The functional significance of genetic variation within the β_1-adrenoceptor

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The β-1 adrenoceptor is an archetypal G-coupled protein receptor that controls sympathetic responses in the heart, kidney and adipocytes. It has been widely exploited as a drug target with the development of antagonists to treat cardiovascular diseases such as hypertension, angina and heart failure. Signalling through the receptor is modulated by desensitization and β1- adrenoceptor down-regulation. It is also affected by *in vitro* substitution of specific amino acid residues within the β-1 adrenoceptor. Amino acid substitutions also occur naturally due to polymorphic variation within the human β-1 adrenoceptor gene itself. Since these variants are common (typically being present in >5% of the population), the pharmacogenetic implications are enormous. A number of these variants have been identified, although two have been the particular focus of recent publications: a serine to glycine substitution at position 49 (49S > G) and an arginine to glycine at position 389 (389R > G). The data on the *in vitro* behaviour of these two receptor variants is reviewed here, along with the evidence that they may affect both the risk of cardiovascular disease and the therapeutic response to β-1 adrenoceptor antagonists.

Introduction

G-protein coupled receptors (GPCRs) with ~750 members represent one of the largest superfamilies of genes in the genome. They allow integrated and dynamic signalling to maintain homeostasis in response to physiological and pathological events. It is now clear that single nucleotide polymorphisms (SNPs) or variations are frequent within the genes for these receptors. Coding SNPs (cSNPs) that affect the amino acids encoded into the GPCR receptor protein are of particular interest, since they can potentially affect the responses to drugs or endogenous agonists and therefore alter the pathophysiology of specific disease states. This is especially important for the β_1-adrenoceptor (AR), which mediates many of the effects of endogenous catecholamines that regulate key physiological events involving the heart, kidney and adipocytes: namely heart rate and contrac-

tility, renin release and lipolysis [1–3]. The β_1-AR gene is reported to contain up to 12 cSNPs within its single coding exon [4]. However, recent interest has focused on the two common polymorphisms for this receptor, 389Arg > Gly (389 R > G) and 49Gly > Ser (49 G > S). This review discusses the work establishing a functional role for these β_1-AR polymorphisms and the part they may play in the pathophysiology of complex disease traits involving the β_1-AR. This pharmacogenetic view of the β_1-AR could improve therapeutic targeting for these diseases and patient risk stratification.

The β_1-adrenoceptor (β_1-AR)

The β_1-AR is a typical example of the TM-7 family of adrenergic receptors that were originally classified on the basis of tissue responses to archetypal sympathomimetic agonists [5, 6]. It is activated by enveloping the

agonist into a pocket allowing conformational changes to the integral part of the receptor, the G-protein binding domain. The resulting complex regulates effector molecules in close proximity to the binding domain leading to tissue responses [7, 8]. Activation can lead to beneficial physiological responses, but sustained stimulation plays a key role in the development and progression of cardiovascular disease. To some extent the deleterious effects of chronic adrenergic signalling are modulated by desensitization and down regulation of the β_1-AR [9]. However, pharmacological blockade of the β_1-AR has become routine in the treatment of ischaemic heart disease, hypertension and heart failure reflecting the pathophysiological importance attached to this receptor.

The β_1-AR was first cloned from a human placenta cDNA library and localized to chromosome 10 (10q24-q26) using somatic cell hybrid analysis and *in situ* hybridization [10, 11]. The subsequent construction of chimeric β_1/β_2 receptors and the mutation of individual residues or distinct regions of the β_1-AR have led to a detailed understanding of its various functional domains. For example, transmembrane spanning region IV appears to be the most important determinant of receptor subtype specificity [12]. The third intracellular loop, or more specifically a proline rich region of 24 amino acids within it, is responsible for the less efficient coupling of the β_1-AR to Gs (compared with the β_2-AR) and also affects agonist promoted sequestration [13]. Various levels of constitutive activity are also produced by the replacement of the C-terminal residue of the third intracellular loop (L322) with amino acids differing in their physico-chemical properties [14]. The C-terminal domain itself determines subtype specific (β_1 *vs.* β_2) desensitization patterns when expressed in Chinese hamster and murine fibroblasts [15]. The N-terminal is also functionally important in addition to its phosphorylation and association with cytoplasmic proteins. Hence, mutation of the asparagine residue at position 15 (15 N > A) affects β_1-AR surface expression and dimerization compared with wild type β_1-AR confirming it as a key N-glycosylation site [16].

Variability of response to β_1-AR agonists and antagonists

The responsiveness to β_1-AR antagonist treatment, endogenous catecholamines and susceptibility to cardiac disease show considerable variability between individuals [17–20]. This was initially assumed to be due to disease status or post-translational modification of receptor function [21], coupled with changes in the β_1-AR signal transduction during progression of diseases such as heart failure [22]. However, it is now apparent

that molecular genetics provides at least a partial understanding of interindividual differences, which remain even allowing for obvious confounders such as age, sex, pharmacokinetics, and aetiology/severity of cardiac disease. This situation parallels the impact that pharmacogenetics has had on the closely related β_2-AR [23].

Polymorphisms within the β_1-AR gene (ADRB1)

A polymorphism was actually detected in the β_1-AR gene shortly after it was first cloned using the same 2.4kb clone to Southern blot *Bgl*I digests of genomic DNA from healthy subjects [24]. However it was another decade before the human β_1-AR gene was subjected to systematic mutation scanning and sequencing [25–28]. Currently, the NCBI SNP database reports 12 cSNPs of which eight are nonsynonymous and result in an amino acid substitution (see Table 1). However, it must be emphasized that most of these SNPs have not been formally verified and exact allele frequencies are unavailable. Nevertheless, it is interesting that all of the nonsynonymous SNPs are located within regions that are highly conserved across species (Table 2). A further 16 SNPs have been reported by Podlowski from mutation scanning of patients with idiopathic dilated cardiomyopathy (IDCM) and normal volunteers without evidence of heart disease [29]. These included one located in the N-terminal: G > T exchange at position 175 (59Ala > Ser). The remainder were identified within the C terminus: 1195C > T (399Arg > Cys), 1205 A > G at 1205 (402His > Arg), 1210 A > G (404Thr > Ala) and 1252C > G (418Pro > Ala). However, they are all rare (frequencies of 1–2%) and have not been verified in other studies. Finally, in the 5′ flanking region, a single individual displayed a homozygous C-T exchange at position −38. This same group has reported three further variants in the 5′ flanking region of ADRB1 in patients with IDCM and coronary heart disease (−93C > T, −210C > T and −2146C > T), but it is not known whether these affect gene expression [30]. These data suggest extensive variation within the human ADRB1 gene, but until it is resequenced in a large enough number of subjects and across different racial groups the exact status of many of these SNPs is uncertain.

β_1-AR genetic variation at codon 389

The polymorphism that has aroused most interest has been the C to G switch (1165C > G) that substitutes an arginine for glycine (389R > G) in the C-terminal region of the receptor [25, 26]. Since the substitution lies within the putative G-protein binding domain, it was likely to be functionally important. The 389R residue is

Table 1
The reported SNPs within the coding region of the β₁-adrenoceptor gene (ADRB1)

dbSNP reference*	SNP functionality	Amino acid position	Base change	Position in codon	Amino acid switch	Type of domain
rs1801252	nonsynonymous	49	A > G	1	Ser > Gly	Extracellular
rs7921133	synonymous	105	G > T	3	Leu > leu	Cytoplasmic loop
rs238742	synonymous	252	G > A	3	Gln > Gln	Cytoplasmic loop
rs2773468	synonymous	268	C > G	3	Gly > Gly	Cytoplasmic loop
rs238741	nonsynonymous	318	C > A	1	Arg > Ser	Cytoplasmic loop
rs622397	nonsynonymous	324	A > G	2	Lys > Arg	Transmembrane
rs180897	nonsynonymous	343	G > A	1	Ala > Thr	Transmembrane
rs189429	nonsynonymous	352	G > T	3	Glu > Asp	Extracellular loop
rs1801253	nonsynonymous	389	G > C	1	Gly > Arg	Cytoplasmic tail
rs171170	nonsynonymous	400	G > T	2	Arg > leu	Cytoplasmic tail
rs365722	synonymous	436	C > T	3	Val > Val	Cytoplasmic tail
rs238740	nonsynonymous	460	C > A	3	Asp > Glu	Cytoplasmic tail

The current status of each SNP can be checked using the following url: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?r_s = #, where # is the corresponding rs number.

Table 2
Species alignment of ADRB1 gene to show highly conserved regions containing the reported nonsynonymous human SNPs from Table 1

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                        41                   51              61
Human       SLLPPASE  G/S  P ----EPLSQQWT  A/S  G MGLLMALIVL
Macacque    SLLPPASE   G   P ----EPLSQQWT   A   G MGLLMALIVL
Mouse       SLLPPASE   G   S ----APLSQQWT   A   G MGLLVALIVL
Rat         SLLPPASE   G   S ----APLSQQWT   A   G MGLLLALIVL
Pig         SLLTPASE   G   S ----VQLSQQWT   A   G MGLLMALIVL
Cow         SLLTSASE   G   P ----PLPSQQWT   A   G MGLLMAFIVL
Cat         SPLTPTSE   G   P ----APLSQQWT   A   G IGLLMALIVL
Sheep       SPLRLAAD   L   G QRGTPLLSQQWT   V   G MGLLVALIVL

                            321                 331            341              351
Human       VAL  R/S  EQ KAL  K/S  TLGIIM GVFTLCWLPF FL  A/T  NVVKAFH R  E/D  LVPDR
Macacque    VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   E   LVPDR
Mouse       VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   D   LVPDR
Rat         VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   D   LVPDR
Pig         VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   D   LVPDR
Cow         VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   D   LVPDR
Cat         VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   D   LVPDR
Sheep       VAL   R   EQ KAL   K   TLGIIM GVFTLCWLPF FL   A   NVVKAFH R   D   LVPDR

                        381                391            401
Human       PDFRKAFQ  G/R  L LCCARRAARR  R/L  HAAHGDRPRASGCLAR
Macacque    PDFRNAFQ   R   L LCCARRAARR   R   HAAHGDRPRASGCLAR
Mouse       PDFRKAFQ   R   L LCCARRAACR   R   RAAHGDRPRASGCLAR
Rat         PDFRKAFQ   R   L LCCARRAACR   R   RAAHGDRPRASGCLAR
Pig         PDFRKAFQ   R   L LCCARRVARG   S   CAAAGDGPRASGCLAV
Cow         PDFRKAFQ   R   L LCCARRAACG   S   HAAAGDPPRALGCLAV
Cat         PDFRKAFQ   R   L LCFARRAARG   G   HAAAGDRPRASGCLPG
Sheep       PDFRKAFQ   R   L LCCARRAACG   S   HGAAGDPPRAAGCLAV
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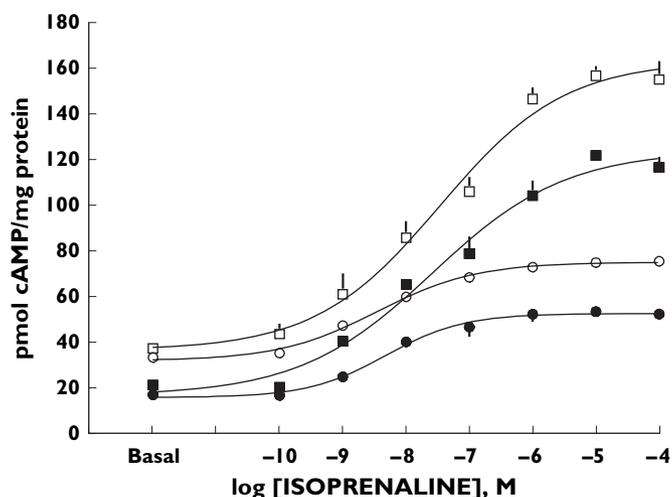
Figure 1

Isoprenaline-stimulated cAMP generation by HEK 293 cells expressing GR, SR, GG or SG haplotypes of the β1-AR. Maximum forskolin stimulated cAMP production was not significantly different between the four haplotypes (GR 388 ± 39 (□), SR 402 ± 22 (■), SG 390 ± 27 (●) and GG 375 ± 32 (○) pmol mg–1 protein). (From [32] with permission)

also highly conserved across species suggesting that 389R and not 389G is the ancestral or wild type of the receptor (Table 2). In fact, expression in a rodent cell line has demonstrated significant differences in the coupling of the 389R and 389G β_1-AR variants to G-proteins in terms of both agonist-promoted receptor binding to Gs and adenylyl cyclase activity [25]. Expression in a human cell line (HEK 293) has produced similar differences in the coupling behaviour of the 389 receptor variants (Figure 1). Although, in HEK cells there were no differences in their constitutive receptor activity (provided they carried the same variant at the 49 position, see below). This may be due to the cell type used (CHW-1102 *vs*. HEK293) containing different postreceptor components, differences in β_1-AR expression density (159 *vs*. 1100 fmol mg^{-1}) or the postreceptor signalling molecule being studied (AC *vs*. cAMP) [31]. Nevertheless, the reduced agonist-induced coupling to G$_s$ for the 389G variant explains the reported absence of high affinity binding sites in competition curves performed with Gpp(NH)p [22, 32].

β_1-AR genetic polymorphism at codon 49

The second polymorphism to arouse interest (145G > A) causes nonconservative substitution of glycine for serine (49G > S) in the N-terminus of the β_1-AR [25, 33]. By analogy with N-terminal substitutions of the β_2-AR, it was expected to affect receptor traffick-

ing. This was confirmed by studying recombinant 49S and 49G receptors and their response to long-term receptor activation (18–24 h). Under these conditions the 49S variant is relatively resistant to agonist-promoted down regulation [34]. The effect of adding cycloheximide to block receptor synthesis suggests that this difference is accounted for by a reduction in the rate of degradation of the 49S receptor after internalization. Immunoblotting of both receptors has shown that the 49S receptor is present in a highly N-glycosylated form that is not found for the 49G variant. This altered glycosylation state may explain the differences in agonist-promoted down regulation, since glycosylation has been reported to affect the down regulation of other G-coupled proteins such as the β_2-AR and gastrin releasing peptide receptor [35, 36]. However, the impact of the 49S > G substitution is more extensive, because there are also differences in the rate of desensitization following short-term agonist exposure (10–20 min), with the 49S variant again being relatively resistant *vs*. the 49G one [37]. Coupling of the receptor variants to adenylyl cyclase is also affected as shown by the constitutive activity of the 49G receptor (see Figure 1). This constitutive activity is almost certainly related mechanistically to its sensitivity to desensitization as well as its increased affinity for β_1-adrenoceptor agonists and inverse β_1-adrenoceptor antagonists [37].

Haplotype responses

Both the 49S > G and 389R > G polymorphisms are common and native β_1-AR function will reflect the variants carried simultaneously at these loci (the receptor haplotype). Individual polymorphisms may act independently to modulate receptor function, although interaction between common polymorphisms is well established, for example, amongst the N-terminal coding variants of the β_2-AR [38]. By expressing the four possible haplotypes for the two common β_1-AR polymorphisms in HEK 293 cells, it is clear that the four haplotypes demonstrate a functional continuum in terms of their coupling efficiency, basal constitutive activity and agonist-stimulated trafficking [32] (Figure 1). Basal activity was affected by the amino acid at position 49 with the magnitude of differences for isoprenaline evoked cAMP and competition binding between the 389R and 389G receptors similar regardless of 49 genotype. The differences in basal receptor function between the GR and the SR haplotypes persisted throughout the range of agonist-stimulated responses, a pattern found also for the GG and SG haplotypes. This work also confirmed increased desensitization for the 49G and 389R recep-

tors compared with the 49S and 389G receptors, respectively, with enhanced changes for the GR haplotype. This has clear implications for attempts to assign functional roles to these polymorphisms *in vivo*. Indeed, the focus on single polymorphisms may partly explain the lack of a consistent pharmacogenetic effect of the 49S > G and 389R > G polymorphisms *in vivo* as discussed below.

Frequencies of β₁ AR genetic polymorphisms

Both the 389R > G and 49G > S genetic polymorphisms are common with allele frequencies amongst healthy individuals of 72/0.28 for 389R > G and 0.85/0.15 for 49S > G [25, 26, 39, 40]. Depending on the type of cardiovascular disease, the frequencies differ slightly as outlined below. More striking are the marked ethnic differences, with black African-Americans having a significantly lower frequency of the 389R allele than other ethnic groups: Chinese 74%, Caucasians 72%, Hispanics 67% and African-Americans 58% [39]. Heart failure is more common in African-Americans compared with otherwise similar cohorts of Caucasians, and the 389R > G polymorphism may provide a genetic basis for this increased incidence. A separate study has confirmed ethnic differences for the 389R > G polymorphism, but failed to identify similar ethnic differences for the 49S > G polymorphism between Caucasians, Chinese and African-Americans [41]. A novel polymorphism in the 5′ flanking region of the β₁-AR gene (−2146T > C) exists in almost complete linkage disequilibrium with the 49S > G polymorphism with reported frequencies for both mutations of 0.108 for controls, 0.163 for IDCM patients and 0.130 for CHD patients [30]. There is also data suggesting linkage disequilibrium between the 49S > G and 389R > G polymorphisms. A study that genotyped almost 700 women found none with the 49G/389G haplotype, despite it being the expected haplotype for 52 of them [42].

Associations of β₁-AR polymorphisms with cardiac function

Despite a consensus in the literature on the functional implications of the 389R > G and 49S > G polymorphisms for the β₁-AR expressed *in vitro*, publications looking at the *in vivo* impact of the polymorphism in humans have been more variable. Several studies have investigated the effects of these polymorphisms on resting haemodynamics and the incidence of hypertension. A Scandinavian study using genotype-discordant sibs, reported that sibs homozygous for the 389R allele had significantly higher diastolic blood pressure and heart rates than siblings carrying either one or two copies of the 389G allele. A case-control study from the same group also found that 389R homozygotes were more likely to be hypertensive with an age and body mass index adjusted odds ratio for hypertension of 1.9 [33]. Of note, there was no effect of the 49S > G polymorphism on blood pressure or heart rate in this study. Humma *et al.* [43] also found that 389R homozygotes had higher blood pressure and heart rate than heterozygotes, although this only applied to the Caucasian subjects in their mixed cohort. Other groups have failed to confirm an effect on resting BP or heat rate from the 389R allele [44–46], and in a large cohort of Chinese and Japanese subjects it was the 49S > G polymorphism that independently affected heart rate and not the 389R > G one [44].

From the *in vitro* behaviour of the dose–response curves for the 389R and 389G receptors it might be expected that *in vivo* differences would be more obvious under high rates of sympathetic activation (and hence receptor occupancy). However, both studies using exercise-induced heart rates or other markers of β₁-AR activation (QS_{2c} and plasma renin release) failed to find an effect of the 389R > G genotype in normal healthy volunteers [39, 40]. Disease status may be a more reliable way of separating the effect of genotype. In the failing myocardium, the loss of β₁-AR coupling efficiency may impact more on the 389G receptor with its already lower coupling efficiency. Two clinical studies support this. Wagoner *et al.* [47] looked at β₁-AR polymorphisms and exercise responses in 263 patients with heart failure and found that despite matched left ventricular function 389R homozygotes had higher peak VO₂ and exercise capacity than 389G homozygotes; heterozygotes were intermediate suggesting a gene dose effect. Analyzing the results as a 389/49 haplotype showed a graded response across the five haplotypes with homozygous 389G/49S having the lowest and homozygous 389R/49G carriers the highest VO₂ and exercise capacity. These physiological variables appear to be more sensitive to β₁-AR genotype since peak heart rate or blood pressure was not affected by genotype.

Another study has reported an effect of the 389R > G genotype on LV mass. In a group of patients attending a renal clinic 389G homozygotes had significantly higher raw LV mass, LV mass index and LV mass indexed-to-height compared to carriers of the 389R allele. This effect persisted even when patients on β-adrenoceptor blockers or renal replacement therapy were removed from the analysis. The 389G homozygotes did have slightly higher blood pressures (~2 mmHg) but this cannot explain their substantially heavier ventricles [48]. It appears that the 389 allele can

affect myocardial cell growth directly, but here it is the 389G and not 389R receptor that confers a gain-of-function.

β₁-AR polymorphisms and responses to antagonists

The discovery of polymorphisms within the β₁-AR has raised considerable interest in the part they might play in determining the clinical response to β₁-AR antagonists. The first study to look for a pharmacogenetic effect analyzed retrospectively the response of a cohort of 147 untreated hypertensive patients randomly assigned to β₁-AR blockade with either bisoprolol or atenolol. In this study, the 389R > G genotype had no influence on the blood pressure or heart rate responses measured 4 weeks from baseline [45]. Similar findings were reported in patients with heart failure recruited into the MERIT-HF trial and treated with metoprolol CR/XL [49]. The 389R > G genotype influenced neither the reduction in heart achieved nor the mortality/morbidity benefit seen in patients randomized to treatment with metoprolol. Another outcome study of 479 patients with early heart failure (mean LVEF <45%) treated with either bisoprolol or carvedilol found that neither the 389R > G or 49S > G polymorphisms affected the event-free survival [50].

In contrast to these findings, small-scale prospective trials have generally reported a highly significant pharmacogenetic effect. Johnston *et al.* [51] found greater reductions in 24 h and daytime diastolic blood pressure in 389R homozygotes compared with 389G carriers. Inclusion of the 49S > G polymorphism actually showed a gradation to the hypotensive response, which was greatest for double homozygotes (389R/49S) and negligible in double carriers of the G alleles (389G/49G). A multivariate analysis further suggested 389R and 49S as the only independent predictors of daytime diastolic blood pressures. Two other studies have reported on the effect of atenolol or metoprolol on basal and exercise-induced blood pressure and heart rate. Sofowora *et al.* [46] reported marked differences in subjects who are 389R homozygotes *vs.* 389G homozygotes: the mean fall in systolic pressures were, respectively, ~9 and ~0 mmHg. Interestingly, there was no difference in the effect on basal heart rate and diastolic BP or the change in any of these variables with exercise. The study by Liu *et al.* [52] reported similar, but smaller, differences in systolic BP reductions after metoprolol in 389R *vs.* 389G homozygotes. This study also found no difference in diastolic BP reductions, although reductions in heart rate were different both at baseline sand after exercise in the two groups. Since β₁-AR antagonists lower blood pressure by different

mechanisms after acute and chronic dosing, it is notable that both of these studies looked at responses to acute dosing with a β₁-AR antagonist. Finally, in a cohort of heart failure patients dose titrated with carvedilol, only 389R homozygotes had a benefit in terms of LVEF: the mean rise was 8.7% *vs.* < 1% in 389G homozygotes [53]. The differences in trial design and use of dose titration may explain why a pharmacogenetic effect of the 389R > G polymorphism in particular was seen in these studies but not in the earlier retrospectives ones.

Ex vivo effect of β₁-AR polymorphisms

A complementary strategy to define the functional importance of receptor polymorphisms is the use of human cells or tissues expressing native β₁-ARs of a particular genotype. A tissue that has been particularly useful for this purpose has been the atrial appendage; which can be recovered relatively easily from patients undergoing cardiac surgery and allows direct assessment of myocyte function *ex vivo*. Hence, atrial appendages recovered from homozygous 389R patients are reported as having increased sensitivity to noradrenaline compared with 389G homozygotes, although maximal contractile responses were intact [54]. This behaviour contrasts with experience in transgenic cell lines where both the sensitivity and maximal responses differed in 389R *vs.* 389G receptors (see Figure 1). However, a further report by Molennar *et al.* [55] has failed to show any *ex vivo* differences at all in the responses of atrial appendages across both the 389R > G and 49G > S polymorphisms. It is not clear whether patient selection or other technical differences between these studies explains this conflict.

Since β₁-ARs are also endogenously expressed on adipocytes this has been an obvious cell type to study. The effect of the 389R > G polymorphism on human fat cell lipolysis was examined in 298 subjects who covered a wide range of body mass indices [56]. Using noradrenaline as the stimulus for glycerol release, this group failed to detect differences in the responses for subjects carrying the 389R *vs.* the 389G receptor. Analyzing either all subjects together or as subgroups based on BMI and gender did not affect the result. The frequency distribution of the 389R > G polymorphism was also similar between lean and obese subjects implying that the 389R > G polymorphism does not influence body mass. However, this interpretation may have been confounded because antilipolytic α-effects were not taken into account and adipocytes express β₂ and β₃-ARs that can also mediate lipolysis. So in summary, the *ex vivo* study of native β₁-ARs has not provided any more clar-

ity about the functional impact of the β_1-AR receptor polymorphisms than the *in vivo* trials.

Disease risk for β_1-AR polymorphisms

Another strategy is to determine whether the β_1-AR genotype shows association with some aspect of cardio-vascular disease such as its prevalence, severity or progression. This approach has been applied to heart failure and ischaemic heart disease, although results have been generally disappointing. For example, in the Cardigene population of clinically well-characterized patients with idiopathic dilated cardiomyopoathy (IDCM), the 389R > G allele frequency was similar to age and sex matched controls [57]. This is surprising given that patients with heart failure have high circulating catecholamine concentrations, the *in vitro* effect of the polymorphism is substantial, there is well described down-regulation of the β_1-AR in heart failure and there is clear therapeutic benefit of β_1-AR blockade. In another study, heart failure patients carrying the 49G allele were associated with decreased mortality risk when followed for 5 years, but again they had a similar incidence of the variant compared with controls [58]. What is lacking to date is a study that addresses haplo-type response rather than the 389R > G and 49S > G taken in isolation. Their impact also appears to be modulated by other adrenoceptor polymorphisms, since the α_{2c}-AR acts synergistically with the 389R allele to increase the risk of heart failure especially amongst Black subjects [59].

The β_1-AR has been extensively implicated in the progression of ischaemic heart disease and myocardial infarction. The reduced function of the 389G variant *in vitro* also effectively produces a naturally β_1-AR blocked form of the β_1-AR suggesting it may be a protective allele. Yet, there was no evidence for this in the longitudinal data set from the WOSCOPS study [60]. Here men with a coronary event were matched for age and smoking status with two control subjects from the same cohort, and 389R > G genotype frequencies compared in the two groups. There were no differences and to date just one study has reported a protective effect of the 389G allele [43]. Since this was actually a protective effect against ventricular arrhythmias in patients with IDCM, it seems unlikely that it can be explained by an influence on IHD alone.

The last association study involved a cohort of 931 Caucasian women genotyped for 389R > G polymorphism, which reported an association of the 389R allele with greater body weight and body mass index [44]. The higher frequency of the 389R variant in obese subjects was hypothesized to lead to increased lipolysis and vis-ceral fat deposition, but is not supported by the *ex vivo* findings in adipocytes [45].

Use of transgenic animals

An obvious genomic tool to use to explore the functional significance of the polymorphisms is targeted transgenesis. This was first reported by Mialat-Perez *et al.* [46] utilizing transgenic mice to over express either 389R or 389G β_1-ARs in ventricular tissues. Hearts from young 389R mice possessed enhanced receptor function and contractility compared with 389G hearts. Presumably reflecting the chronic enhanced adrenergic drive possible through the 389R receptor, the differences in contractile responses to dobutamine reversed in older mice. The protective loss of β_1-AR density was also significantly lower in 389R hearts, although they were more sensitive to the effects on contractility of receptor blockade with propranolol. These results may not be directly applicable to human hearts, which have an intrinsically lower adrenergic tone and level of β_1-AR expression compared with rodents. Nevertheless, the same authors have reported that clinical benefit from β_1-AR blockade of patients with heart failure is more likely in carriers of the 389R receptor. Hence, transgenic models based on the 49S > G polymorphism or haplotype extremes of 389R > G/49S > G could provide further important clues about the role of β_1-AR pharmacogenetics in human heart failure.

Conclusions

The search for functional evidence of the importance of the two common β_1-AR polymorphisms, 389R > G and 49S > G has suggested substantial effects both *in* and *ex vivo*. But a functional comparison especially outside of transgenic cell lines has been very inconsistent, and this lack of consistency needs to be addressed. Nevertheless, it seems likely that these polymorphisms will impact on clinical practice, both in terms of predicting cardiovascular disease risk as well as in the pharmacological management of cardiac disease with β_1-AR antagonists.

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