The impact of \( \beta \) 2 adrenergic receptor polymorphisms on the outcomes in cardiovascular diseases

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Abstract

Cardiovascular diseases (CVD) include a heterogeneous group of multifactorial conditions and represent the major health problem in the western society. Many studies have evidenced that inter-individual variability affects the prognosis and the response to pharmacological treatment in patients with CVD. The identification of genetic markers to select patients more susceptible to develop cardiovascular complications has a therapeutic interest for undertaking individualized therapeutic approach. The sympathetic nervous system acts through adrenergic receptor subtypes and plays a key role in the development and prognosis of CVD. In particular, \( \beta \) 2 adrenergic receptors (\( \beta \) 2ARs), expressed in a wide variety of tissues, are critical regulators of cardiac output, peripheral vascular resistance and metabolism. Several variations with multiple single-nucleotide polymorphisms have been identified in \( \beta \) 2AR gene. There are 3 common \( \beta \) 2AR polymorphisms characterized in more detail for their influence on functional receptor activity. In particular, the changing an arginine for a glycine at position 16 of the receptor protein (Arg16Gly) is associated with increased agonist-induced down-regulation; the substitution of glutamine with glutamic acid at position 27 (Gln27Glu) leads to resistance to down-regulation; the substitution of threonine with isoleucine (Thr164Ile) at position 164 causes receptor uncoupling from the G protein. Many studies have indicated the association of \( \beta \) 2AR polymorphisms with various cardiovascular and metabolic diseases and have contributed to indicate the \( \beta \) 2AR gene variants an appropriate target for investigating possible links between receptor polymorphisms, drug responses and susceptibility to CVD. However, the reports on the association of \( \beta \) 2AR polymorphisms with clinical outcomes of CVD have been contradictory. In this review, we will illustrate the effects of \( \beta \) 2ARs genetic variability on the management of CVD.

Introduction

Cardiovascular diseases (CVD) include a heterogeneous group of multifactorial conditions and represent the major health problem in the western society.1,2 Despite recent advances in the medical treatment, mortality and morbidity rates in CVD remain elevated.4 Identification of prognostic factors is an important aspect of CVD management that has been the focus of intense clinical and basic research.5 The variability of prognosis and drug responses in patients with CVD is only partially explained by factors such as medication adherence, demographic status, functional condition and behavioral attributes.6 Several studies have suggested an important role of inheritance as prognostic factors on risk and variable clinical course of CVD.7,8 Pharmacogenetic is the science that studies the role of genetic variability in determining inter-individual (between-subject) differences in the response to a pharmacologic therapy.9 In particular, the purpose of pharmacogenetic research is the use of individual genetic profiles to develop a personalized medical therapy capable to obtain an optimal drug response in subjects with a given disease.9 The sympathetic nervous system (SNS) has a wide variety of cardiovascular actions. Most of SNS functions are mediated by \( \beta \) ARs, G-protein-coupled receptors with a pivotal role in the regulation of cardiac output and peripheral vascular resistance. The gene encoding for the \( \beta \) 2AR is highly polymorphic in humans and these variants may influence the sensitivity to adrenergic signal transduction.10 Moreover, \( \beta \) 2AR gene polymorphisms are possible candidates for risk factors and predictors of response to treatment of CVD.11 In particular, Arg16Gly variant is associated with an increase of agonist-promoted down-regulation,1 while the Glu27 variant was associated with complete resistance to down-regulation. The Thr164Ile polymorphism is represented within the ligand-binding pocket and its presence causes receptor uncoupling from the G protein12. These polymorphisms have been implicated in various cardiovascular and metabolic phenotypes.13 To date, various case-control studies have been conducted to investigate the relationship between \( \beta \) 2AR gene polymorphisms and cardiovascular risk in different population groups,1,15 but the results have been conflicting and inconclusive. One reason for this inconsistency may be the typically small sample size of the individual studies, which may mean that there was insufficient statistical evidence to reach an agreement.

\( \beta \) adrenergic receptors

The \( \beta \) ARs are members of G-protein-coupled receptor family16 which play a critical role in the regulation of cellular functions.17 The \( \beta \) ARs share a common structure with seven hydrophobic transmembrane segments, an extracellular amino terminus and a cytoplasmic carboxy terminus that contribute to the binding pocket occupied by the ligand.18 Three different \( \beta \) AR subtypes have been pharmacologically identified and they are encoded by distinct genes. The genes are 40-50% similar and encode single polypeptide chains of between 400 and 500 amino acids. The genes encoding \( \beta \) 1ARs and \( \beta \) 2ARs are intronless, whereas those encoding the \( \beta \) 3ARs have introns with part of the C terminus encoded by the second exon.19 Agonist-induced activation of \( \beta \) ARs catalyzes the exchange of guanosine triphosphate for guanosine diphosphate on the \( \mathrm{G}_{\alpha} \) sub-unit of G proteins, resulting in the dissociation of the heterotrimer into active \( \mathrm{G}_{\alpha} \) and \( \mathrm{G}_{\beta \gamma} \) sub-units, which are competent to signal independently.19 Prolonged SNS activation induces the \( \beta \) ARs desensitization, a complex of autoregulatory processes that causes a decrease of \( \beta \) AR density without variation of intracellular cAMP concentration.20 The non-agonist-specific \( \beta \) AR desensitization is a rapid process in which protein kinase A phosphorylates agonist activated \( \beta \) ARs at serines in the third intracellular loop and the proximal cyto-
plasmic tail, leading to the uncoupling of the receptor from its signal-transducing G protein Gs. On the other hand, the agonist-specific, or homologous desensitization of βARs is mediated by members of the family of serine/threonine kinases (GRKs) that enhances the affinity of the receptor for interaction with cytosolic proteins known as the β-arrestins.2 The binding between β-arrestin and βAR serves to uncouple the receptor from Gs,2,22 promote internalization of desensitized βARs that can lead to one of several outcomes, including receptor degradation or receptor recycling back to the sarcolemmal membrane.22,23 Prolonged agonist exposure causes a net loss of cellular receptors (down-regulation) with the activation of degradation mechanisms (ubiquitination) that are independent of receptor phosphorylation.14 To restore the membrane compartment of βAR is now required the transcription at the βAR gene level and post-translation conversion of mRNA to protein.22,21

**βAR gene polymorphisms**

The human βAR gene, located on the long arm (q31–32) of chromosome 5,24 is highly polymorphic, with over 80 polymorphisms including 49 single nucleotide polymorphism (SNP) and 4 insertion/deletion variants (Table 1). The βAR-SNPs are organized into combinations that are inherited together into 24 haplotypes that each have a frequency of >1%.25 Many studies have demonstrated the presence of eight SNPs within the 1.5-kb 5'-untranslated region (UTR) upstream from the ATG start codon.22,25 This region contains a short open reading frame for a 19 amino acid leader peptide, called the β upstream peptide (BUP) or the 5'-leader cistron (LC), that controls the βAR gene expression at translational level.25 Moreover, two of the eight 5'-UTR SNPs create and ablate restriction enzyme sites of enzymes such as the PvuII and Bsp1266I, respectively25) and were therefore intensively investigated. Another 5'-UTR SNP, which appears potentially to be important, results from a base change (TC) at –367 bp from the start codon.22,25 It interrupts consensus AP-2 site 7 base pairs downstream of an overlapping Sp1/AP-2 site, a region also containing strong positive promoter activity that can alter gene expression through differences in transcription factor transactivation.28 The analysis of βARs gene also showed that the 3'-UTR, implicated in mRNA stability and translation, contained a poly-C repeat of variable length (11, 12, 13 or very rarely 14C), which is interrupted by polymorphisms at position 1269, giving rise to additional genetic variation.

Gly16Arg and Gin27Glu are two common βAR SNPs in the general population and their allele frequencies vary with ethnicity.27 The Thr164Ile SNP is rare and exists only in the heterozygous state; the frequency of heterozygosity was 3-5% in all populations studied.28

There is tight linkage-disequilibrium within the βAR gene.29 As a result we have common haplotypes: Arg-19 is always associated with Gly16, while Cys19 is associated with either Arg16 or Gly16.23,30 Gin27 is almost always associated with Gin16, whereas Gin27 is associated with either Arg16 or Gin16. Finally, Ile164 is closely associated with Gin16 and Gin27. Accordingly, the WT-β2AR consists of Cys19-Cys–Arg16–Gin27Gln–Thr164Thr.15,31,32

To characterize the functional role of βAR polymorphisms on agonist induced responses, βAR constructs expressing the SNP at position 16, 27, and 164 were assessed in specialized cell lines.24,36 In vitro studies have demonstrated that the Gin16 and Gin27 variants do not alter basal or agonist-induced ligand binding and adenylyl cyclase activity.35 However, the Arg16Gly and Gin27Glu variants affect agonist-stimulated receptor down-regulation.35,36 Gin16 genotype enhanced agonist induced downregulation of the βAR compared with the wide subtype;37 moreover, the Arg16Gly genotype has similar patterns of agonist-induced down-regulation, implying that Arg16 seems to be a recessive allele.27 On the other hand, the Gin27 genotype showed attenuation of βAR agonist-promoted desensitization in comparison with those with the Gin27 genotype.32

To investigate the role of Gin27 βAR on cardiac hypertrophy,13,38 Iaccarino et al.14 used HEK293 lines overexpressing Gin27 and Gin27 variants of the human βAR and assessed that the Gin27 βAR variant magnifies the catecholamine induced expression of ANF promoter or a cardiac cell hypertrophy indicator.41 In COS-7 cells transfected with Arg19Cys genotypes McGraw et al. showed that Cys19 (β upstream peptide, also known as 5 leader cistron) allele leads to a consistently greater βAR expression as compared with the Arg19 variant.34

Moreover, the Gin27Glu polymorphism’s association with βAR agonist-induced receptor desensitization may be explained, at least in part, by its association through linkage disequilibrium with Arg19 Cys since Gin27 is co-inherited with Arg19Gly and Gin27 is co-inherited with Cys19.34,35

Also 3'-UTR poly-C repeats polymorphisms alter βAR expression levels. Caucasians with the Arg16 genotype present three different haplotypes defined by the length of the poly-C repeats, with haplotype frequencies ranging from 14% to 43%.24 An in vitro study showed that cells transfected with the Arg16–11C haplotype presented lower mRNA and receptor expression, more extensive mRNA degradation and a greater tendency for βAR down-regulation as compared with the other two haplotypes.43 Such differences in Arg16 genotype may result in important phenotypic variation in the in vivo responses to βAR agonists, and may in part, explain the discrepancies in clinical studies investigating the relationship between treatment responses and Arg16–Gly polymorphism alone.30 To date, variations of the poly-C repeat have yet to be investigated in clinical studies, and it is highly likely that assessment of the effects of poly-C polymorphism in conjunction with other βAR polymorphisms or haplotypes would better predict therapeutic responses to βAR agonists. The effect of the Thr164Ile polymorphism on βAR binding affinity and coupling to Gs has been studied in CHW-1102 cells. In these cells, Thr164Ile polymorphism exhibited decreased receptor binding affinity with epinephrine, isoproterenol, and norepinephrine.44 Furthermore, Ile164-Arg-16 showed diminished reduced basal and agonist-induced activation of the adenylyl cyclase, implying a diminished βAR-G protein interaction.45

The impact of the βAR 16Gly and Gin27 variants on agonist-induced desensitization, have been investigated in studies in vitro, and data were quite controversial.28 Some studies have evaluated the effect of the Gin16Gly βAR on the cardiovascular response to exercise showing that homoyzgote Gin16 βAR subjects have an increased cardiac stroke volume and cardiac output both at rest and during exercise compared with homozygote βAR Arg16.46,47 On the other hand, in healthy subjects, other reports have found that the increase of heart rate, contractility and blood pressure (BP) such as are not significantly affected by the Arg16 and Gin27 variants genotypes.47,49
Other investigations have demonstrated that isoprenaline induced increases in forearm blood flow or dilation of hand vein and found that volunteers homozygous Gly16 exhibited larger vasodilatory responses than did volunteers homozygous Arg16.46,50

The Gly16 variant of β2AR causes resistance to down-regulation and therefore attenuation of agonist promoted functional desensitization.41,51 It can be considered a gain-of-function mutation because of the longer duration of stimulation. Indeed, subjects who are homozygous for Gly16 have a significantly higher maximal forearm vasodilation to intra-arterial isoproterenol than those who are homozygous for Glu27, regardless of the amino acid present at position 16.46 It is therefore conceivable to speculate that cardiac Glu27 β2AR drives an exaggerated hypertrophic response to catecholamines.48 Some other reports in the literature contradict these findings, showing that other β2AR-dependent physiologic responses are depressed in the presence of the Glu27 polymorphism in vivo. Interestingly, Bruck et al. found that volunteers homozygous for Gly16 β2AR exhibited a slowed onset in desensitization of cardiac responses or in down-regulation of lymphocyte β2AR density, and this occurred although volunteers carried two or one allele Gly16.46,51 There is not a consensus on the reasons for the discrepancies between in vitro and in vivo. One possible explanation is the antagonizing effect on desensitization of the Gly16 polymorphism, which is in linkage disequilibrium with Glu27, although this viewpoint is also challenged by recent evidence showing that the Gly16 allele may lead to enhanced physiologic responses in vivo.12

The Thr164Ile variant of the β2AR occurs only rarely and is found only in the heterozygous form,27 the majority of subjects carrying the Thr164Alle polymorphisms are also carriers of Gly16 variant in combination with the Glu27 variant in vivo.29,52

Thr164Ile variant does alter ligand binding and G protein coupling. In cells transfected with cDNA that mimics this SNP, the Ile164 receptor displays a lower binding affinity for β2AR agonists, a 50% reduction in agonist-induced adenylyl cyclase activity, and uncoupling of the receptor from the G protein compared with the wild-type receptor.54,55 In transgenic mice the expression of Ile164 receptor in myocardium tissue impairs resting heart rates and inotropic and lusitropic indices.52 The Ile164 variant of the β2AR gene in endothelial cells loses the ability to mediate cell specific responses to catecholamine.53,54 To gain better insight on the role of Ile164 on atherosclerosis, Piscione et al. showed that adenoviral (Ad) β2AR-Ile164 infection reduces isoproterenol mediated Vascular smooth muscle cells (VSMC) proliferation.55 In humans, vascular responses and heart contractility are altered in subjects carrying the ile164 variant of the β2AR gene compared with wild-type volunteers.32,54,56,57

The analysis of effects of β2AR polymorphisms is therefore inevitably complicated by the strong Linkage Disequilibrium among SNPs which results in the occurrence of several common haplotypes resulting in multilocus effects.29 These limitations make most likely that genetic-epidemiological data alone give details in relevant functional alterations of polymorphic β2AR.12,58,59 Taken together, the available data demonstrate that the β2AR polymorphism might affect functional responsiveness in vitro, ex vivo and in vivo and appear to be associated with cardiovascular disease states in which β2AR are considered to be important.

β2AR gene polymorphisms in hypertension

Hypertension is a multifactorial condition that represents the most important risk factor for cerebral ictus, myocardial infarction and heart failure (HF), as well as the one with the highest incidence in the population, peaking at 60-70% at advanced age. Several studies have identified genetic factors that influence BP and metabolic responses to β-blockers, thiazide diuretics, and rennin-angiotensin system antagonists. Whether such pharmacogenetic differences translate to differences in the clinical outcome of antihypertensive therapy is less clear, particularly when patients receive multiple drugs that are titrated to a target BP. A pharmacogenetic approach to treating hypertension could not only reduce the number and cost of medications but also reduce morbidity and mortality if the outcome of drug treatment differs by genotype. Given the pivotal role of β2ARs in the regulation of cardiac output and peripheral vascular resistance, several studies proposed β2AR gene polymorphisms as strong candidates for risk factors and predictors of response to treatment of hypertension.1,53 The association between hypertension and Gly16 variant was found in Africans,62 but no association either for Arg16 or Gly16 with hypertension, and may result in renal failure and respective increased mortality.66 In recent study of Litovkina et al. observed that β2AR16 Arg variant is associated to development of hypertension and accelerated decline in kidney function in Russian patients suffering from CGN.57

The β-blockade treatment induces a vascular remodeling that correlates with changes in left ventricular wall thickness in hypertensive patients.67 Based on these observations lacarrino et al.68 assessed the impact of the β2AR polymorphisms on cardiac and vascular target organ damage in a population of untreated essential hypertensive patients after evaluation of clinical, anamnestic and biochemical data. This study showed that β2AR gene polymorphisms had no effect of BP values, but affects cardiac remodeling in response to hypertension. In particular, the presence of Glu27 variant is associated to a significantly higher risk of cardiac hypertrophy compared to patients with the Glu27 allele. The effects of Glu27 polymorphism are more predominant in younger patients while it seems to fade with age. The effect of the presence of Glu27 polymorphism on the cardiac remodeling might be related to the gain-of-function in signal transduction induced by the mutation on β2AR gene.21 In a subsequent study lacarrino et al.69 investigated the effects of β2AR variants on the left ventricular mass index (LVMI) regression when BP is reduced with β-blockers (Atenolol), which are unable to completely block β2AR,67 rather than with angiotensin-converting enzyme (ACE) inhibitors (Enalapril), which in hypertension reduce the whole sympathetic discharge.72 This study showed that patients harboring Glu27 β2AR showed a larger regression of LVMI when treated with enalapril rather than atenolol. These results have supported suggested that in Glu27 patients an important effect of anti hypertensive therapy on regression of left ventricular hypertrophy (LVH) is mediated through a non-βBP-dependent mechanism, but depends on enhanced hypertrophic effect of the sympathet ic system.67 ACE inhibitors, which reduce the sympathetic discharge overall,42 are also able to
reverse LVH through the reduction of the hypertrophic effect of catecholamines.\textsuperscript{24} This property may be particularly relevant in Glu27 patients, because by reducing sympathetic activation, it may prevent the more marked catecholamine-mediated hypertrophy stimulus induced by the Glu27 β2AR variant.

Few studies have investigated the effect of Thr164Ile on hypertension. Pereira et al.\textsuperscript{75} reported increased systolic BP in Thr164Ile heterozygotes compared to non carriers in a ethnically mixed Brazilian population.\textsuperscript{75} Furthermore, no significant association between Thr164Ile genotype and hypertension was found in a linkage study with 638 participants from 212 Polish pedigrees with clustering of hypertension.\textsuperscript{76} The lack of consistency amongst these studies may be attributable to ethnic differences in study subjects. Another explanation could be that analyses were not stratified by gender in any of these previous studies, probably due to the loss of power resulting from the reduction in sample size. Thr164Ile heterozygosity was associated with increased diastolic BP in women, but not in men in the Copenhagen City Heart Study.\textsuperscript{77}

\textbf{β2AR polymorphisms in coronary artery disease}

Coronary artery disease (CAD) is one of the leading causes of death in the world, despite the improvement of diagnostic and therapeutic tools. Elevated levels of catecholamines lead to increased βAR activity that results in progressive coronary atherosclerosis and deterioration of cardiac functions.\textsuperscript{59} β2ARs play a pivotal role in the control of myocardial contractility of the failing heart\textsuperscript{56} and several studies have proposed that β2AR polymorphisms may be important determinant of CAD. However, the impact of β2AR polymorphisms on coronary atherosclerosis and cardiovascular clinical events is highly controversial. In the study of Yamada et al., none of the β2AR polymorphisms was associated with increased risk form of myocardial infarction in a Japanese population.\textsuperscript{90} On the other hand, a large series of studies support the association with atherosclerosis. In an observational cohort study in the elderly the Glu27 allele of the β2AR was associated with a lower risk of incident coronary events in this elderly population.\textsuperscript{91} Analysis of the patient cohort from the Physicians’ Health Study demonstrated that only specific haplotype combinations (Ile164-Glu27)-Thr164 and Gly16-Gln27-Ile164) increased the risk for myocardial infarction but this association disappeared after adjustment for other polymorphisms.\textsuperscript{92} Furthermore, Barbato et al. demonstrated that that prevalence of Glu27 variant is higher among CAD patients in central European population and the presence of this allele should be considered an independent disease risk factor for coronary artery disease.\textsuperscript{93} Zak et al. observed a significantly higher prevalence of the Arg allele of Arg16 Gly polymorphism in coronary artery disease (CAD) patients than healthy controls.\textsuperscript{76} A significant correlation between 27Glu allele carrier state and CAD was noted in patient population in Saudi Arabia.\textsuperscript{84} Although the rare incidence, individuals with the Ile164 allele and normal LV function show blunted hemodynamic responses to adrenergic stimulation.\textsuperscript{28} Barbato et al. showed that Thr164Ile polymorphism negatively modulates β2-agonist-mediated myocardial contractile performance in patients with normal and failing myocardium and this β2AR variant is associated with adverse long-term prognosis of patients with congestive HF due to idiopathic cardiomyopathy.\textsuperscript{66} Moreover, Piscione et al. found a relationship between β2AR Ile164 polymorphism and coronary and peripheral artery disease in a prospective study in which were enrolled 330 patients undergoing elective or urgent percutaneous coronary intervention (PCI) for CAD documented.\textsuperscript{85} Interestingly, this study evidenced that Ile164 polymorphism frequency was higher in CAD (12.1% vs 3%, P=0.008) than the control population; β2AR Ile164 mutant is associated with an earlier and more aggressive CAD, and it adversely affects prognosis in patients with severe CAD undergoing PCI. This evidence also showed that a group of patients with peripheral artery disease exhibited a higher prevalence of the Ile164 genotype (7%) with a more severe clinical phenotype than those with Thr164.\textsuperscript{55} These data support the concept that β2AR polymorphism may predict prognosis in CAD.

Nevertheless studies that address the association of β2AR polymorphisms and outcomes in patients with ischemic heart disease present conflicting results. First of all, McLean et al.\textsuperscript{56} showed that specific genetic variations present in the β2AR genes would predict LV remodeling in patients chronically treated with a β1 selective antagonist following a first ST elevation myocardial infarction (STEMI).\textsuperscript{57} Specifically, the Glu27Glu variant was associated with an approximately seven-fold increased risk of LV end systolic dilatation, and a four-fold risk of end diastolic volume enlargement and LV ejection fraction decline at 6 months when compared to the full cohort.\textsuperscript{55} Coronary artery spasm (CAS) is also associated to a wide spectrum of ischemic heart diseases, ranging from variant angina pectoris to acute myocardial infarction and even sudden cardiac death.\textsuperscript{58} Given the important role of the SNS in CAS pathophysiology, some studies have evaluated whether genetic polymorphisms affecting autonomic activity contribute to the pathogenesis of CAS.\textsuperscript{57} Among the numerous polymorphisms related to the SNS, Park et al. have found that β2AR Glu27 allele homozygote state were associated with CAS in a Korean population.\textsuperscript{89} On the other hand, Zhou et al. failed to confirm these data in Chinese population.\textsuperscript{99}

\section*{β2AR gene polymorphisms and heart failure}

One complication of myocardial infarction is the development of adverse left ventricular remodeling and progression to HF. Increased cardiac adrenergic activity is one of the major determinants of the progression of LV dysfunction and the poor outcomes of the patients with HF. Acute and long-term therapy with β-blockers has become a standard following acute myocardial infarction and HF Therapy with β-blockers reduce infarct size and mortality among myocardial infarction and HF patients, most likely by decreasing cardiac energy requirements and modifying arrhythmia risk. Some studies suggested that genetic polymorphisms may mediate differential therapeutic end points of β-blocker treatment, including left ventricular ejection fraction improvement, survival, and hospitalization due to HF exacerbation. However, the association of genetic β2AR polymorphisms and therapeutic end points of β-blocker treatment is object- of controversy. Pacanowski et al. investigated the influence of β2AR and β2AR haplotype variation on the incidence of death, nonfatal myocardial infarction, and nonfatal stroke as well as the pharmacogenetics of β-blocker (atenolol) and calcium channel blocker (verapamil) based antihypertensive therapy in the International Verapamil SR/Trandolapril Study - GENetic Substudy (INVEST-GENES).\textsuperscript{90} Authors showed that patients with the β2AR haplotype containing the Arg16 and Gln27 alleles would be at relatively higher risk for cardiovascular events and that atenolol would be beneficial as compared with sustained-release verapamil (verapamil SR). Pharmacogenetic analysis revealed that the risk for the primary outcome was significantly higher in Gly16-Glu27 haplotype in verapamil SR-treated patients but not in atenolol-treated patients. The analysis revealed that patients with at least one copy of the Ser49-Arg389 β2AR haplotype and zero copies of the Gly16-Glu27 β2AR haplotype (representing 42% of the study population) had better outcomes when treated with atenolol than with verapamil SR (HR 0.42, 95% CI 0.21-0.82, P=0.01). Comparing this result to the HR of 0.64 when considering the β2AR gene alone suggests that a consideration of both genes may be even more informative for identifying those most likely to benefit from β-blocker therapy.\textsuperscript{99} In another study per-
formed on 80 HF patients treated with the nonselective β-blocker carvediol, Kaye et al. demonstrated that subjects carriers of the Glu27 allele were more likely to have an increase in ejection fraction or fractional shortening than those who were homozygous for the allele encoding the Gln27 variant (63 vs 26%, P=0.003) thus suggesting that determination of β2AR status may be of value for tailoring individual therapy in patients with HF.

In contrast, De Groot observed that β2AR polymorphisms did not explain the interindividual variability in the response to β-blocker therapy.

In a recently published study, a β2AR haplotype (Arg16Arg26/Gln27Gln) was associated with increased risk for death or heart transplantation in 220 patients, 95 (80%) of whom were on an ACE inhibitor/angiotensin receptor blocker and a β-blocker at baseline. When considered relative to β-blocker use, this association was most strongly driven by those not on a β-blocker (HR of 3.52 vs HR of 1.55). These results suggest that certain genotypes/haplotypes may be at increased risk of adverse outcomes and that β-blockers may attenuate the risk associated with that genotype/haplotype. Interestingly, these findings are consistent with those from an acute coronary syndrome population, in which the Arg16Gln27 haplotype was also associated with adverse outcomes, even among those treated with a β-blocker. Collectively, these data may suggest that the Arg16Gln27 haplotype of the β2AR may be a high-risk haplotype group deserving of more aggressive therapy. Confirm to this view derives from Troncoso et al., who have evaluated the influence of Gln27Glu β2AR polymorphism on the variable response to treatment with carvedilol in patients with chronic HF. The results of this study showed that chronic HF patients with the Glu27/β2AR allele have a better response to carvedilol.

For the more clinically relevant outcome of survival in HF patients, the results are mixed. Brodde et al. observed that HF patients with the Arg16Arg-Gln27Glu-β2AR seem to have a more pronounced adverse outcome (heart transplantation) and increased risk for sudden cardiac death. In a prospective study on large cohort of clinically treated HF patients who had been prescribed metoprolol or carvedilol, Schnerert et al. failed to found significant effect of β2AR genotypes on incidence of critical end point of survival in β-blocker–treated HF patients. Similar results were obtained by de Groot et al. that found no association between functional βAR polymorphisms and survival in patients with stable HF. However, the authors demonstrated, with a univariate analysis, a possible association between the combined β2AR Gly16Gly/β1AR Gln27Gln genotype and survival.

Recently Petersen et al. showed that β2AR Arg389-homozygous and β2AR Gly389-carrier HF patients treated with carvedilol present a two-fold major risk of mortality relative to all other genotype combinations. There was no difference in survival in metoprolol-treated HF patients between genotype groups. The data indicate that patients with β2AR and β2AR genotypes may benefit more from metoprolol than carvedilol treatment.

In HF, the Thr164Ile polymorphism is characterized by reduced exercise tolerance and higher mortality. However, pathophysiologic mechanisms contributing to the poor outcome of these patients are not clear and it is unclear whether the poor outcome is related to direct effects of the Ile164 polymorphism on the myocardial contractile performance or to systemic hemodynamics. Preliminary study showed that in chronic HF-patients, terbutaline-induced increases in heart rate, but not in contractility, were not different in patients with the Thr164Thr or the Thr164Ile variant of the β2AR.

On the other hand, Wagoner et al. assessed in chronic HF-patients either heterozygous Thr164Ile or homozygous Thr164Thr exercise capacity and found that patients with the Thr164Ile variant of the β2AR 8 had a lower peak V O2 than patients homozygous Thr164Thr. Moreover, Liggett et al. genotyped 259 patients with HF due to ischemic or dilated cardiomyopathy and found that the allele frequencies for the Arg16Gly, Glu27Glu and Thr164Ile polymorphisms of the β2AR did not differ with those assessed in 212 healthy controls. However, those patients carrying the Thr164Ile polymorphism had much more rapid progression to transplantation or death; these data is challenged by the observation from Leineweber in 2006, showing that the frequency of the Ile164 allele is almost identical in healthy controls, chronic HF-patients and heart transplantation-patients.

Several factors contribute to ventricular tachyarrhythmias and sudden cardiac death (SCD) in HF patients and most of them are still completely understood. In this setting, the modulation of β2AR activity with pharmacologic treatment plays a protective role against cardiac arrhythmias. Given their properties to modulate β2ARs activity and β-blockers efficacy, β2AR gene polymorphisms might contribute to the risk stratification for ventricular tachyarrhythmias and appropriate implantable cardioverter defibrillator (ICD) shocks.

It has been observed that Glu27 homozygous patients have an increased risk of SCD and that the Arg16Gln27Glu haplotype may significantly increase the risk of adverse outcomes in HF patients. Recently, Pezzali et al. have indicated for the first time that β2AR polymorphisms affect reverse remodeling and arrhythmic events after cardiac resynchronization therapy (CRT) in patients with HF. This study shows that carriers of the Gln27Glu β2AR variant is associated to greater improvement in LVEF after CRT compared with Glu27 homozygous patients. In addition, Glu27 homozygotes which had a higher incidence of arrhythmic events presented an increased rate of appropriate ICD shocks and cardiac events compared with the other patients.

β2AR polymorphisms and stroke

Stroke is the second most common cause of death and disability worldwide. It is a multifactorial disease influenced by both environmental and genetic factors. The age-related decline in β2AR receptor function and subsequent cyclic AMP generation is a common factor underlying atherosclerosis, vascular insufficiency and hypertension. Stroke is a multifactorial, polygenic disorder, influenced by both environmental and genetic factors. There are several risk factors has been discovered for stroke, such as hypertension, diabetes, smoking, dyslipidemia etc., however these risk factors do not explain why some individual are more susceptible to these environmental determinants in comparison to others with same given risk factors. Some studies have shown that age-associated decline in β-adrenergic receptor sensitivity in the cardiovascular system. A growing body of evidence, suggesting that genetic variant may predispose to developing stroke. Few studies have addressed the association of β2AR polymorphism with ischemic stroke and both positive and negative associations have been reported. A case control study, reported from Italy, indicated a positive association between Gln27Glu polymorphism and ischemic stroke. A previous study by Heckbert et al., failed to show significant association of β2AR variant with the incidence of stroke. A prospective cohort study included 25,225 women showed that the different haplotype combination of β2AR gene variant did not affect the incidence of ischemic stroke in women. A case control study report ed by Zhao et al. did not find significant association of Gln27Glu polymorphism with risk of ischemic stroke, however, higher frequency of variant Glu27 allele in ischemic stroke patients than controls has been observed. A recent case control study confirmed the relationship between β2AR Glu27Glu polymorphism and ischemic stroke in North Indian subjects, suggesting that Glu27Glu polymorphism may confer higher risk of large vessel disease stroke in this population. These preliminary evidences allow to hypothesize that the identification of a subgroup of patients, who are carrier of β2AR receptor polymorphism, may have implication for planning their future stroke treatment strategies.
Role of $\beta_2$AR polymorphisms in metabolic disturbances

The adrenergic system controls glucose and lipid metabolism in the liver, adipose tissue, and skeletal muscle, in part through the $\beta_2$AR. The catecholamines stimulate hepatic glucose production to some extent through the $\beta_2$AR, and also regulates pancreatic insulin secretion. Therefore, the $\beta_2$AR may also constitute a potential candidate gene to explain part of the genetic predisposition to dyslipidemia and related traits. Several investigations have identified associations between $\beta_2$AR polymorphisms and metabolic disorders such as hypertension, insulin resistance, and obesity.114,115 Landsberg116 and Julius117 have proposed that hyperinsulinemia and insulin resistance stimulate sympathetic nervous activity and thermogenesis to limit further weight gain in obese subjects.118 On the other hand, some longitudinal studies observed that sustained sympathetic activity was the prime mover for future weight gain in originally non-obese, normotensive subjects, and that insulin resistance was more an accessory factor.119,120 The $\beta_2$AR is the dominant lipolytic receptor in adipose tissue112,113 and in skeletal muscle.121 The $\beta_2$AR activation increases the intracellular cyclic AMP levels that activate protein kinase A, which in turn promotes activation of hormone-sensitive lipase that catalyses the rate-limiting step in lipolysis.122,123 Recent evidence indicates that a genetic component is important in the pathogenesis of obesity-related hypertension and insulin resistance. In particular, it has aroused great interest the observation that $\beta_2$AR polymorphisms cause marked variations in catecholamine-induced lipolysis in fat cells.128 Some investigation have observed a strong evidence for the linkage between $\beta_2$AR polymorphisms, heightened sympathetic nervous system activity, obesity, hypertension, and the development of insulin resistance.129 Current studies allow the speculation that the Glu27 variant might be associated with higher indices of obesity, higher body fat, larger fat cell volume and higher fasting insulin levels when compared with the Glu27Glu allele.130 However, although the Glu27Variant has been associated with obesity131,132 and Type II diabetes,133 the findings have not been replicated in all studies.129,130

Masuo et al.130 observed that originally nonobese, normotensive subjects carrying the Gly16 allele is associated to high plasma norepinephrine levels and to increase of weight gain and BP. In a weight loss study, the $\beta_2$AR the Gly16 allele of Arg16Gly was associated with resistance to long term significant weight loss, and the Glu27 allele was linked to resistance to short-term weight loss moreover, lean normotensive men carrying the Gly16 allele of Arg16Gly had a higher frequency of insulin resistance.131 Ishiyama-Shigemoto et al. assessed the role Gln27Glu substitution in the development of obesity and obesity-related metabolic disorders and observed that Gln27Glu substitution was higher in obese subjects and in patients with Type II (non-insulin-dependent) diabetes mellitus than control subjects.132 Moreover, this study indicated that the obese subjects carrying the Gln27Glu variant allele had higher concentrations of serum triglyceride than obese subjects homozygous for the wild type allele.133 Conversely, the frequency of Gly16 homozygotes was lower in obese women when compared with non-obese women, although the association was not present in male subjects.134 These results were not confirmed by another study on general population of northern France, in which the Gln27Glu variant did not significantly influence body mass index (BMI).135 The phenotypic effect of two common $\beta_2$AR polymorphisms on body weight and/or plasma lipid and lipoprotein abnormalities were also examined by Ehrenborg et al. in a homogenous and representative population of Swedish healthy men.136 This study observed BMI, such as VLDL cholesterol and triglyceride concentrations were significantly increased in individuals carrying the Gln27Glu variant.137 Laccarino et al. tested the hypothesis that in hypertensive patients a given polymorphism of $\beta_2$ARs might predict the occurrence of metabolic adverse events during $\beta$AR blocking treatment.138 In particular, in this study were evaluated the effects of $\beta_2$AR polymorphism in hypertensive population, which are involved in glucose and lipid metabolism, on the occurrence of diabetes and dyslipidemia observed after long-term treatment with $\beta$-blockers. The $\beta_2$AR Glu27 variant resulted associated with a larger occurrence of dyslipidemia due to increased serum triglycerides, independently from treatment.139 Treatment with $\beta$-blockers in these patients associates with a further significant increase of elevated serum triglycerides and combined dyslipidemia. On the contrary, $\beta$-blockade in patients harboring this polymorphism did not change the occurrence of diabetes or low HDL. This result is particularly noteworthy, because it allows to identify a subpopulation where the occurrence of dyslipidemia after $\beta$-blockade is very likely, with an incidence that is above 60%. The identification of this subpopulation makes safer the long-term treatment with $\beta$-blockers in patients who do not carry the polymorphism and who represent the majority of hypertensive patients.140 Another study observed that metoprolol succinate or carvedilol treatments were associated with a significant increase of baseline insulin and triglycerides levels in hypertensive patients with the Glu27Glu and Gly16Gly genotypes compared with the Gln27Gln and Arg16Arg genotypes; moreover the magnitude of triglycerides elevation over time following $\beta$-blocker treatment was significantly higher among the subjects with the Arg16Arg genotypes, despite lower initial concentrations.139 These data are in line with those of Iwamoto et al.,141 who described the same association between the $\beta_2$AR Glu27 variant and hypertriglyceridemia in unselected populations. According with these data, Daghestani et al.142 observed that in overweight and obese subjects, while did not differ in the genotype and allele frequencies of Glu27, the subjects Glu27 homozygote have greater BMI, waist and hip circumference, higher triglyceride, insulin and leptin levels.143 Similarly in another study Kunnas et al.,144 observed significantly higher amount of visceral fat in women with Glu allele compared to Gln allele homozygote.145 Ishiyama et al. have suggested an association of polymorphisms in the $\beta_2$AR receptor gene with obesity, hypertriglyceridemia, and diabetes mellitus in Japanese subjects.146 They observed that the Gln27Glu heterozygotes were twice as common in obese subjects, and that the frequency of the Glu27 allele was also higher in patients with type 2 diabetes mellitus than in non-diabetic subjects.147 Another Japanese study reported that the Gln27Glu $\beta_2$AR receptor variant was associated with obesity in males.148 Ukkola et al., found that gene-to-gene interactions among the $\alpha_2C$, $\beta_2$, and $\beta_2$AR receptor genes contributed to the phenotypic variability in abdominal obesity and plasma lipid and lipoprotein in a family study in Quebec.149 Though, these and other studies show an association between the Glu27Glu, however, there is considerable debate on this association, because there have been several studies that have failed to show the significant association between $\beta_2$AR gene polymorphism and obesity.150-152 Hayakawa et al., reported that Gln27Glu and Arg16Gly polymorphisms of the $\beta_2$AR gene are not a major contributing factor to obesity, blood pressure, serum lipid levels, uric acid, or free fatty acid levels in 210 Japanese men.153 Oberkoller et al., reported that Gln27Glu polymorphisms in the $\beta_2$AR gene is not a major factor contributing to morbid obesity in Austrian women.154 Kim et al., found that the Gln27Glu and Arg16Gly polymorphisms of the $\beta_2$AR gene are not major factor contributing towards obesity in Korean subjects.155 In a study on 397 Taiwanese subject Glu27 $\beta_2$AR allele contributes to the risk of obesity and predict obesity-related metabolic traits such as BMI, triglyceride and systolic PB.156 Furthermore, a few studies report gender-specific association between $\beta_2$AR polymorphism and obesity. Although the reasons for this discrepancy among studies are unclear, they may be due to the difference in the degree of obesity among study subjects or...
Table 1. β-AR gene polymorphisms.

| Genetic data SNP | AA position | Common | Published allele frequencies of the rare allele |
|------------------|-------------|--------|-----------------------------------------------|
|                  |             |        | Caucasians | African-Americans | Asians | Latino-Hispanic |
| T−3459C          | 5' Up stream |        | 0.42       | 0.16            | NR     | NR             |
| A−3308C          | 5' Up stream |        | 0.001      | 0.03            | NR     | NR             |
| T−3291C          | 5' Up stream |        | 0.44       | 0.45            | NR     | NR             |
| A−3287T          | 5' Up stream |        | 0.44       | 0.45            | NR     | NR             |
| C−3264T          | 5' Up stream |        | 0.001      | 0.004           | NR     | NR             |
| C−3251A          | 5' Up stream |        | 0.44       | 0.44            | NR     | NR             |
| T−3207C          | 5' Up stream |        | 0.008      | 0               | NR     | NR             |
| T−3159C          | 5' Up stream |        | 0.14       | 0.19            | NR     | NR             |
| C−2633T          | 5' Up stream |        | 0.44       | 0.43            | 0.11   | 0.05           |
| G−2547T          | 5' Up stream |        | 0.002      | 0               | NR     | NR             |
| T−2387C          | 5' Up stream |        | 0.45       | 0.45            | NR     | NR             |
| T−2274C          | 5' Up stream |        | 0.44       | 0.45            | NR     | NR             |
| C−1265G          | 5' Up stream |        | 0          | 0.002           | NR     | NR             |
| −2239 D/I        | 5' Up stream | Poly-A | NR         | NR              | NR     | NR             |
| −2051 D/I        | 5' Up stream |        | 0          | 0.05            | NR     | NR             |
| A−1818T          | 5' Up stream |        | 0.38       | 0.24            | NR     | NR             |
| G−1786C          | 5' Up stream |        | 0.001      | 0.002           | NR     | NR             |
| C−1738T          | 5' Up stream |        | 0.004      | 0.002           | NR     | NR             |
| G−1653A          | 5' Up stream |        | 0.001      | 0.007           | NR     | NR             |
| C−1531T          | 5' Up stream |        | 0.17       | 0.32            | NR     | NR             |
| T−1429A          | 5' Up stream |        | 0.18       | 0.32            | NR     | NR             |
| G−1343A          | 5' Up stream |        | 0.44       | 0.45            | NR     | NR             |
| G−1023A          | 5' Up stream |        | 0.44       | 0.45            | 0.12   | 0.10           |
| G−895A           | −31         | Met→Ile | 0.005      | 0               | NR     | NR             |
| C−706A           | -93         | Val→Val | NF         | NF              | 0.10   | 0.27           |
| G−654A           | −112        | Glu→Lys | 0.39       | 0.23            | NR     | NR             |
| C−468G           | −174        | His→Asp | 0.44       | 0.19            | 0.45   | 0.40           |
| C−406T           | −194        | Pro→Pro | 0.001      | 0.06            | 0.29   | NR             |
| −376 D/I         | 25-base D/I |        | 0          | 0.02            | NR     | NR             |
| T−367C           | −207        | Pro→Pro | 0.44       | 0.16            | 0.30   | 0.13           |
| −T262C           | −242        | Glu→Glu | 0.003      | 0.05            | NR     | NR             |
| T−47C            | −19 BUP     | Cys→Arg | 0.44       | 0.19            | NR     | NR             |
| T−20C            | −5          | Tyr→Tyr | 0.44       | 0.19            | NR     | NR             |
| G46A             | 16          | Gly→Arg | 0.38       | 0.48            | NR     | NR             |
| C66T             | 22          | His→His | 0.004      | 0.007           | NR     | NR             |
| C79G             | 27          | Glu→Glu | 0.44       | 0.19            | NR     | NR             |
| G252A            | 84          | Leu→Leu | 0.17       | 0.32            | NR     | NR             |
| C491A            | 164         | Thr→Ile | 0.02       | 0.004           | 0.01   | 0.03           |
| C523A            | 175         | Arg→Arg | 0.15       | 0.32            | NR     | NR             |
| C559G            | 220         | Ser→Cys | 0          | 0.03            | NR     | NR             |
| G1053C           | 351         | Gly→Gly | 0.26       | 0.35            | NR     | NR             |
| T1059C           | 366         | Tyr→Tyr | 0.004      | 0               | NR     | NR             |
| G1239A           | 413         | Leu→Leu | 0.32       | 0.44            | NR     | NR             |
| 1269 D/I         | Poly-C      |        | 0.36       | 0.23            | 0.41   | 0.1            |
| C1275A           | 3 UTR       |        | 0.29       | 0.54            | NR     | NR             |
| C1277A           | 3 UTR       |        | 0.003      | 0.03            | NR     | NR             |
| C1278A           | 3 UTR       |        | 0.005      | 0.05            | NR     | NR             |
| C1289T           | 3 UTR       |        | 0.01       | 0               | NR     | NR             |
| G1676A           | 3 UTR       |        | 0.003      | 0               | NR     | NR             |

Position of single nucleotide polymorphism (SNP) relative to ARB/β gene (NCHI Single Nucleotide Polymorphism database is online at http://www.ncbi.nlm.nih.gov/SNP/). AA, amino acid; A, adenine; C, cytosine; G, guanine; T, thymine; D/I= insertion/deletion; NR, not reported; BUP, upstream peptide, also known as 5' leader cistron. Modified from Taylor and Bristow, 2006; and Hawkins et al., 2006.
may be due to genetic variations between ethnic groups. However, further studies in larger populations are required to verify these results. Our findings provide strong evidence on the influence of the Gln27Glu genetic variants on lipid phenotypes, insulin and leptin levels in overweight/obese Saudi subjects.

The mechanism by which the β2AR Glu27 variant is associated with a larger incidence of dyslipidemia is presently unknown. The increased resistant to agonist-induced downregulation and degradation observed in β2AR Glu27 variant in vitro experiments might contribute, in part, to an increase in plasma triglyceride levels. Moreover, Glu27 allele is in linkage disequilibrium with a mutation in the promoter region, resulting in an increased synthesis of the protein with ensuing increase in lipolysis in adipocytes. However, these mechanisms are all contradicted by the fact that stimulation of the β2AR results in no change or a decrease in plasma triglyceride level, and a low β2AR sensitivity is linked to hypertriglyceridaemia. Regarding the reasons why β-blocker treatment is associated with an increased incidence of dyslipidemia in patients with the β2AR Glu27 variant, it can be hypothesized that the β2AR β-blockade induced by atenolol or metoprolol, two rather selective β1 antagonists, may result in the preferential activation of β2ARs. The consequence of this phenomenon would be even larger in patients with the β2AR genetic variant resulting in dyslipidemia. The relevance of this finding includes the possibility to predict those patients that are highly likely to develop this side effect and consequently to extend to the majority of the patients the benefits of chronic β-blockade.

Therefore, despite the large number of previous studies report the influence of the Arg16Gly and Gln27Glu β2AR variants on the risks of obesity, hypertension and type 2 diabetes, how these polymorphisms contribute to the development of these pathological conditions remains unclear. Further large-scale analysis with well-characterized traits will be necessary to elucidate the full effect of β2AR variants on metabolic disturbance.

Conclusions

The exact molecular mechanism of the effects of polymorphisms on cardiovascular phenotype and outcome include unique interaction between genotypes in conformation and downstream signaling of β2AR. Although the association studies relating polymorphisms to CVD often result in controversial findings, it is now evident that they have an important impact. It has been suggested that the use of relaxed selection criteria may increase background noise and mask possible genotype-phenotype relationships. For this reason, restrictive inclusion criteria, such as those requiring similar race, age, body dimension, duration, and severity of hypertension, as well as no previous pharmacologic treatment, are required for the identification of those patients in which the impact of β2AR variants will have the most impact. The evidence gathered in so far are promising for the tailoring of individualized therapy for CVD patients.

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