β_1 -adrenoceptor Arg389Gly polymorphism and heart disease: marching toward clinical practice integration

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Keywords: β_1 -adrenergic receptor • β -blocker • cardiovascular disease • dobutamine • drug response • GWAS • heart failure • hypertension • polymorphism

The β_1 -adrenergic receptor (AR) is a G protein-coupled receptor (GPCR) abundantly expressed in the plasma membranes of human cardiac myocytes. It is the primarily responsible AR for mediating the catecholamine-induced positive inotropy and chronotropy in the heart [1]. The human β_1 AR gene (*Adrb1*) encodes a 477-amino acid functional protein through a nucleotide sequence that has at least nine nonsynonymous variations reported to date. Out of these, the Gly/Arg polymorphism at amino acid position 389 (cytosine or guanine at nucleotide 1165 of its coding region) is by far the best-characterized variation to date affecting function and signaling of the encoded receptor [2,3]. This amino acid position is located in the intracellular C-terminus of the β_1 AR, a region that purportedly participates in the receptor's interactions with the G_s protein, and also gets phosphorylated by GPCR-kinases (GRKs), which induces the subsequent binding of β -arrestins that terminates G-protein signaling (receptor desensitization) [1]. Consequently, this C-terminal region is crucial for both activation and termination of the β_1 AR-dependent pro-contractile signaling in the heart [1]. Indeed, the Arg variant, substantially less prevalent in African Americans than in Caucasians (58 vs 73%, respectively), is presumed to favor/stabilize the active state of the human β_1 AR [2], rendering the Arg389 β_1 AR hyperfunctional compared with the Gly389 receptor. *In vitro* studies in heterologous cell systems (cell lines with the two variants exogenously overexpressed) have

confirmed the higher activity of the Arg389 receptor in terms of second messenger cAMP (cyclic adenosine monophosphate) accumulation, G_s protein coupling efficiency and high affinity ligand binding in the absence of guanine triphosphate (GTP) [2,3]. Accordingly, it has also been found to display enhanced homologous (agonist-induced) desensitization versus the Gly variant [4]. In physiologically relevant cell systems, in other words, murine cardiomyocytes isolated from cardiac-specific overexpressors of the two variants, increased contractility and receptor desensitization (in old age) were displayed by the Arg variant-overexpressing mice [5], and the beating frequency of cardiomyocytes isolated from these mice was also higher than that of Gly β_1 AR-overexpressing myocytes [6]. However, studies on the native variant receptors in explanted human heart tissue failed to confirm differences in activity/signaling between the two variants, possibly because the human heart tissue studied was not normal (explanted from heart failure patients) [4]. Thus, the overall cardiac status may very well affect or mask the functional status of the polymorphic β_1 AR [7]. Of course, inter-species differences (murine vs human) in cardiomyocyte physiology and cardiac β_1 AR biochemistry might be another reason for these discrepancies. Finally, the agonist-activated Arg389 variant was recently shown to undergo GRK-dependent hyperphosphorylation leading to enhanced β -arrestin2 binding (relative to the Gly β_1 AR) in heterologous HEK293 cells *in vitro*,

Katie A McCrink

Laboratory for the Study of Neurohormonal Control of the Circulation, Department of Pharmaceutical Sciences, Nova Southeastern University College of Pharmacy, Ft Lauderdale, FL, USA

Anastasios Lymperopoulos

Author for correspondence:
Laboratory for the Study of Neurohormonal Control of the Circulation, Department of Pharmaceutical Sciences, Nova Southeastern University College of Pharmacy, 3200 S. University Dr., HPD (Terry) Bldg/Room 1338, Ft Lauderdale, FL 33328–2018, USA
Tel.: +1 954 262 1338
Fax: +1 954 262 2278
al806@nova.edu

consistent with the elevated homologous desensitization of the Arg389 variant [8]. On the other hand, the agonist-activated Arg389 β_1 AR seems to preferentially bind β -arrestin2 over β -arrestin1 when overexpressed in neonatal rat ventricular myocytes (NRVMs), while the Gly389 β_1 AR shows no preference for either of the two β -arrestins (LYMPEROPOULOS A, UNPUBLISHED DATA). Given that β -arrestin2-dependent signaling in the heart may very well be beneficial for cardiac function, contrary to the cardiotoxic and negative for contractility β -arrestin1-dependent signaling [9], this preference of the Arg variant for β -arrestin2 interaction in NRVMs may underlie the hyperactivity of the Arg389 β_1 AR toward elevated contractility/cardiac function. This finding also suggests that the β -arrestin1/2 (and GRK) complements of the human cardiomyocyte, available for interaction with the β_1 AR, must be taken into account when studying the cardiac effects of the Arg389Gly β_1 AR polymorphism.

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Given that the β_1 AR is mainly expressed in the heart, where it stimulates cardiac output, and in the juxtaglomerular apparatus of the kidneys, where it induces renin release activating the renin-angiotensin-aldosterone system (RAAS) [2], it comes as no surprise that most studies have focused on associations of the Arg389Gly polymorphism with heart failure and hypertension. Indeed, large cohort studies have indicated that the Arg allele is associated with hypertensive risk, while the Gly allele confers lower risk for (essential) hypertension [10]. However, larger (in >1000 subjects) genome-wide association studies (GWAS) have failed to confirm this association [2]. Likewise, studies exploring heart failure association, albeit smaller than those on hypertension risk, have also failed to consistently link the Arg389Gly variation (or any other *Adrb1* locus variation) with risk for heart failure [2]. Nonetheless, this polymorphism has been associated with birth weight in Europeans, which may play a role in hypertension development later in adulthood [11]. Finally, other cardiovascular conditions potentially associated with the Arg389Gly polymorphism include acute ST-segment elevation myocardial infarction (STEMI) [12], idiopathic dilated cardiomyopathy (DCM) in a small Mexican population [13], arrhythmogenesis in Japanese DCM patients [14] and small vessel disease underlying ischemic stroke in a North Indian population [15]. Of course, these associations await confirmation in

large GWAS's as well as mechanistic delineation in functional *in vitro* studies.

With regards to drug response, the Arg389 homozygotes exhibit larger responses to the β_1 AR-agonist and positive inotrope drug dobutamine than Gly carriers, in terms of both cardiac function and plasma renin activity [16–18]. Similar observations have been made for several β -blockers (β_1 AR-antagonists): Arg389 carriers show greater reductions in cardiac hemodynamic parameters, heart rate, blood pressure and plasma renin activity, in response to various β -blockers (metoprolol, bisoprolol, esmolol, atenolol) than Gly carriers [2]. This is consistent with the Arg389 β_1 AR being more responsive to another β -blocker, carvedilol, *in vitro* [6], and suggests that the Arg allele normally, in other words, in healthy carriers, confers more robust responses to β -blocker drugs. Nevertheless, the majority of studies on the effects of this polymorphism on β -blocker responses are in heart failure patients, as expected, since β -blockers are nowadays part of the mainstay chronic heart failure drug regimen [9]. Consistent with reports from normal individuals, β -blocker-treated homozygous Arg389 patients show substantially larger improvements in their left ventricular indices than Gly389-carrying patients treated with the same drug [19]. In addition, important genetic subgroup studies of the BEST (β -blocker Evaluation of Survival) clinical trial have provided convincing evidence for better efficacy of the β -blocker bucindolol (pharmacologically very similar to carvedilol) in Arg389 homozygotes, especially if the Ins322–325Del *Adra2c* gene (the gene for the α_{2C} AR) variant co-exists [20].

In summary, the more common (thereby considered ‘wild type’) Arg389 allele appears to encode a β_1 AR that, compared with its Gly389 counterpart, is hyperfunctional at the molecular and signaling levels, and quite likely at the physiological level (i.e., in the cardiovascular system), as well. This seems to translate into better responses of the Arg allele carriers (both healthy individuals and patients) to β_1 AR ligand drugs, either agonists (dobutamine) or antagonists (β -blockers). In contrast, whether the Arg389Gly variation influences cardiovascular disease risk (mainly, heart failure or hypertension) remains an open question. Several obstacles need to be overcome by future studies in order to reach some consensus on addressing this question. First and foremost, the sample size of the association studies needs to be as large as possible. Quite frequently in pharmacogenomics, small candidate gene association studies report exciting and promising results, which soon turn out to be biased, false positive and irreproducible in larger GWAS's. In fact, the rule of thumb appears to be that the sample size

of a study and its likelihood of confirming an association, are inversely proportional. The recent incorporation of very large consortia, in the order of several thousands of individuals, in the design of GWAS's will undoubtedly increase both the sensitivity and specificity of the reported associations in the future. Apart from sample size considerations and from taking the much more scientifically rigorous GWAS, instead of the candidate gene study, approach, another big roadblock is the multitrait status/complexity of most diseases (especially heart diseases). Heart failure and hypertension are extremely multifaceted disease entities, develop over long periods of time and their pathophysiologies involve hundreds (maybe even thousands) of genes. Moreover, the disease state itself may reciprocally modify the pathophysiological effects of the polymorphism (e.g., different effects of the Arg389Gly β_1 AR polymorphism in healthy vs diseased myocardium, see above). Additional complicating factors are the potential for linkage disequilibrium (i.e., involvement in a haplotype) of the studied polymorphism (the Arg389Gly variation is in linkage disequilibrium with the Ser49Gly variation, also within the *Adrb1* coding region) and the 'dosage' effect of the polymorphism, in other words, whether the phenotypic effects of the two variants are equalized by each other and the polymorphism requires both alleles to manifest itself. This may very well be the case also with the Arg389 β_1 AR polymorphism, since phenotypic effects are observed almost exclusively in Arg/Arg homozygotes. Finally, with regards to functional studies, it is always better to conduct these studies in physiologically relevant cell systems (i.e., in the Arg389Gly β_1 AR case, cardiac myocytes), with physiology as close to human as possible, and which express physiologically relevant levels of the (receptor) variants. Heterologous murine or rat cell lines, transfected to express supraphysiological β_1 AR variant levels, most likely do not represent true human cardiomyocytes with endogenous β_1 AR levels very well.

Given all of these hurdles above, it should come as no surprise that the Arg389Gly β_1 AR polymorphism has not been able to be definitively associated with heart failure, hypertension or other heart diseases, and it may very well be unrealistic to expect it ever will. Nevertheless, and despite the caveats and difficulties in interpreting and reconciling the ever-increasing number of complex studies in this field, the following conclusions about the presence of glycine (instead of arginine) at position 389 of the human β_1 AR can be drawn with a significant amount of certainty: Gly389 results in impaired efficacy of the β -blocker treatment in chronic heart failure (and in other heart disease) patients, and Gly389 results in reduced β_1 AR function and signaling *in vitro*, as well as in suppressed cardiac and blood pressure responses of the β_1 AR *in vivo*. Thus, it is quite plausible that, soon, β_1 AR genotyping of the cardiovascular patient for the Arg389Gly variation will allow for personalization of his/her drug therapy, since the presence of Arg389 homozygosity will identify the patient as a good responder to β -blockers (or β -agonists/cardiostimulants), whereas Arg/Gly heterozygosity or Gly389 homozygosity will signal to the clinician that a dose escalation of the prescribed β -blocker (or β -agonist drug) is necessary. The ball is now in the court of researchers performing very large GWAS's to firmly establish these inferences, so that they can be incorporated into the clinical practice sooner rather than later. The hope is that another success story for pharmacogenomics will soon be delivered, this time for the benefit of cardiology.

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