympathetic nervous activity	Neumeister et al., 2005
Association with heart failure in dilated cardiomyopathy in 90 case subjects and 119 control subjects in Japan	No effects on investigated parameters	Nonen et al., 2005
Association with heart failure in 260 case subjects and 230 control subjects in Italy	No effects on investigated parameters	Metra et al., 2006
Plasma catecholamine levels; blood pressure; heart rate in 224 healthy individuals	No effects on investigated parameters	Kurnik et al., 2007
Association study of this and the *ADRB1* G389R variant in 54 heart failure patients treated with metoprolol.	D associated with greater negative chronotropic response.	Lobmeyer et. al., 2007
Hemodynamic dexmedetomidine responses in 73 healthy subjects	No effects on investigated parameters	Kurnik et al., 2008a
Blood pressure and heart rate response to cold pressure test in 79 healthy subjects	DD genotype associated with greater positive chronotropic response	Kurnik et al., 2008b
Registry study with 637 heart failure patients treated with metoprolol or carvedilol	No difference in survival rates	Sehnert et al., 2008

physiological expression levels; however, the Gly49 variant is more susceptible to agonist-induced down-regulation, leading to lower receptor densities in vivo (Table 6) (Levin et al., 2002; Rathz et al., 2002). An enhanced susceptibility toward agonist-induced down-regulation may not only manifest during extended treatment with exogenous agonists but also in the presence of endogenous agonists, particularly during states of chronically enhanced sympathoadrenal activity. In contrast, the Arg389 variant seems to couple more effectively to $G\alpha_s$ protein and adenylyl cyclase stimulation; hence, it is considered to be a gain of function variant (Table 6) (Mason et al., 1999).

A range of polymorphisms in the human β_2-AR gene (*ADRB2*) has been identified, among which the R16G, Q27E, and T164I have received the most attention (Fig. 4). More than a dozen additional SNPs have been reported, but many of them are rare, and the available knowledge on their functional relevance is limited for all of them, particularly with regard to drug-response studies (Brodde, 2008). The genotypes Gly16 and Glu27 are frequent in whites (approximately 50%), but a somewhat different prevalence may exist in other ethnicities (Table

1) (Hawkins et al., 2006; Lima et al., 2006; Wu et al., 2006). Genotype-phenotype association studies for the frequent R16G and Q27E variants have largely remained inconclusive with regard to arterial hypertension and other cardiovascular diseases (Hahntow et al., 2006; Rosskopf et al., 2007). Neither of the variants in position 16 or 27 is associated with major alterations in ligand affinity or in vitro signaling potency. However, the Gly16 variant has been shown to be more susceptible to agonist-induced down-regulation, whereas the Glu27 variant is relatively resistant to agonist-mediated desensitization processes (Table 7) (Green et al., 1994). If the two variant alleles occur in combination, the Gly16 effects dominate the phenotype; i.e., a Gly16/Glu27 double mutant also exhibits enhanced agonist-induced down-regulation; in contrast, the Arg16/Glu27 double mutant was completely resistant to down-regulation (Green et al., 1994). The T164I polymorphism has been consistently associated with altered receptor function both in vitro and in vivo (Table 7). Clinical phenotypes have been reported much more consistently, but because of its low prevalence (approximately 3% in whites; never

TABLE 5
Association of the GNB3 C825T variant with different cardiovascular and pharmacogenomic phenotypes

| Parameters Analyzed	Results	Reference
Association study with hypertension; functional analyses of variant	T allele associated with hypertension, occurrence of altered splice variant and increased signal transduction in association with T allele	Siffert et al., 1998
Coronary vasoconstriction in response to α_2-AR agonist azepexole in 48 patients with coronary artery disease	T allele, increased coronary blood flow reduction	Baumgart et al., 1999
Coronary vasoconstriction in response to methylergonovine in 131 patients with healthy coronary arteries	T allele, increased susceptibility to vasoconstriction	Meirhaeghe et al., 2001
Hemodynamic parameters in 70 volunteers upon administration of propranolol and α-methylnorepinephrine	T allele, higher stroke volume & lower peripheral resistance; propranolol evokes greater fall in stroke volume. No effect on hand vein constriction.	Schäfers et al., 2001
Effects of endothelin-1, angiotensin II, and norepinephrine on skin microcirculation in 25 volunteers.	T allele, enhanced vasoconstriction to endothelin-1, angiotensin II and norepinephrine	Wenzel et al., 2002
Hemodynamic clonidine responses in 30 young healthy individuals	T allele, greater fall in blood pressure & total peripheral resistance; slowing of pulse wave velocity.	Nürnberger et al., 2003
Interactions of endothelin A receptor antagonist darusentan with endothelin-1, norepinephrine and angiotensin II in 37 volunteers	No effects on systemic or local responses to endothelin A receptor blockade	Mitchell et al., 2004
Interactions of angiotensin II receptor antagonists with endothelin 1, norepinephrine and angiotensin II in 25 volunteers	T allele, more pronounced inhibition by valsartan of angiotensin II- or endothelin 1-mediated vasoconstriction in the microcirculation.	Mitchell et al., 2006
Blood pressure and heart rate response to cold pressure test in 79 healthy subjects	TT genotype: greater positive chronotropic response	Kurnik et al., 2008b
Hemodynamic dexmedetomidine responses in 73 healthy subjects	No effects on investigated parameters	Kurnik et al., 2008b
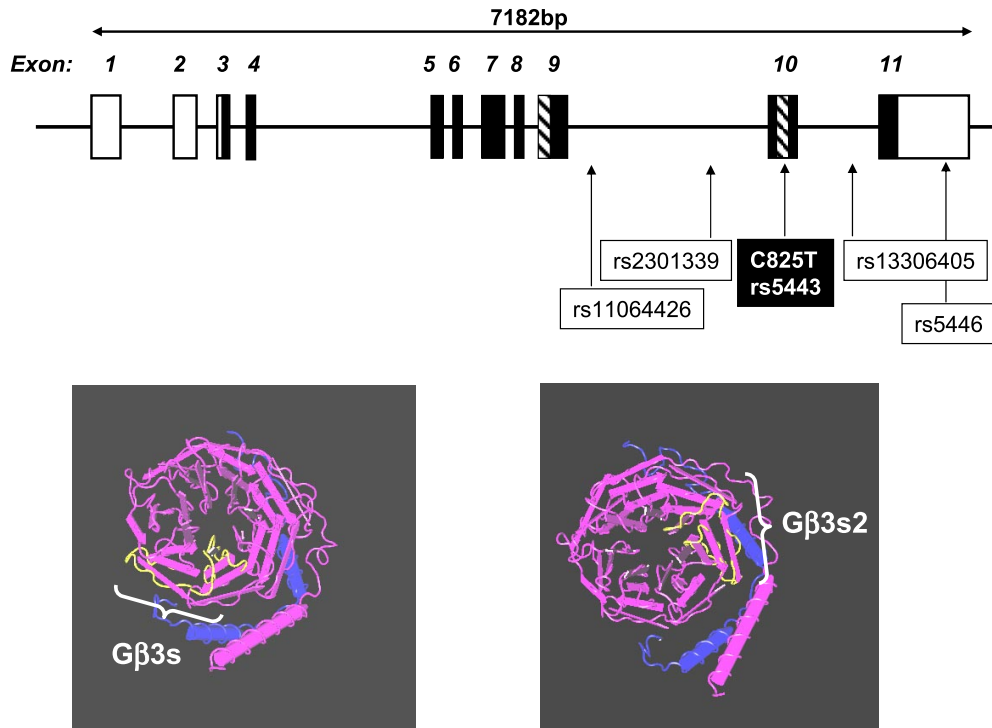
FIG. 3. Gene structure and proposed three-dimensional structure of Gβ3 and its splice variants, Gβ3s and Gβ3s2. Top, the Gβ3 gene consists of 11 exons; coding exons are indicated in black. The frequently investigated SNP C825T (rs5443) is almost completely linked to four additional variants (rs11064426, rs2301339, rs13306405, and rs5446). Pre-mRNA transcripts carrying the 825T allele favor alternative splicing at two sites indicated by hatched boxes. Bottom, Gβ proteins belong to the superfamily of propeller proteins. They comprise seven "propeller blades," each formed by the equivalent of a WD protein domain. Shown is the entire G$\beta\gamma$ dimer composed of a Gβ subunit (cyan) and a Gγ subunit (blue). Splicing of the 5′ part of exon 9 of the Gβ3 gene leads to transcripts for Gβ3s. Deletion of an internal part of exon 10 gives rise to transcripts for Gβ3s2. In both cases, the exact equivalent of one WD domain is missing, which affects different propeller blades in Gβ3s and Gβ3s2, as denoted. Propeller proteins with 4 to 12 WD domains have been described. Hence, Gβ3s and Gβ3s2 may form a six-blade structure. Free G$\beta\gamma$ dimers play important roles in the coordination of multiprotein complexes. Together with a Gα subunit in its GDP state, they form a stable heterotrimer. It has been hypothesized that Gβ3 variants are less capable of stabilizing the Gα subunit in its GDP state, thus giving rise to enhanced signal transduction.
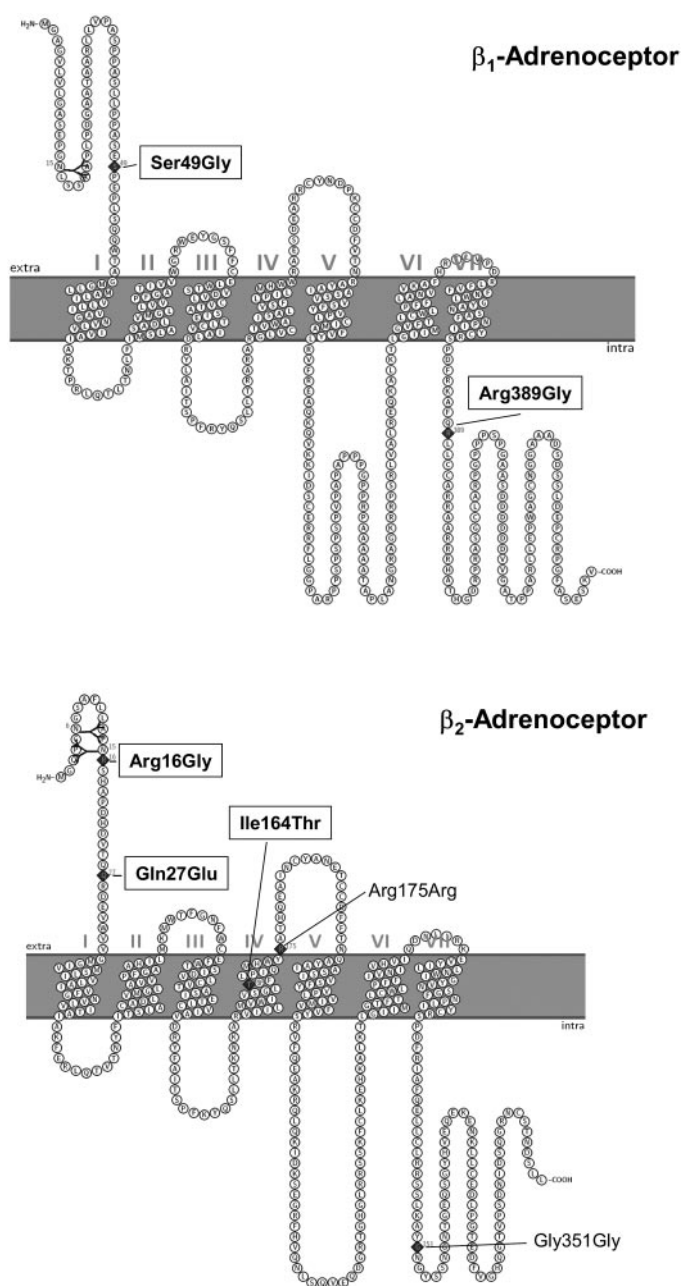
FIG. 4. Top, topological model of the β_1-AR. The gene is located on chromosome 10q24-q26 and consists of only one exon. Glycosylation sites are denoted by "y-like" structures at the external loop. Variants to the coding sequence are indicated by diamonds. Common and well characterized variants are the S49G polymorphism (rs1801252), with the Ser allele occurring in 70 to 80% of all persons, and the G389R variant (rs1801253), with the Arg allele occurring in 60 to 70% of all persons. Bottom, topological model of the β_2-AR. The gene is located on chromosome 5q31-q32 and consists of only one exon. Glycosylation sites are denoted by y-like structures at the external loop. Variants to the coding sequence are indicated by diamonds. Common and well characterized variants are the R16G polymorphism (rs1042713), with the Arg allele occurring in 25 to 40% of all persons, and the Q27E variant (rs1042714), with the Glu allele occurring in 50 to 60% of all white persons. The I164T variant (rs1800888) is associated with a strong and stable phenotype. Additional common variants of unknown functional relevance are R175R (rs1042718) and G351G (rs1042719).

found homozygously, so far), it has been studied less extensively (Michel and Büscher, 2008).

The β_3-AR plays a much smaller role in the regulation of cardiovascular functions than the other two β-AR subtypes. Whereas some investigators have proposed presence and functional role of β_3-AR in the human heart, the overall evidence in this regard remains equivocal (Brodde and Michel, 1999). β_3-AR may also contribute to the regulation of vascular tone in some mammals, but only little data on humans are available (Rozec and Gauthier, 2006). Among polymorphisms in the β_3-AR gene (*ADRB3*), only the W64R variant has been investigated in greater detail, predominantly in the context of metabolic regulation (Arch, 2008). Hence, little information is available addressing the potential effects of this SNP on drug responses in the cardiovascular system, at least partly reflecting the general paucity of data with β_3-selective ligands, which have been investigated in humans (Vrydag and Michel, 2007). Therefore, the following will focus on the pharmacogenomics of human β_1- and β_2-ARs.

1. β-Agonist Responses. With regard to agonist responses, most studies on β_1-AR polymorphisms have investigated a possible role of the R389G variant. Using right atria from patients undergoing coronary bypass grafting, one group of investigators demonstrated that the Gly389 variant was associated with smaller cAMP and inotropic responses (Table 6) (Sandilands et al., 2003), but other investigators did not find such differences (Table 6) (Molenaar et al., 2002; Sarsero et al., 2003). Four different in vivo studies reported that the R389G polymorphism does not affect heart rate or inotropic or plasma renin activity responses to bicycle exercise, which presumably reflects responses to the endogenous agonist NE (Table 6) (Büscher et al., 2001; Xie et al., 2001; Liu et al., 2003; Sofowora et al., 2003). Likewise, two studies published in abstract form reported that this SNP does not affect chronotropic or renin responses to infusion of the agonist dobutamine, although one of them reported an effect on cardiac contractility (Leineweber et al., 2004). On the other hand, carriers of the β_1-AR Gly389 or Ser49 variants had a higher need for an increase in heart failure medication during metoprolol therapy (Terra et al., 2005). Likewise, the demand for inotropic catecholamine support after coronary artery bypass grafting was higher in homozygous carriers of the Gly389 variant compared with homozygous carriers of the Arg389 variant, both treated with metoprolol (Table 6) (Leineweber et al., 2007). In both studies, however, it remains unclear whether these genotype-dependent effects reflect altered agonist and/or antagonist responses.

With regard to variants in the β_2-AR gene ex vivo studies from genotyped subjects using circulating mononuclear cells (mostly lymphocytes), airway smooth muscle cells, lung mast cells, or adipocytes demonstrated that neither the R16G nor the Q27E SNP affected β_2-AR expression (Lipworth et al., 1999) or basal or maximally stimulated adenylyl cyclase activity (Table 7) (Large et al., 1997; Lipworth et al., 1999; Moore et al., 2000; Bruck et al., 2003a). In line with findings from transfected cells,

TABLE 6
Association of the ADRB1 variants with different cardiovascular and pharmacogenomic phenotypes

Variant	Parameters Analyzed	Results	Reference
G389R	Expression of receptor variants in cell lines	Arg389, gain of function; increased adenylyl cyclase activation	Mason et al., 1999
G389R	β-Blocker responses in 147 patients with hypertension	No effects on investigated parameters	O'Shaughnessy et al., 2000
G389R	Exercise-induced heart rate response in 24 volunteers	No effects on investigated parameters	Xie et al., 2001
G389R	Exercise-induced heart rate response in 17 volunteers	No effects on investigated parameters	Büscher et al., 2001
S49G	Expression of receptor variants in cell lines	Gly49, higher basal and stimulated adenylyl cyclase activity; more sensitive toward inhibition by metoprolol; higher desensitization	Levin et al., 2002
S49G	Expression of receptor variants in cell lines	Similar ligand binding affinities; Gly49 enhanced down-regulation	Rathz et al., 2002
S49G/G389R	Norepinephrine actions on atrial muscle from 87 patients undergoing coronary artery bypass surgery	No effects on investigated parameters	Molenaar et al., 2002
G389R	Exercise-induced heart rate response in 16 volunteers	No effects on exercise-induced heart rate; enhanced response to metoprolol in Arg389	Liu et al., 2003 R
G389R	Analysis of receptor function in human atria from 54 patients	Arg389 enhanced cAMP and inotropic responses	Sandilands et al., 2003
S49G/G389R	Analysis of receptor function in human atria	No effects on investigated parameters	Sarsero et al., 2003 G
G389R	Exercise-induced heart rate response in 34 volunteers	Arg389 larger decrease in resting blood pressure upon atenolol; no effect on exercise-induced responses	Sofowora et al., 2003
G389R	Treatment of 40 patients with hypertension with metoprolol	Arg389 larger reduction in diastolic blood pressure	Johnson et al., 2003
G389R	Hemodynamic parameters and mortality during metoprolol treatment in 600 patients	No effects on investigated parameters	White et al., 2003
S49G/G389R	Treatment of 101 patients with hypertension with left ventricular hypertrophy with atenolol	No effects on investigated parameters	Karlsson et al., 2004
S49G/G389R	Analysis of 61 patients with heart failure on therapy with metoprolol	No difference in rate of cardiac decompensation; Gly389 and Ser49 required more heart failure medications	Terra et al., 2005
S49G/G389R	199 heart failure patients treated with β-blockers	No differences in heart rate and ejection fraction before and after β-blocker therapy	de Groote et al., 2005
S49G/G389R	Metoprolol titration in 54 untreated patients with hypertension	Ser49 higher baseline resting heart rates; no effect on response to β-blocker	Beitelshees et al., 2006
G389R	Analysis in cell transfected cells; outcome to bucindolol in 1040 heart failure patients	Agr389 carriers treated with bucindolol no clinical response	Liggett et al., 2006
G389R	Demand for catecholamine support in heart surgery patients	Arg389/Arg389 patients required less adrenaline support	Leineweber et al., 2007
G389R	Outcome of heart failure with carvedilol in 135 patients	Arg389/Arg389 greater improvement in left ventricular ejection fraction after 1.5 years of treatment	Chen et al., 2007
S49G/G389R	Patient registry study with 637 heart failure patients treated with metoprolol or carvedilol	No difference in survival rates	Sehnert et al., 2008
S49G/G389R	Follow-up of 5895 coronary artery disease patients treated with atenolol or verapamil	Increased mortality risk in verapamil group with Ser49-Arg389; no effect in atenolol group	Pacanowski et al., 2008

the Gly16 genotype exhibited greater agonist-induced desensitization in human airway smooth muscle cells (Table 7) (Moore et al., 2000), although that was not confirmed in a study on human lung mast cells (Table 7) (Chong et al., 2000). Likewise, a greater agonist-induced desensitization was reported for the Glu27 variant in human airway smooth muscle cells (Moore et al., 2000), whereas less desensitization was found for the same genotype in human lung mast cells (Table 7) (Chong et al., 2000). These data suggest the possibility that the effects of these SNPs in the amino terminus of the β_2-AR may depend on the cell type in which it is expressed. In contrast, the Ile164 variant, even if present in the heterozygote state only, was consistently associated with lower potency and/or maximal responses to various β-AR agonists in lymphocytes (Büscher et al., 2002), human lung mast cells (Kay et al., 2003), or adipocytes (Table 7) (Hoffstedt et al., 2001).

Most in vivo studies in volunteers reported a lack of effect of the R16G or the Q27E SNP on chronotropic and inotropic responses to agonists, such as terbutaline (Table 7) (Gratze et al., 1999; Hoit et al., 2000; Bruck et al., 2003a). In light of the limited role of β_2-AR in the human heart (Brodde and Michel, 1999), this is not surprising. Studies on vascular function have reported that the Glu27 variant is associated with higher responsiveness (Cockcroft et al., 2000; Dishy et al., 2001), whereas reports on the R16G SNP are equivocal with lower responsiveness of the Gly16 allele (Gratze et al., 1999; Hoit et al., 2000), no difference (Dishy et al., 2001), or higher responsiveness in carriers of the Gly16 allele being reported (summarized in Table 7) (Cockcroft et al., 2000; Garovic et al., 2003). This apparent contradiction with the in vitro data could be explained by the finding that β_2-AR are always exposed to some down-regulation

TABLE 7
Association of the ADRB2 variants with different cardiovascular and pharmacogenomic phenotypes

Variant	Parameters Analyzed	Results	Reference
R16G/Q27E	Expression of receptor variants in cell lines	Arg16, increased agonist-induced receptor down-regulation; Gln27 completely resistant to down-regulation	Green et al., 1994
T164I	Functional analysis including transgenic animals	Ile164 is dysfunctional variant	Turki et al., 1996
R16G/Q27E	Expression of β_2 receptors on lymphocytes; receptor coupling	No effect observed	Lipworth et al., 1999
R16G	Salbutamol application in 57 volunteers	Gly16Gly16 decreased vasodilation	Gratze et al., 1999
R16G/Q27E	Functional analysis of human lung mast cells	Gly16 and Glu27 resistant to agonist-induced down-regulation	Chong et al., 2000
R16G/Q27E	Functional analysis of human airway smooth muscle cells	Glu27 associated with increased down-regulation	Moore et al., 2000
R16G	Hemodynamic terbutaline responses in 20 volunteers	No major differences; increased limb blood flow at highest terbutaline doses in Gly16 carriers	Hoit et al., 2000
R16G/Q27E	Brachial artery infusion with isoproterenol in 127 volunteers	Heterozygous Gln27 and homozygous Arg16 lower baseline blood flow, attenuated isoproterenol effect	Cockcroft et al., 2000
R16G/Q27E	Isoproterenol-mediated venodilation in 26 volunteers	Arg16 increased agonist-mediated desensitization; Glu27 increased agonist-mediated responsiveness	Dishy et al., 2001
R16G/Q27E	Down-regulation of β_2-AR on lymphocytes upon treatment with terbutaline for 2 weeks in 23 healthy volunteers	No difference in extent of down-regulation; delayed onset in homozygous carriers of Glu27	Bruck et al., 2003a
T164I	Hand vein responses upon isoproterenol and phenylephrine in 26 volunteers	Ile, 5-fold reduction in sensitivity to vasodilatation and increased vasoconstriction	Dishy et al., 2004
T164I	Hemodynamic terbutaline responses in 16 volunteers	Ile, blunted terbutaline-induced heart rate increase and contractility; reduced desensitization	Bruck et al., 2003b
R16G/Q27E	Hemodynamic terbutaline responses in 32 volunteers	Glu27 homozygotes desensitize more slowly	Bruck et al., 2003c
Q27E	Outcome in 80 heart failure patients treated with carvedilol	Gln27 homozygotes lower proportion of good carvedilol responders	Kaye et al., 2003
R16G/Q27E	Terbutaline effects on hand vein dilation in 27 volunteers at baseline and after two terbutaline treatment	Desensitization Arg16Gln27>Arg16Gln27>Gly16Glu27	Bruck et al., 2005
R16G/Q27E	199 heart failure patients treated with β-blockers	No differences in heart rate and ejection fraction before and after β-blocker therapy	de Groote et al., 2005
T164I	Cardiac contractile responses to terbutaline in 55 subjects	Ile, blunted terbutaline-induced contractile response; adverse course in heart failure	Barbato et al., 2007
R16G/Q27E	Registry study with 637 heart failure patients treated with metoprolol or carvedilol	No difference in survival rates	Sehnert et al., 2008
R16G/Q27E	Follow-up of 5895 coronary artery disease patients treated with atenolol or verapamil	No difference in event rates	Pacanowski et al., 2008

stimuli by endogenous agonist and that the relative resistance toward agonist-induced down-regulation may result in greater responses (see below). In agreement with the in vitro data, the rare Ile164 variant of the β_2-adrenoceptor was associated with reduced heart rate and contractile in vivo responses to terbutaline in healthy volunteers (Bruck et al., 2003b) and in patients with heart failure (Table 7) (Barbato et al., 2007).

Based upon the mentioned in vitro findings of altered susceptibility to agonist-induced desensitization in some (Moore et al., 2000) but not other cell types (Chong et al., 2000), other studies have assessed whether any of the above genotypes is also associated with alterations in in vivo desensitization upon extended agonist treatment. The results of such trials have remained equivocal. Thus, the Arg16 rather than the Gly16 variant of the β_2-AR was reported to be associated with enhanced short-term agonist-induced desensitization in studies using the dorsal hand vein technique (Dishy et al., 2001). In other studies, volunteers were treated orally with terbutaline for up to 2 weeks with subsequent assessment of chronotropic and inotropic response to intravenous terbutaline (Bruck et al., 2003c). In these experiments, neither the R16G nor the Q27E polymorphism affected the extent of agonist-induced

desensitization, but the Glu27 homozygotes exhibited a slower desensitization than the other groups. The same group of investigators also reported similar findings for the in vivo down-regulation of lymphocyte β_2-AR (Bruck et al., 2003a, 2005).

Although β-AR agonists have a well established place in obstructive airway disease—which is out of the scope of this review—and may cause cardiovascular side effects such as tachycardia, their primary use for the treatment of cardiovascular disease is largely limited to settings of acute heart failure (e.g., immediately after cardiac surgery or in shock). With possible exception of the rare β_2-AR T164I polymorphism, the overall, although not unequivocal, evidence suggests only a minor role, if any, of β-AR polymorphisms in short-term agonist responses. The case for an effect of β_2-AR SNPs on susceptibility to agonist-induced desensitization is more convincing; however, its clinical relevance for agonist use in cardiovascular medicine remains unclear because such drugs typically are not administered long term. When such agonists are used long term in pulmonary medicine and exhibit cardiovascular side effects, the role of polymorphisms affecting desensitization also remains unclear in light of the frequent concomitant administra-

tion with glucocorticoids, which can counteract agonist-induced β_2-AR desensitization (Postma et al., 2008).

2. β-Antagonist Responses. The interpretation of pharmacogenomic β-AR antagonist data are less straightforward than that of agonist data in several ways. For example, a functionally relevant polymorphism in a β_2-AR may affect prejunctional control of NE release and, hence, may modulate antagonist responses by making it compete with altered amounts of agonist. Moreover, responses to β-AR agonists have mostly been studied upon short-term administration, whereas responses to antagonists, particularly in patients, have mostly been investigated upon long-term use. The latter implies the possibility that genetic variants may not primarily influence the drug response itself but rather homeostatic mechanisms occurring during prolonged drug treatment. Furthermore, clinical medicine uses β-AR antagonists that either have similar affinity for β_1- and β_2-AR or exhibit some degree of selectivity for β_1-AR. In most cardiovascular indications, β_1-selective and nonselective antagonists are similarly effective, indicating that blockade of β_1-AR may be sufficient to reach a therapeutic response. Nevertheless, in some indications, such as congestive heart failure, not all β-AR antagonists are similarly effective (Brodde, 2007). Although the reasons for such compound-specific effects are not fully clear, they may relate to a phenomenon called ligand-directed signaling or biased agonism (Michel and Alewijnse, 2007). In this regard, it was reported that the β-AR antagonists bisoprolol and metoprolol exhibited similar inverse agonism at Gly389 and Arg389 variants of the β_1-AR, whereas carvedilol showed significantly greater inverse agonism at the Arg389 variant (Rochais et al., 2007). Within the same study, carvedilol also had greater inhibitory effects on spontaneously beating cardiomyocytes transfected with the Arg389 compared with the Gly389 variant. Other investigators reported enhanced negative propranolol effects on inotropy in Arg389 transgenic mice (Mialet Perez et al., 2003). Thus, in vitro and animal data demonstrate that findings obtained with one β-AR antagonist are not necessarily predictive for those with other representatives from that class. Finally, it is being debated whether polymorphisms related to drug-metabolizing enzymes (i.e., CYP2D6 poor metabolizer state in the context of metoprolol or carvedilol administration) may alter clinical effects of β-AR antagonists in a way that overshadows those of polymorphisms in the receptors or their signal transduction pathways (Kirchheiner et al., 2004; Zineh et al., 2004; Terra et al., 2005; Ismail and Teh, 2006). Although some of these arguments on theoretical grounds should also be applicable to at least some β-AR agonists, until now they have been considered mainly in the context of antagonist exposure.

Several groups of investigators have studied clinical responses to β-AR antagonists depending on β_1-AR genotype, most of which were performed in patients with hypertension. Two studies with short-term single dose administration of atenolol (Sofowora et al., 2003) or metoprolol (Liu et al., 2003) to healthy volunteers reported larger reductions of blood pressure and/or resting heart rate in homozygous Arg389 compared with homozygous Gly389 carriers (Table 6). Likewise, a patient study reported that long-term treatment with metoprolol yielded greater blood pressure reductions in Arg389 carriers (Johnson et al., 2003). However, four other studies did not confirm greater hemodynamic effects of β-AR antagonists in patients with an Arg389 compared with those with a Gly389 genotype (summarized in Table 6) (O'Shaughnessy et al., 2000; Filigheddu et al., 2004; Karlsson et al., 2004; Beitelshees et al., 2006).

Given that variants in the AR genes affect hemodynamic responses in β-blocker therapy and that such variants also affect the risk of congestive heart failure as proposed for β_1- and α_{2C}-AR variants (Small et al., 2002)—although both notions are not without controversy— one major question is whether β-blocker therapy affects primary endpoints of patients with heart failure in a genotype-dependent fashion. In a substudy of the MERIT HF trial (Metoprolol CR/XL Randomized Intervention Trial in Chronic Heart Failure), 307 patients on metoprolol and 293 patients on placebo were genotyped for the *ADRB1* G389R variant. After a follow-up period of 12 months, the *ADRB1* variants were associated with neither the degree of heart rate reduction nor with all-cause death or hospitalization (Table 6) (White et al., 2003). One problem of this study is that outcomes were compared between *ADRB1* genotypes irrespective of the respective treatment assignment, making it difficult to draw conclusions about β-blocker therapy. In contrast, the BEST study (β-blocker Evaluation of Survival Trial) enrolled 1040 patients with heart failure who were treated with bucindolol or placebo in addition to standard heart failure therapy. The patients were genotyped for the *ADRB1* G389R variant. This phenotype did not affect hospitalization rates and survival in the placebo group. However, homozygous carriers of the Arg389 variant treated with bucindolol exhibited a significant reduction in mortality and hospitalizations, whereas β-blocker treatment with bucindolol was without effect in carriers of the Gly389 variant, indicating a strong pharmacogenomic effect (Liggett et al., 2006). Bucindolol is a nonselective β-blocker with additional sympatholytic effects (Liggett et al., 2006). It displays substantially higher intrinsic sympathomimetic activity (partial agonism) than metoprolol and carvedilol. However, depending on the examined tissue, it can also behave as a partial inverse agonist (Maack et al., 2000). Whereas metoprolol, bisoprolol, or carvedilol (i.e., β-blockers devoid of intrinsic sympathomimetic activity but with inverse agonistic activity) markedly reduced mortality in patients with heart failure, this was not the case for bucindolol (The Betablocker Evaluation Survival Trial Investigators, 2001). Together, the situation with bucin-

dolol may not be representative for other more frequently used compounds. An observational trial with 227 heart failure patients on standard therapy, including β-blockers, reported that carriers of two copies of the *ADRB2* Arg16-Gln27 haplotype had a 2.5-fold increase in risk for adverse outcomes within 4 years. Variants in *ADRB1*, *ADRA2C*, and in components of the RAAS (*ACE, AGTR1, AGT*) had no effect on mortality (Shin et al., 2007). The problem of this work is that it does not allow us to classify observed pharmacogenomic effects to a definite class of drugs. In another study on the role of *ADRB2* variants in heart failure therapy with carvedilol, homozygous and heterozygous carriers of the Glu27 allele had an almost 3-fold higher chance compared with homozygous carriers of the Gln27 allele to belong to the responder group defined by an improvement in ejection fraction of more than 10% (Table 7) (Kaye et al., 2003). In contrast, *ADRB2* variants did not affect the degree of improvement in left ventricular ejection fraction during 18 months of carvedilol therapy in patients with heart failure in another study. Here, improvement in left ventricular ejection fraction was best in patients who were homozygous carriers of the gain-of-function Arg389 variant in *ADRB1* (Tables 6 and 7) (Chen et al., 2007).

In a recent study, 637 patients with heart failure from two registries were discharged on a therapy with metoprolol or carvedilol in addition to standard RAAS inhibition and diuretic therapy. They were genotyped for the *ADR1B* variants S49G and G389R, the *ADRB2* polymorphisms G16R and Q27E, and the *ADRA2C* variant del322-325 and followed for approximately 3 years. None of the investigated variants affected all-cause mortality or transplant-free survival (Sehnert et al., 2008). A major problem of such a registry study is that adherence of the patients to drug therapy was tested only 60 days after enrollment. Furthermore, no adjustments for β-blocker dose was made. Nevertheless, this study is in accordance with a smaller trial that did not observe an effect of any of these variants on improvement in left ventricular ejection fraction upon β-blocker therapy in patients with heart failure (de Groote et al., 2005). In another recent study, 5895 patients with coronary artery disease and hypertension were treated with either atenolol or verapamil (sustained release) for blood pressure control. Patients with the *ADRB1* Ser49-Arg389 haplotype (i.e., the β_1-AR variant with intrinsic gain of function) exhibited an 8-fold higher mortality if treated with verapamil. However, if patients with this haplotype were treated with atenolol, their mortality risk increased by 2-fold only compared with the other haplotypes, a nominal increase that was not significant. Polymorphisms in *ADRB2* had no conclusive effects (Tables 6 and 7) (Pacanowski et al., 2008). Together, this would favor a concept where β-blocker therapy could abolish risks induced by β_1-AR variants. It further indicates that pharmacogenomic analysis of certain target genes

may have special relevance for drugs not affecting these targets, and it further underscores the close association between pharmacogenomics and disease-modifying genes (Büscher and Grasemann, 2006).

Whereas most pharmacogenomic studies on β-AR ligands focused on direct drug targets (i.e., AR genes), several groups have now addressed the impact of genetic variants in proximate signaling pathways. β-AR couple to $G\alpha_S$, and a common silent polymorphism in the $G\alpha_S$ gene (*GNAS*) has been identified (rs7121; I131I; T393C; for allele frequencies, see Table 1) that is located in exon 5 (Jia et al., 1999). The T allele of this variant was associated with the risk for and the level of hypertension. Hypertensive carriers of the C allele responded better to β-blocker therapy. These observations, however, have not yet been widely replicated or have only been reproduced for hypertension with certain environmental interactions, including alcohol consumption and smoking (Abe et al., 2002; Chen et al., 2003; Yamamoto et al., 2004; Lu et al., 2006). In a small study, *GNAS* rs7121 alleles were associated with blood pressure-lowering effects of the β-blocker timolol applied as an ophthalmic preparation (Nieminen et al., 2005). There is also evidence that *GNAS* rs7121 alleles affect the activity of the autonomic nervous system (Tabara et al., 2002; Yasuda et al., 2004). $G\alpha_S$ is expressed ubiquitously, and recent reports suggest that the rs7121 variant is also a predictor for cancer survival (Frey et al., 2005, 2006) and psychiatric traits (Zill et al., 2002; Minoretti et al., 2006). How the silent polymorphism in rs7121 translates into these phenotypes is difficult to explain. The *GNAS* gene is genetically highly complex, has four different first exons, and gives rise to at least six different transcripts. *GNAS* belongs to the class of imprinted genes, and some transcripts are transcribed maternally, paternally, and biallelic, partially in a tissue-dependent fashion (for review, see Weinstein et al., 2004, 2006). The rs7121 is located in a recombination hotspot of *GNAS* centered on exons 4 and 5, separating two haploblocks (Yang et al., 2004). It has been demonstrated that rs7121 is in close linkage disequilibrium with rs6123837, a polymorphism located in the putative promoter region of the start exon, that is involved in the generation of $G\alpha_S$ (Frey et al., 2008). A demonstration that these *GNAS* variants are actually associated with altered $G\alpha_S$ protein expression is lacking. The potential role of variants in the *GNB3* on α_2-AR signaling has already been reviewed in section II.B. In the context of β-AR ligands, only minor evidence exists for a role of these variants on the antihypertensive response to β-blockers (Filigheddu et al., 2004). In a clinical trial with young healthy volunteers, carriers of the *GNB3* 825T allele exhibited a significantly elevated stroke volume and a reduced peripheral resistance at baseline. Administration of propranolol resulted in a significantly greater fall in stroke volume in carriers of the T allele (Table 5) (Schäfers et al., 2001).

An important element in β-AR signaling—and also for many other GPCRs—is the function of G protein-coupled receptor kinases (GRKs) that were originally discovered by their ability to phosphorylate the activated forms of β-AR and related GPCR. Phosphorylation is followed by binding of arrestin proteins, which prevent receptors from activating downstream heterotrimeric G protein pathways while allowing activation of arrestin-dependent signaling pathways (e.g., cascades involved in cell proliferation or receptor trafficking) (for review see Premont and Gainetdinov, 2007; Moore et al., 2007). A L41Q variant has been identified in the gene of GRK5 (Liggett et al., 2008). GRK5 and GRK2 (also known as β-AR kinase) are involved in the phosphorylation of β-ARs and contribute to β-AR desensitization. The GRK5 Leu41 variant was more potent in uncoupling isoproterenol-stimulated responses than the GRK5 Gln41 variant. The GRK5 Leu41 variant occurs predominantly in persons with African descent. In patients treated with β-blockers for heart failure or cardiac ischemia, mortality was reduced in carriers of the GRK5 Leu41 variant (Liggett et al., 2008).

Other groups have analyzed variants in various genes not directly involved in β-AR signaling, including those for angiotensinogen (AGT), the AT₁ receptor, and transforming growth factor β1 and for diverse outcome parameters, such as alterations of left ventricular mass index (for review, see Shin and Johnson, 2007). However, in most cases in which associations between variants and β-blocker responses were identified, the pathophysiological explanations remain unclear, and in most cases these reports await independent confirmation.

In conclusion, the clinical response to β-AR antagonist may be modulated by heterogeneity in a range of genes, but most of the proposed polymorphism effects in genes other than the β-AR remain to be confirmed. Among β-AR polymorphisms, the Arg389 variant of the β₁-AR is hyperfunctional in preclinical studies. Although this has been proposed to translate into altered β-AR antagonist responses by some investigators, the majority of patient studies have not confirmed this finding.

III. Angiotensin II Receptor Ligands and Inhibitors of Angiotensin-Converting Enzyme

Inhibition of the RAAS is a mainstay of current cardiovascular therapy. Estimates based on prescription data suggest that approximately 10% of the population in industrialized countries such as Germany is treated long term with these compounds (Schwabe and Paffrath, 2008). ACE inhibitors and blockers of the AT₁ receptor are used for the treatment of hypertension, congestive heart failure, and diabetic nephropathy, among other indications. Aliskiren, the first oral renin-inhibitor, has increased the armamentarium of RAAS inhibition. Furthermore, blockers of the mineralocorticoid receptor, such as spironolactone and eplerenone, which are now frequently used in the therapy of heart failure, also belong to this functional class of drugs. AT is an agonist at two GPCRs, the AT₁ and AT₂ receptors. AT₁ antagonists and AT are ligands of these receptors. Both AT₁ receptor function and the generation of AT are modulated by common genetic polymorphisms, which are reviewed in section III.A.

A. Angiotensin-Converting Enzyme Inhibitors and Genetic Variants in the Angiotensin-Converting Enzyme Gene

Given the key importance of the RAAS, many groups searched for genetic variants in the genes of its compounds upon cloning of the respective genes. One of the first widely investigated common variants in cardiovascular medicine is the I/D polymorphism in the ACE gene (ACE, rs1799752; allele frequencies see Table 1; Fig. 5). The deletion comprises 287 base pairs in intron 16 of ACE corresponding to an Alu repeat genetic element. The D allele of this polymorphism is associated with an enhanced enzyme activity (Marre et al., 1994). This I/D polymorphism itself may not directly cause increased ACE activity but it is in linkage disequilibrium with other polymorphisms directly affecting enzyme activity. In this regard, the A2350G SNP shows a tighter correlation with ACE activity than the I/D polymorphism (Narita et al., 2003). However, only little information is available on the role of this SNP in drug responses, and most studies still focus on the above I/D polymorphism.

The ACE I/D polymorphism has been associated with the risk for many cardiovascular disorders, although many observations remained unconfirmed. Cardiovascular and cardiometabolic disorders allegedly associated with the ACE alleles comprise atrial fibrillation (Ravn et al., 2008), impaired glucose tolerance (Bonnet et al., 2008), diabetes (Feng et al., 2002), diabetic nephropathy (Ng et al., 2005; Hadjadj et al., 2007), myocardial infarction (Niu et al., 2002), hypertension (Agerholm-Larsen et al., 2000), increased carotid artery wall thickness (Sayed-Tabatabaei et al., 2003), left ventricular hypertrophy (Kuznetsova et al., 2004), and Alzheimer's disease (Meng et al., 2006).

Associations between ACE genotype and clinical response to ACE inhibitors have been tested for a range of diseases. Although most studies have focused on blood pressure lowering, many other studies have investigated preventive effects in (diabetic) nephropathy or other indications of this drug class. Associations between I/D genotype and blood pressure responses have been tested for a range of ACE inhibitors, including benazepril, captopril, enalapril, fosinopril, imidapril, lisinopril, and quinapril. With regard to the clinical response of blood pressure lowering, a systematic review has reported that of 11 retrieved studies, the D allele was associated with greater ACE inhibitor-induced blood pressure lowering in three studies, whereas it was associated with smaller blood pressure lowering in four other studies; four studies found no difference between genotypes
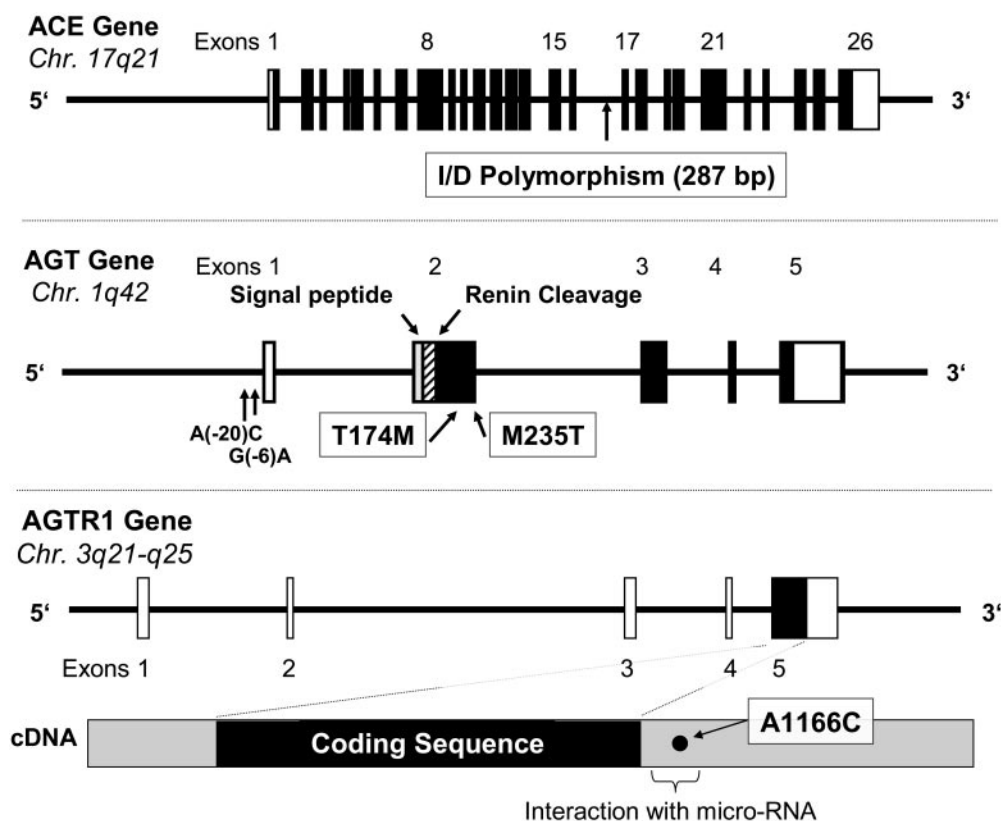
FIG. 5. Top, gene structure of the ACE gene consisting of 26 exons and 25 introns. The I/D polymorphism (rs1799752) affects intron 16. Arrows denote regions where SNPs are in linkage disequilibrium with the I/D polymorphism. Middle, gene structure of the AGT gene. Denoted is the localization of the common M235T (rs699) and T174M (rs4762) variants. The M235T polymorphism is in linkage disequilibrium with promoter SNPs that affect transcription factor binding sites, as indicated. Bottom, gene structure of the gene for the AT receptor type I (AGTR1). Denoted is the common A1166C polymorphisms that has been associated to altered micro RNA binding.

(Koopmans et al., 2003). Using a somewhat different search strategy, other investigators yielded similar conclusions (Mellen and Herrington, 2005). Many of the original studies had been based upon post hoc genotyping, and only few had been specifically designed to test genetic effects. Another systematic review analyzed randomized placebo-controlled trials of ACE inhibitors across a range of cardiovascular and renal indications (Scharplatz et al., 2005). Although a trend for greater antihypertensive responses in white DD carriers compared with II carriers was observed, this analysis also failed to detect consistent effects of the *ACE* I/D polymorphism in antihypertensive treatment. More recent clinical trials did not change this inconclusive picture (Harrap et al., 2003; Arnett et al., 2005; Bleumink et al., 2005; Schelleman et al., 2005).

This notion of inconclusive effects of the *ACE* I/D polymorphism also extends to other indications of ACE inhibitors, such as coronary artery disease and surrogate markers thereof (Tsikouris and Peeters, 2007). In a large study on 2089 Chinese patients with type 2 diabetes, carriers of the II and DI genotypes benefited significantly more from inhibition of the RAAS with regard to development of diabetic nephropathy (So et al., 2006). Despite these inconsistent results, it has been proposed that genotyping for the I/D polymorphism may be cost-

effective for treatment allocations in the prevention of end-stage renal disease (Costa-Scharplatz et al., 2007).

A series of studies has addressed the effect of the *ACE* I/D variant on therapeutic responses to AT_1 inhibitors. The rationale for these analyses is the hypothesis that increased generation of AT could compete with AT_1 blockers at the AT_1 receptor. Because diuretics sensitize the RAAS, some investigators have analyzed whether the *ACE* I/D variant affects blood pressure-lowering effects of diuretics. Whereas the number of available studies in these contexts is smaller than that on ACE inhibitors, they also yielded rather inconsistent results (Kurland et al., 2001, 2002, 2004; Koopmans et al., 2003; Coto et al., 2005). A study in 93 patients with chronic heart failure suggests a modulatory effect of the I/D polymorphism on the left ventricular ejection fraction during spironolactone therapy (Cicoira et al., 2004).

Another key component of the RAAS is AGT. A M235T (rs699) polymorphism in the *AGT* gene was the first and the most scrutinized candidate variant linked to essential hypertension (Fig. 5). It is in linkage disequilibrium with a T174M (rs4762) polymorphism, also frequently addressed (allele frequencies given in Table 1). Available evidence suggests that the T allele of the M235T variant is associated with higher circulating AGT levels (~20% in homozygous carriers) and increased blood pressure

(Jeunemaitre et al., 1992; Staessen et al., 1999). The AGT gene comprises ~12 kb on chromosome 1 and contains five exons. AGT expression is controlled by a 1.2-kb promoter region, and it is further augmented by an enhancer immediately downstream of a second polyadenylation site in the 3′-flanking region. The sole exchange of methionine by threonine at position 235 is not a likely mechanism causing hypertension. This exchange is located distantly from the renin cleavage site, and in vitro renin-mediated angiotensin I generation is kinetically not different for the 235T variant. Currently, a promoter polymorphism at position −6 (G-6A) in strong linkage disequilibrium (−6G−235M; −6A−235T) is a more plausible candidate for differing AGT transcriptions (Inoue et al., 1997; Morgan et al., 1997). Many additional polymorphisms, however, have been identified in the AGT gene, including an additional promoter polymorphism at −20. Several groups have addressed whether variants in AGT affect the response to ACE inhibitor therapy. One trial described an association between "poor responder" status to ACE inhibitors and the *AGT* Met allele (Hingorani et al., 1995). Two other studies refuted this finding (Dudley et al., 1996; Mondorf et al., 1998). Two endpoint studies have analyzed whether variants in *AGT* affect the protective effects of ACE inhibitors. In one trial, the *AGT* 235T allele was associated with a stronger reduction of the risk for nonfatal stroke in users of ACE inhibitors than in users of other antihypertensive drugs, whereas there was no difference in the risk of nonfatal MI (Bis et al., 2003). Following more than 4000 hypertensive subjects of the Rotterdam Study, it was observed that carriers of the *AGT* 235MM genotype treated with ACE inhibitors had a lower risk for myocardial infarction than carriers of the 235MT or 235TT genotypes with ACE inhibitors (Schellemann et al., 2007). In contrast to the study by Bis et al. (2003), no effect on stroke risk was observed here. In summary, the contribution of the *ACE* I/D polymorphism and the *AGT* M235T or T174M variants to the observed variances in cardiovascular drug responses is modest at best and genotyping of these polymorphisms does not aid routine treatment regimens.

B. Variants in the AT_1 Receptor Gene and Responses to AT_1 Blockers

Many groups have intensively searched for genetic variants in the AT_1R gene and potential associations with cardiovascular diseases or pharmacogenomic effects. Meanwhile more than 600 mostly rare variants have been identified in the AT_1 receptor gene (Oro et al., 2007). However, most studies focused on the only common variant of the AT_1R gene, the A1166C SNP located in the 3′-UTR (Table 1; Fig. 5). Its functional significance has long been a matter of debate. Recent data indicate that this SNP interrupts the repression of receptor expression by microRNA-155 (Martin et al., 2007). This could contribute to the regulation of in vivo expression levels of the AT1R. Among the many other polymorphisms that have been described within the AT1R gene, functional implications have been demonstrated only for one very rare coding SNP of the AT1R gene (Hansen et al., 2004).

Similar to the studies on ACE gene polymorphisms, those for the A1166C SNP in the AT1R gene yielded conflicting results. Compared with the C allele carriers of the A1166C SNP, homozygous AA carriers showed blunted responses to the AT_1 receptor antagonist losartan in one study (Miller et al., 1999). A similarly reduced response was observed in AA carriers for irbesartan-induced regression of left ventricular hypertrophy (Kurland et al., 2002). On the other hand, the opposite (i.e., a significantly greater blood pressure-lowering response to losartan) was reported for AA carriers in another study (Coto et al., 2005). Finally, the blood pressure lowering or reduction of cardiac hypertrophy in response to irbesartan was not related to the A1166C or four other SNPs in the AT1R gene in three other reports (Kurland et al., 2001, 2004; Liljedahl et al., 2004).

Similar to studies in which the effect of ACE gene polymorphisms had been tested for responses to AT receptor antagonists, some studies have also assessed the role of *AT1R* polymorphisms on ACE inhibitor responses. An effect of the A1166C SNP on ACE inhibitor responses was found in one study (Benetos et al., 1996) but remained unconfirmed (Hingorani et al., 1995). In three other studies, the A1166C SNP did not affect the response to diuretics (Mellen and Herrington, 2005).

In conclusion, a possible relationship between polymorphisms of the AT1R gene and clinical responses to drugs, which work via such receptors or which may involve such receptors indirectly, have not yielded consistent support for the relevance of these variants. Again, it should be noted that almost of all of these studies represent post hoc genotyping and, in most cases, involved only relatively small patient numbers. Although components of the RAAS are obvious candidate genes for the pharmacogenomics of ACE inhibitors, AT_1 blockers, diuretics—and given the effects of β-blockers on renin secretion—of other antihypertensive drugs, we have to conclude after many studies that the contribution of the known variations in these genes to the observed interindividual variance in drug responses is modest, if present at all. The reason for this sobering notion is most likely that we deal with a highly regulated system. From the many genetically engineered mouse models of the RAAS, it seems that sole changing of the expression levels of RAAS components (the suggested mechanisms of the common RAAS polymorphisms in man), as exemplified by mice harboring 1, 2, 3, or 4 ACE genes, does not change blood pressure as long as the secretion of renin and other pressure regulating systems is adapted to this situation (Bernstein et al., 2005). In this scenario, the effects of the identified variants in the candidate genes of the RAAS are of modest degree, which may easily be counteracted.

IV. Ligands at Other G Protein-Coupled Receptors

The extensive research related to ligands at AR and AT receptors reflects the fundamental importance of the sympathetic nervous system and the RAAS in cardiovascular regulation. Nevertheless, several other GPCR families also contribute in relevant ways to the maintenance of cardiovascular homeostasis and/or the pathophysiology of cardiovascular disease. However, much less is known regarding the pharmacogenomics of ligands at such GPCR. This relative scarcity of pharmacogenomics data, at least, partly reflects that ligands at those receptors are used on a much more restricted scale in cardiovascular medicine compared with those at AR and AT receptors.

A. Muscarinic Acetylcholine Receptors

Muscarinic acetylcholine receptor agonists play only a minor therapeutic role in cardiovascular disease, and data from other indications suggest that commonly used doses of such drugs actually may not be any more effective than placebo (Barendrecht et al., 2007). Although muscarinic receptor antagonists are important in pulmonary and urogenital medicine (Michel and Parra, 2008), their use in cardiovascular medicine is largely limited to the treatment of bradycardiac arrhythmia. Heart rate control by muscarinic receptors is largely mediated by the M_2 subtype of muscarinic receptors (Brodde and Michel, 1999). A rare mutation (C176W) in the M_2 muscarinic receptor gene (CHRM2) has recently been associated with the occurrence and course of dilated cardiomyopathy, potentially mediated by the generation of auto-antibodies against the mutant muscarinic receptor (Zhang et al., 2008). In contrast, common polymorphisms of functional relevance have not been reported for the coding region of the M_2 receptor (Fenech et al., 2001), however, a multiallelic CA tandem repeat polymorphism has been identified in the promoter region of CHRM2 (Fenech et al., 2004). Whereas this polymorphism has major effects on the transcription of CHRM2, its potential impact on tachycardic responses to muscarinic receptor antagonists has not been investigated. The effects of two polymorphisms located in intron 5 (rs324640) or the 3′-untranslated region (rs8191992) of CHRM2, originally identified in a study on alcohol dependence and major depressive syndromes (Wang et al., 2004), were analyzed in volunteers undergoing maximal exercise testing (Hautala et al., 2006). A highly significant difference in heart rate recovery of 12 beats/min was observed in association with the various alleles of these SNPs. The effect increased upon further endurance training. Evidence is lacking whether these variants in CHRM2 also affect chronotropic responses of muscarinic agonists or antagonists or β-blockers.

In summary, the genetic variance of CHRM2 is only incompletely understood. Compared with the efforts to identify and characterize variants in AR genes, we are only at the beginning of research into CHRM2. Whereas apparently functional variants have been identified, detailed pharmacogenomics studies are required to investigate their potential effect on chronotropic responses, not only in response to muscarinic agonists and antagonists but also to β-AR agonists and, of particular importance, to β-blockers.

B. Vasopressin Receptors

Vasopressin acts via two types of receptors, termed V_1 and V_2. V_2 receptors play an important role in the regulation of renal fluid handling; numerous rare mutations of this subtype have been described and play important roles in the pathophysiology of renal diabetes insipidus (Insel et al., 2007). Agonists at V_1 receptors such as terlipressin are sometimes used because of their vasoconstricting properties (e.g., in the emergency treatment of esophageal variceal or other bleedings). Agonists at V_2 receptors such as desmopressin are used in the treatment of enuresis and nocturia (Cvetković and Plosker, 2005). V_2 receptor antagonists such as tolvaptan and lixivaptan, originally developed for the treatment of hyponatremia, are currently undergoing investigation for use in patients with heart failure (Schweiger and Zdanowicz, 2008). However, pharmacogenomic information is not available for any of the vasopressin receptor ligands. Accumulating evidence suggests that brain peptide hormones—including vasopressin and oxytocin—have major functions for the "social brain" for instance by modulation of pair bonding, social and sexual fidelity, assurance, and trust. This has sparked intensive efforts to unravel genetic factors in this system. A functional variant in the V_1 receptor has been described that affects such neuropsychological traits (Walum et al., 2008). With a greater understanding of the genetic diversity in this system pharmacogenomic questions can in turn be readdressed in more detail.

C. Endothelin Receptors

Two subtypes of endothelin receptors exist, termed ET_A and ET_B, and several mutations and polymorphism have been described in the corresponding genes (Rossi and Pitter, 2006). Although some of the ET_B receptor variants may be important in the pathogenesis of Hirschsprung's disease, there are also several reports linking them to cardiovascular disease, such as arterial and pulmonary hypertension or congestive heart failure, but many of these associations are not unequivocal or remain to be replicated (Rossi and Pitter, 2006). The only endothelin receptor ligands used in clinical medicine are antagonists such as bosentan, which are used in the treatment of pulmonary hypertension. However, the genetic heterogeneity in these receptors has not been investigated relative to clinical responses to such drugs. On the other hand, one study in healthy volunteers has explored a role of the C825T polymorphism in the G

protein $\beta3$ subunit (see section II.B) on vascular responses to local administration of endothelin-1 and systemic administration of the ET_A receptor antagonist darusentan (Mitchell et al., 2004). The effect of the same C825T polymorphism on the response to endothelin-1 in one of the two vascular beds (skin microcirculation) was also studied by these investigators in a later study (Mitchell et al., 2006). In one of these studies, carriers of the T allele exhibited enhanced dorsal hand vein vasoconstriction upon endothelin-1 administration (not darusentan-sensitive), whereas skin microcirculation responses to the agonist (darusentan-sensitive) were not affected by genotype in both studies; darusentan-induced blood pressure lowering was not related to genotype. These studies raise the possibility that vascular ET_B but not ET_A receptor agonist responses are affected by the C825T G protein $\beta3$ subunit polymorphism; however, these findings await independent confirmation.

V. Conclusions and Prospects

Until now, reports of altered cardiovascular drug responses based upon genetic polymorphisms in GPCRs and their signaling cascades have remained controversial. Common and frequently studied variants in these genes have only modest effects on gene function. Therefore, their effects on clinical responses are at best small relative to those of other factors affecting drug response, including life style, drug adherence, drug interactions, nutritional factors, hepatic and renal functions, drinking and smoking habits, age, and body composition, to name but a few. Effects of gender and ethnicity have also major impacts on drug responses (Rahemtulla and Bhopal, 2005; Duster, 2007; Franconi et al., 2007; Drake et al., 2008; McNamara, 2008). Although both factors have undoubtedly a genetic background, the involved genes and their mechanisms await further elucidation. Furthermore, the increasing understanding of epigenetic mechanisms at the cross-roads of genetics and environment requires an adjustment of the rather static concepts in current pharmacogenomics (Ingelman-Sundberg et al., 2007).

Together, the presented data do not support the concept that pharmacogenomics will drive the choice of drug and/or dosage in major cardiovascular indications for routine therapy in the immediate future. Nevertheless, the concept of personalized cardiovascular medicine remains attractive and may gain clinical relevance for some drugs. We are experiencing long-awaited and breathtaking new results in the field of complex diseases. Mass genotyping, whole-genome analyses with arrays harboring probes for a million different SNPs, the foundation of powerful multinational consortia with thousands of exquisitely characterized patients and controls, and improved statistical and epidemiological methods have resulted in the discovery of novel genes, loci, or polymorphisms implicated in the pathogenesis of obesity, diabetes, or myocardial infarction, to name but a few (Frayling et al., 2007; Sladek et al., 2007; Helgadottir et al., 2008). Although former genetic research in complex diseases predominantly focused on candidate genes, these new approaches allows for the search of novel "anonymous" genes. This already has resulted in the discovery of novel disease genes and genetic variants, with comparably high predictive power. Further research will show whether these novel disease genes are also suitable for the pharmacogenomic prediction of therapeutic responses. For the recently discovered variants in the transcription factor 7-like 2 gene (*TCF7L2*) in diabetes, clear evidence exists that causal disease variants also determine the therapeutic responses to sulfonylurea (Pearson et al., 2007).

A major problem in pharmacogenomics research is the frequent use of small cohorts with limited power to detect and reproduce relevant genetic factors. Genome-wide association analyses require large numbers of well characterized subjects. Hence, future studies must comprise enough patients and controls to reach sufficient statistical power. Furthermore, meticulous characterization and phenotyping of patients is of utmost importance. Whereas these requirements can be fulfilled in part for scheduled controlled clinical trials for new compounds, the analysis for already introduced substances requires new controlled trials for which it is difficult to find sponsors.

For today's clinical practice in cardiovascular medicine, it seems prudent to focus on well characterized robust genetic variants, the genotyping of which may be advantageous for actual treatment decisions. Genotyping of polymorphisms in *VKORC1* and *CYP2C9* for the dosage finding in oral anticoagulant therapy is a promising approach in this regard. With regard to variants in AR, prospective studies are needed to demonstrate (e.g., the value of genotyping at the β_1-AR G389R locus) for differential therapy in heart failure.

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