British Journal of Pharmacology (2003) 138, 279–282

www.nature.com/bjp

COMMENTARY

Receptor gene polymorphisms: lessons on functional relevance from the β_1-adrenoceptor

*,[1]Martin C. Michel & [2,3]Paul A. Insel

[1]Department of Medicine, University of Essen, Essen, Germany; [2]Department of Pharmacology, University of California San Diego, La Jolla, California, U.S.A. and [3]Department of Medicine, University of California San Diego, La Jolla, California, U.S.A.

British Journal of Pharmacology (2003) **138,** 279–282. doi:10.1038/sj.bjp.0705031

β-Adrenoceptors in general, and β_1-adrenoceptors in particular, are important mediators of the sympathoadrenal system and regulate numerous physiologic events, including cardiac rate and contractility, lipolysis, and renin release. The gene encoding the human β_1-adrenoceptor, which is located on chromosome 10q24–q26, is quite polymorphic with 18 single nucleotide polymorphisms (SNPs), 17 within the coding exon for the receptor, seven leading to amino acid substitutions; it has been proposed that there may be 11 different genotypes (Podlowski *et al*., 2000). To date, though, most effort has focused on two loci: G49S (A for G at nucleotide 145) and R389G (C for G at nucleotide position 1165) (Maqbool *et al*., 1999; Mason *et al*., 1999; Tesson *et al*., 1999). The latter variant, which is located in the carboxy terminal tail portion of this heptahelical G-protein-coupled receptor (GPCR), is of interest because when heterologously expressed in CHW-1102 cells, the 389R variant shows a 'gain of function', i.e., greater basal and isoprenaline-stimulated cAMP levels, which has been attributed to greater coupling of the 389R receptor to G_s (Mason *et al*., 1999).

In this issue of the journal Sandilands *et al*. (2002) report that right atrial appendages isolated from patients homozygous for 389R exhibit significantly enhanced noradrenaline-stimulated cAMP formation and contraction compared to atria from 389G homozygotes. These findings add interesting information to the ongoing debate on the functional relevance of β_1-adrenoceptor subtype polymorphisms. Moreover, they are also an instructive example for potential pitfalls in pharmacogenomics research.

Assessment for SNPs and other genetic variants in GPCRs has become a 'growth industry' in pharmacological research because many believe that such variants, in particular coding sequence SNPs (cSNPs), such as the 389R variant of the β_1-adrenoceptor, will help explain why a given drug acts in a quantitatively or qualitatively different way in different people. Increasing evidence documents that SNPs occur commonly, albeit with varying frequency, among GPCRs. The cSNPs in GPCRs are of particular interest because they are presumed to have a greater tendency to affect the responses to drugs and/or endogenous agonists and hence, to alter the pathophysiology of specific disease states (Rana *et al*., 2001). Such responses can be studied in several different ways: *in vitro* (e.g. upon heterologous expression in suitable cells) to determine possible differences in receptor function and susceptibility to regulatory influences, *in vivo* and *ex vivo* to assess the impact of genetic variants on responses of tissues from normal subjects or patients, and/or at the population level to determine whether a given genotype or allele shows linkage or association with a particular disease state (prevalence, onset, severity, clinical features, progression, etc.) or sensitivity to drug treatment. All of these approaches have been used in the context of β_1-adrenoceptor polymorphisms, in particular the 389R variant, but no clear consensus has emerged.

Heterologous expression with comparison of variant and 'wild-type' receptor is a straightforward approach to determine whether a receptor derived from a polymorphic gene exhibits altered function. Since cellular responsiveness depends on levels of receptor expression, it is important that such studies be performed in host cells that are transfected to yield very similar expression levels. Two groups of investigators have recently reported results for this approach with the G49S polymorphism of the β_1-adrenoceptor (Rathz *et al*., 2002; Levin *et al*., 2002). Although the two groups both found that the G49 variant is more susceptible to agonist-promoted down-regulation, their findings were markedly different with regard to other properties of the variant receptors. Rathz *et al*. (2002) expressed G49 and S49 receptors in both CHW and HEK 293 cells and found that the two types of receptors yielded similar antagonist and agonist binding properties, basal and agonist-stimulated adenylyl cyclase activities and susceptibility to agonist-promoted receptor internalization. In contrast, Levin *et al*. (2002) expressed receptors in HEK 293 cells at up to 10 fold higher density than did Rathz *et al*. (2002) and found that the G49 variant exhibited features of a constitutively active receptor: increased agonist affinity, basal and agonist-stimulated adenylyl cyclase activity, inverse agonism by the antagonist metoprolol and susceptibility to agonist-mediated desensitization. Perhaps these discrepant results are explained by the differences in receptor density and possibly by the use of different batches of HEK cells, which have been noted to differentially express post-receptor components (Lefkowitz *et al*., 2002). In addition, the G49S polymorphism is reportedly in strong linkage dysequilibrium with an as-yet poorly characterized polymorphism ($-2146T>C$) in the 5′ untrans-

*Author for correspondence at: Nephrol. Lab. IG 1, Universitätsklinikum Essen, 45122 Essen, Germany;
E-mail: martin.michel@uni-essen.de

lated region of the β_1-adrenoceptor gene (Wenzel *et al*., 2000). As noted above, when heterologously expressed, the 389R β_1-adrenoceptor was also noted to be constitutively active (Mason *et al*., 1999).

A complementary approach to heterologous expression is the *ex vivo* study of cells or tissues isolated from subjects with defined genotypes. Both Sandilands *et al*. (2002) and Molenaar *et al*. (2002) assessed right atrial appendages from patients undergoing cardiac surgery to determine the functional impact of β_1-adrenoceptor receptor variants. Sandilands *et al*. (2002) found that right atrial appendages from 37 homozygous 389R subjects showed greater inotropic potency of noradrenaline (pEC_{50} 6.92 *vs* 6.36) and greater noradrenaline-stimulated cAMP accumulation compared to atria from 17 homozygous 389G patients. Molenaar *et al*. (2002) studied 87 patients who had been pre-treated with a β-blocker and 20 without such treatment, and found that irrespective of previous β-blocker therapy, neither the G49S nor the R389G polymorphism affected the potency or maximum responses of noradrenaline with respect to contractile force, time to reach peak force or time to reach 50% relaxation. Similarly, Ryden *et al*. (2001) have studied lipolytic responses to several β-adrenoceptor agonists in isolated subcutaneous adipocytes from 298 subjects covering a wide range of body mass indices but failed to detect differences in response for subjects with either 389R *vs* 389G.

A third approach to assess the functional consequences of receptor gene polymorphisms is to conduct *in vivo* studies. With regard to the R389G polymorphism, Xie *et al*. (2001) and Büscher *et al*. (2001) simultaneously reported that healthy subjects homozygous for either genotype have very similar heart rate responses to exercise, a β_1-adrenoceptor-mediated response. Büscher *et al*. (2001) also found that exercise (β_1-adrenoceptor)-promoted cardiac contractility and plasma renin activity were similar for subjects with the two different amino acids at the 389 position. Another study was that by O'Shaughnessy *et al*. (2000) who found that 4 week treatment with a β_1-selective blocker yielded similar heart rate and blood pressure responses in hypertensive subjects homozygous for the 389G and 389R genotypes. By contrast, Humma *et al*. (2001) assessed haemodynamics in 142 patients (72 who were 389R homozygotes and 70 who were either homozygous or heterozygous for 389G) undergoing stress echocardiography with the β-adrenoceptor agonist dobutamine and observed significantly higher resting heart rate, diastolic blood pressure (BP), and 'double product' (heart rate $\times$ systolic BP) in 389R homozygotes; systolic BP was also greater in Caucasian subjects who were 389 homozygotes.

Taken together, the present literature is thus quite inconsistent with respect to functional effects of β_1-adrenoceptor gene polymorphisms. The only consistently reported alteration is an enhanced susceptibility to agonist-promoted down-regulation of the G49 genotype. For all other effects the G49 and the R389 genotype were reported to exhibit enhanced responsiveness in some, but not other, studies. The overall balance of data suggests an enhanced function of those two alleles, but the magnitude of the enhancement does not appear to be sufficient to allow consistent detection.

If variants at one or both of the polymorphic sites in the β_1-adrenoceptor gene alter functional responsiveness, this could affect not only responses to physiologic stimuli and drug treatment but also might alter disease susceptibility or manifestations, such as ethnic variations in such features. Studies in Caucasian, Chinese or African–American subjects indicate a frequency of 85–89% for the 49S allele (Maqbool *et al*., 1999; Moore *et al*., 1999; Borjesson *et al*., 2000; Wenzel *et al*., 2000; Molenaar *et al*., 2002). In contrast, the 389R allele is found in 71–78% of Caucasian and Chinese subjects, but in only 58% of African–Americans (Maqbool *et al*., 1999; Tesson *et al*., 1999; Mason *et al*., 1999; Moore *et al*., 1999; Büscher *et al*., 2001; Xie *et al*., 2001; Molenaar *et al*., 2002).

Because of the key role of β_1-adrenoceptors on inotropic and chronotropic activity, a few studies have assessed the potential contribution of genetic variants in those receptors to common cardiovascular disorders, in particular congestive heart failure and coronary heart disease. Two studies found that the frequency of the 49G allele did not significantly differ between 184 patients with congestive heart failure and 77 age-matched controls (Borjesson *et al*., 2000) or between 98 idiopathic dilated cardiomyopathy (DCM) patients and 102 control subjects (Wenzel *et al*., 2000). However, Borjesson *et al*. (2000) found that patients with the 49G allele exhibited a greater mortality (risk ratio 2.34) during a 5 year follow-up period. In two other studies, one with about 160 and the other with about 400 idiopathic DCM patients and age- and sex-matched control subjects (Iwai *et al*., 2002), the frequency of the 389R allele was similar in controls and those with disease (Tesson *et al*., 1999). Iwai *et al*. (2002) reported, however, that the G389 allele was more frequent in patients without ventricular tachycardia (odds ratio 0.29 compared to R389 homozygotes). Ranade *et al*. (2002) studied more than 1000 subjects of Chinese and Japanese descent and found that the G49S, but not the R389G, polymorphism was associated with a few beats increase in resting heart rate. In spite of this observation, Kanki *et al*. (2002), in a study of 66 patients, found that neither of the β_1-adrenoceptor variants was associated with an altered susceptibility to acquired long QT syndrome.

Two studies have found that neither the R389G nor the G49S polymorphism are associated with coronary arterial events: White *et al*. (2002), who assessed in 1554 individuals from the West of Scotland Coronary Prevention Study and Wenzel *et al*. (2000) who examined a smaller number of German subjects. In contrast to the above studies, which all have relied on cross-sectional comparisons, Bengtsson *et al*. (2001) have performed both a cross-sectional and a sibling study to determine the association of the R389G polymorphism with essential hypertension. In both types of study, the authors found a higher blood pressure in subjects with at least one 389R allele compared to homozygous 389G subjects.

As stated above, Ryden *et al*. (2001) reported that lipolytic response to β-adrenergic stimulation was not affected by the R389G polymorphism. Those authors also failed to observe an association between genotype and body mass index, findings that contrast with those of Dionne *et al*. (2002), who observed an association of the 389R allele with body weight, body mass index and fat mass.

Because of limited data available and the contradictory results reported in the association studies, we believe that it is too early to draw conclusions about the role of polymorphisms of the β_1-adrenoceptor gene in disease states or in inter-

subject variation in physiologic, pathophysiologic, or pharmacologic responses. As has been noted previously (Rana *et al*., 2001), one must use multiple levels of investigation to define the true 'importance' of a particular polymorphism, especially one in a GPCR. Firstly, functional consequences of a receptor gene polymorphism need to be studied, ideally in parallel studies, at the cell, tissue, organism and population level. Results obtained at any one of those levels require independent confirmation. The increasing ease with which genotyping can be undertaken has led to a profusion of cross-sectional studies, probably the least informative type of investigation, especially when undertaken with relatively small numbers of subjects of poorly defined ethnicity. Results from such studies have limited statistical power, which is not even reported in most articles. Jones & Montgomery (2002) have recently questioned whether the funding of small studies of a single polymorphism should continue, and instead have suggested a need for large national and international collaborative efforts to obtain adequate numbers of subjects. From a genetic point of view, studies of receptor variants that utilize multiple family members, such as twins, multi-generational assessments, large pedigrees, or discordant siblings are also likely to be more informative than cross-sectional studies. *In vivo* pharmacodynamic studies are likely to prove very important in defining the impact of GPCR variants, such as the R389 β_1-adrenoceptor. Assessment of *ex vivo* cells from genetically well-characterized subjects will be critical to define the molecular mechanisms by which such variants modulate receptor signalling and regulation. Thus, the combined use of multiple approaches is likely to prove essential to define the 'true' physiologic and pharmacologic importance of receptor polymorphisms, such as the R389G β_1-adrenoceptor.

Note added in proof

A recent study, based on 159 heart failure patients and 189 controls, reported that presence of the 389R genotype does not affect the risk to have heart failure, but in combination with a deletion mutatnt of the α_{2C}-adrenoceptor it significantly increases the odds range to 10.11 in African–Americans (Small *et al*., 2002). These findings elegantly highlight the importance of studying haplotypes rather than isolated SNPs.

Work in the authors' laboratories related to this topic is supported by grants from National Institutes of Health and Deutsche Forschungsgemeinschaft.

References

BENGTSSON, K., MELANDER, O., ORHO-MELANDER, M., LINDBLAD, U., RANSTAM, J., RASTAM, L. & GROOP, L. (2001). Polymorphism in the β_1-adrenergic receptor gene and hypertension. *Circulation,* **104,** 187–190.

BORJESSON, M., MAGNUSSON, Y., HJALMARSON, A. & ANDERSSON, B. (2000). A novel polymorphism in the gene coding for the β_1-adrenergic receptor associated with survival in patients with heart failure. *Eur. Heart J.,* **21,** 1853–1858.

BÜSCHER, R., BELGER, H., EILMES, K.J., TELLKAMP, R., RADKE, J., DHEIN, S., HOYER, P.F., MICHEL, M.C., INSEL, P.A. & BRODDE, O.-E. (2001). In-vivo studies do not support a major functional role for the Gly389Arg β_1-adrenoceptor polymorphism in humans. *Pharmacogenetics,* **11,** 199–205.

DIONNE, I.J., GARANT, M.J., NOLAN, A.A., POLLIN, T.I., LEWIS, D.G., SHULDINER, A.R. & POEHLMAN, E.T. (2002). Association between obesity and a polymorphism in the β_1-adrenoceptor gene (Gly389Arg ADRB1) in Caucasian women. *Int. J. Obes. Relat. Metab. Disord.,* **26,** 633–639.

HUMMA, L.M., PUCKETT, B.J., RICHARDSON, H.E., TERRA, S.G., ANDRISIN, T.E., LEJEUNE, B.L., WALLACE, M.R., LEWIS, J.F., MCNAMARA, D.M., PICOULT-NEWBERG, L., PEPINE, C.J. & JOHNSON, J.A. (2001). Effects of β_1-adrenoceptor genetic polymorphisms on resting hemodynamics in patients undergoing diagnostic testing for ischemia. *Am. J. Cardiol.,* **88,** 1034–1037.

IWAI, C., AKITA, H., SHIGA, N., TAKAI, E., MIYAMOTO, Y., SHIMUZU, M., KAWAI, H., TAKARADA, A., KAJIYA, T. & YOKOYAMA, M. (2002). Suppressive effect of the Gly389 allele of the β_1-adrenergic receptor gene on the occurrence of ventricular tachycardia in dilated cardiomyopathy. *Circ. J.,* **66,** 723–728.

JONES, A. & MONTGOMERY, H. (2002). The Gly389Arg β_1 adrenoceptor polymorphism and cardiovascular disease: time for a rethink in the funding of genetic studies? *Eur. Heart J.,* **23,** 1071–1074.

KANKI, H., YANG, P., XIE, H.G., KIM, R.B., GEORGE, JR. A.L. & RODEN, D.M. (2002). Polymorphisms in β-adrenergic receptor genes in the acquired long QT syndrome. *J. Cardiovasc. Electrophysiol.,* **13,** 252–256.

LEFKOWITZ, R.J., PIERCE, K.L. & LUTTRELL, L.M. (2002). Dancing with different partners: PKA phosphorylation of seven membrane spanning receptors regulates their G protein coupling specificity. *Mol. Pharmacol.,* **62,** 971–974.

LEVIN, M.C., MARULLO, S., MUNTANER, O., ANDERSSON, B. & MAGNUSSON, Y. (2002). The myocardium-protective Gly-49 variant of the β_1-adrenergic receptor exhibits constitutive activity and increased desensitization and down-regulation. *J. Biol. Chem.,* **277,** 30429–30435.

MAQBOOL, A., HALL, A.S., BALL, S.G. & BALMFORTH, A.J. (1999). Common polymorphisms of β_1-adrenoceptor: identification and rapid screening assay. *Lancet,* **353,** 897.

MASON, D.A., MOORE, J.D., GREEN, S.A. & LIGGETT, S.B. (1999). A gain-of-function polymorphism in a G-protein coupling domain of the human β_1-adrenergic receptor. *J. Biol. Chem.,* **274,** 12670–12674.

MOLENAAR, P., RABNOTT, G., YANG, I., FONG, K.M., SAVARIMUTHU, S.M., LI, L., WEST, M.J. & RUSSELL, F.D. (2002). Conservation of the cardiostimulant effects of (-)-norepinephrine across Ser49Gly and Gly389Arg beta$_1$-adrenergic receptor polymorphisms in human right atrium in vitro. *J. Am. Coll. Cardiol.,* **40,** 1275–1282.

MOORE, J.D., MASON, D.A., GREEN, S.A., HSU, J. & LIGGETT, S.B. (1999). Racial differences in the frequencies of cardiac β_1-adrenergic receptor polymorphisms: analysis of c145A>G and c1165G>C. *Hum. Mutat.,* **14,** 271.

O'SHAUGHNESSY, K.M., FU, B., DICKERSON, C., THURSTON, D. & BROWN, M.J. (2000). The gain-of-function G389R variant of the β_1-adrenoceptor does not influence blood pressure or heart rate response to beta-blockade in hypertensive subjects. *Clin. Sci.,* **99,** 231–232.

PODLOWSKI, S., WENZEL, K., LUTHER, H.P., MÜLLER, J., BRAMLAGE, P., BAUMANN, G., FELIX, S.B., SPEER, A., HETZER, R., KOPKE, K., HOEHE, M.R. & WALLUKAT, G. (2000). Beta1-adrenoceptor gene variations: a role in idiopathic dilated cardiomyopathy? *J. Mol. Med.,* **78,** 87–93.

RANA, B.K., SHINA, T. & INSEL, P.A. (2001). Genetic variations and polymorphisms of G protein-coupled receptors: functional and therapeutic implications. *Annu. Rev. Pharmacol. Toxicol.,* **41,** 593–624.

RANADE, K., JORGENSON, E., SHEU, W.H., PEI, D., HSIUNG, C.A., CHIANG, F.T., CHEN, Y.D., PRATT, R., OLSHEN, R.A., CURB, D., COX, D.R., BOTSTEIN, D. & RISCH, N. (2002). A polymorphism in the β_1-adrenergic receptor is associated with resting heart rate. *Am. J. Hum. Genet.,* **70,** 935–942.

RATHZ, D.A., BROWN, K.M., KRAMER, L.A. & LIGGETT, S.B. (2002). Amino acid 49 polymorphisms of the human β_1-adrenergic receptor affect agonist-promoted trafficking. *J. Cardiovasc. Pharmacol.,* **39,** 155 – 160.

RYDEN, M., HOFFSTEDT, J., ERIKSSON, P., BRINGMAN, S. & ARNER, P. (2001). The Arg 389 Gly β_1-adrenergic receptor gene polymorphism and human fat cell lipolysis. *Int. J. Obes. Relat. Metab. Disord.,* **25,** 1599 – 1603.

SANDILANDS, A.J., O'SHAUGHNESSY, K.M. & BROWN, M.J. (2002). Greater inotropic and cyclin AMP responses evoked by noradrenaline through Arg389 β_1-adrenoceptors versus Gly389 β_1-adrenoceptors in isolated human atrial myocardium. *Br. J. Pharmacol.,* in press.

SMALL, K., WAGONER, L.E., LEVIN, A., KARDIA, S.L.R. & LIGGETT, S.B. (2002). Synergistic polymorphsims of $\beta_1$and α_{2C}-adrenergic receptors and the risk of congestive heart failure. *New Engl. J. Med.,* **347,** 1135 – 1142.

TESSON, F., CHARRON, P., PEUCHMAURD, M., NICAUD, V., CAMBIEN, F., TIRET, L., POIRIER, O., DESNOS, M., JULLIERES, Y., AMOUYEL, P., ROIZES, G., DORENT, R., SCHWARTZ, K. & KOMAJDA, M. (1999). Characterization of a unique variant in the β_1-adrenoceptor gene and evaluation of its role in idiopathic dilated cardiomyopathy. *J. Mol. Cell. Cardiol.,* **31,** 1025 – 1032.

WENZEL, K., FELIX, S.B., BAUER, D., HEERE, P., FLACHMEIER, C., PODLOWSKI, S., KOPKE, K. & HOEHE, M.R. (2000). Novel variants in 3 kb of 5′UTR of the β_1-adrenergic receptor gene (-93C > T, -210C > T, and -2146T > C): -21a6C homozygotes present in patients with idiopathic dilated cardiomyopathy and coronary heart disease. *Hum. Mutat.,* **16,** 534.

WHITE, H.L., MAQBOOL, A., MCMAHON, A.D., YATES, L., BALL, S.G., HALL, A.S. & BALMFORTH, A.J. (2002). An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals at risk of coronary events. A WOSCOPS substudy. *Eur. Heart J.,* **23,** 1087 – 1092.

XIE, H.G., DISHY, V., SOFOWORA, G., KIM, R.B., LANDAU, R., SMILEY, R.M., ZHOU, H.H., WOOD, A.J.J., HARRIS, P. & STEIN, C.M. (2001). Arg389Gly β_1-adrenoceptor polymorphism varies in frequency among different ethnic groups but does not alter response in vivo. *Pharmacogenetics,* **11,** 191 – 197.

(Received September 25, 2002
Accepted October 7, 2002)