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Beta$_1$-Adrenergic Receptor Gene Polymorphisms and Response to Beta$_1$-Adrenergic Receptor Blockade in Patients with Essential Hypertension

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Summary

Background: Studies suggest that the Ser49Gly and Arg389Gly polymorphisms in the β_1-adrenergic receptor might be of functional importance for the cardiovascular system. Both have been associated with altered receptor activity in vitro, and with hypertension and cardiac failure in vivo.

Hypothesis: The aim of this study was to test whether these polymorphisms were associated with the change in heart rate or blood pressure in patients with essential hypertension and left ventricular (LV) hypertrophy treated with the β_1-adrenergic receptor blocker atenolol.

Methods: Blood pressure and heart rate were measured in 101 hypertensive patients with echocardiographically verified LV hypertrophy, randomized in a double-blind study to treatment with either the β_1-adrenergic receptor blocker atenolol or the angiotensin II type I receptor antagonist irbesartan. Changes in blood pressure and heart rate were evaluated after 12 weeks. Beta$_1$-adrenergic receptor genotyping was performed using polymerase chain reaction and restriction fragment length polymorphism.

Results: We found no significant associations between the changes in the measured variables and either of the two polymorphisms. However, carriers of the 49Gly allele showed a tendency toward a greater reduction in heart rate compared with patients with the Ser/Ser49 genotype ($p = 0.06$).

Conclusions: The Ser49Gly and Arg389Gly β_1-adrenergic receptor polymorphisms do not seem to exert a major effect on the changes in heart rate and blood pressure during 12 weeks of treatment with atenolol in patients with essential hypertension and LV hypertrophy.

Key words: beta$_1$-adrenergic receptor, polymorphism, gene, pharmacogenetics, hypertension, atenolol, adrenoceptor

Introduction

Beta$_1$-adrenergic receptors (β_1-ARs) play a pivotal role in the regulation of the cardiovascular system. In the human heart, both β_1- and β_2-ARs are expressed, but the β_1 is the major subtype and is known to stimulate the rate and strength of cardiac contraction.[1–3] On agonist stimulation, it elicits excitatory reactions in the heart, leading to higher cardiac output through increased cardiac inotropy and chronotropy. Beta-AR blockers, today mainly those selective for the β_1-AR, are widely used in the treatment of hypertension.[4] However, the antihypertensive response to this class of drugs is highly variable, and the individual response cannot be predicted at the present time.

Two frequent polymorphisms in the gene encoding the β_1-AR have been found. The first occurs at nucleotide position 145, where an A is substituted for a G, resulting in a nonsynonymous amino acid substitution of serine to glycine at position 49 in the receptor protein (Ser49Gly).[5–9] The second polymorphism is located at nucleotide position 1165, where a C is substituted for a G, which alters the encoded amino acid from arginine to glycine at amino acid position 389 (Arg389Gly).[5–9] The Ser49Gly variant, located in the extracellular amino-terminal region of the receptor, has been proposed to alter the localization of the receptor in the cell membrane and receptor trafficking.[8] Arginine389Gly is located in the proximal portion of the cytoplasmic tail of the receptor near the seventh transmembrane-spanning domain,[8] and there is reason to be-

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lieve that this polymorphism changes the tertiary structure of the receptor due to a neutral and small Gly residue replacing a basic and large Arg residue.

We have previously shown that the angiotensin-converting enzyme (ACE) I/D and the aldosterone synthase gene polymorphisms were associated with the blood pressure-lowering response to treatment with irbesartan, but not with atenolol, implicating that pharmacogenetics might be used to individualize antihypertensive treatment.[10, 11]

In the present study, our aim was to test whether the two β_1-AR polymorphisms described above were associated with the change in heart rate (HR) and blood pressure in patients with essential hypertension and left ventricular (LV) hypertrophy treated with the β_1-AR blocker atenolol for a period of 12 weeks. As comparison, we studied patients treated with the angiotensin II type I receptor antagonist irbesartan.

Materials and Methods

Patients

The subjects participated in the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial, which has been described in detail earlier.[12] Briefly, Caucasian men and women with mild to moderate essential hypertension and echocardiographically verified LV hypertrophy were enrolled, with the primary goal of evaluating the efficacy of irbesartan compared with atenolol on blood pressure reduction and regression of LV hypertrophy. Left ventricular hypertrophy was considered present if LV mass index was > 131 g/m^2 for men and > 100 g/m^2 for women. After patients rested for at least 10 min in a seated position, blood pressure was measured by trained study nurses using a mercury sphygmomanometer and was determined as the average of three measurements taken 1 min apart. Heart rate was then recorded in the seated position. Inclusion criteria were diastolic blood pressure (DBP) of 90–115 mmHg at two examinations within a week, with values differing by no more than 8 mmHg. During treatment, blood pressure was measured at trough (24 ± 3 h after the last dose). Secondary hypertension was excluded by means of physical examination and routine laboratory tests. All antihypertensive agents were withdrawn before the start of a 4–6 week, single-blind, placebo lead-in period, after which the patients received either irbesartan 150 mg or atenolol 50 mg once daily as monotherapy in a double-blind fashion. The doses were doubled after 6 weeks if DBP was ≥ 90 mmHg. In all, 102 patients completed the first 12 weeks of monotherapy. One patient was excluded in the present study since we were unable to acquire appropriate blood samples.

DNA Extraction

DNA was isolated using QIAamp, DNA Blood Mini Kit (QIAGEN, Germany) according to the manufacturer's Blood and Body Fluid Spin Protocol. Polymerase chain reaction was performed using primers described by Maqbool *et al.*[6] Genotyping was done with restriction fragment length polymorphism, in which the Ser49Gly was enzymatically cleaved by Eco 0109 I and the Gly389Arg by BcgI, as described.[6]

The appropriate university ethics committees approved this study. The participating patients gave their informed consent and the study was completed in accordance with institutional guidelines.

Statistics

Data are presented as mean values ± standard error (SE). The estimated adjusted mean difference in systolic blood pressure (SBP), DBP, and HR change at 12 weeks of monotherapy between Ser49Ser and carriers of the 49Gly allele (two categories) on the one hand, and between Arg389Arg and carriers of the 389Gly on the other, was calculated with the general linear models (GLM) procedure of SAS software (SAS, Cary, N.C., USA) for each treatment group. A p value of < 0.05 was considered significant.

Results

Baseline characteristics stratified by genotype and treatment are presented in Table I. The observed allele frequencies were in Hardy-Weinberg equilibrium and were in accordance with frequencies found in other Swedish/Scandinavian populations.[4, 7] Regarding the Ser49Gly polymorphism, patients with the Ser/Gly and Gly/Gly genotypes were considered as one group, since there were only three patients with the latter genotype (all in the atenolol group). Equally, regarding the Arg389Gly polymorphism, patients with the Arg/Gly and Gly/Gly genotypes were considered as one group, since there were only seven patients with the Gly/Gly genotype (three in the atenolol group and four in the irbesartan group).

As seen in Table II, there was no association between either of the two polymorphisms and changes in SBP, DBP, and HR at 12 weeks. However, among carriers of the 49Gly allele, there was a tendency toward a greater reduction in HR compared with patients with the Ser/Ser49 genotype in the atenolol group ($p = 0.06$). The same was observed for the patients with the Arg/Arg389 genotype, compared with the carriers of the Gly389 allele in the irbesartan group ($p = 0.06$). Adjustment for the potential covariates age, dose, gender, SBP, and DBP at baseline and LV myocardial infarction at baseline did not influence the results significantly (data not shown).

Discussion

The primary aim of this study was to test whether the Ser49Gly and Arg389Gly β_1-AR polymorphisms were associated with the changes in blood pressure and HR in patients with essential hypertension and LV hypertrophy treated with the β_1-AR blocker atenolol. We found no significant association with either of the two polymorphisms in this respect; however, the Gly49 allele was associated with a tendency toward a greater reduction of HR at 12 weeks in the atenolol group.

TABLE I Baseline patient characteristics stratified by treatment and genotype

	Atenolol			Irbesartan		
Ser49Gly	Ser/Ser (n = 35)	Ser/Gly and Gly/Gly (n = 17)	p Value	Ser/Ser (n = 33)	Ser/Gly (n = 16)	p Value
Age (years)	54.8 (1.3)	53.8 (2.6)	0.71	54.5 (1.5)	53.3 (1.7)	0.65
Sex (male/female)	26/9	10/7	0.34	18/15	12/4	0.22
BMI (kg/m^2)	27.5 (0.7)	26.2 (0.7)	0.23	27.0 (0.5)	27.6 (1.0)	0.49
SBP (mmHg)	159.4 (3.1)	163.2 (5.6)	0.53	164.0 (3.3)	163.5 (4.0)	0.92
DBP (mmHg)	103.2 (1.4)	102.6 (1.9)	0.77	104.6 (1.2)	104.3 (1.8)	0.89
HR (beats/min)	65.1 (1.3)	68.5 (2.5)	0.19	70.0 (2.0)	67.0 (2.4)	0.39
LVMI (g/m^2)	147.1 (4.2)	135.1 (6.8)	0.12	151.5 (7.0)	148.0 (5.8)	0.74
Dose (mg)	66.4 (4.7)	78.1 (6.8)	0.17	246.8 (13.6)	260.0 (17.7)	0.57
Arg389Gly	Arg/Arg (n = 34)	Arg/Gly and Gly/Gly (n = 18)	p Value	Arg/Arg (n = 21)	Arg/Gly and Gly/Gly (n = 28)	p Value
Age (years)	52.7 (1.6)	57.8 (1.7)	0.046	54.4 (1.8)	53.8 (1.5)	0.80
Sex (male/female)	22/12	14/4	0.53	12/9	18/10	0.77
BMI (kg/m^2)	26.8 (0.6)	27.6 (0.9)	0.47	27.5 (0.7)	26.9 (0.6)	0.52
SBP (mmHg)	164.0 (3.4)	154.2 (4.6)	0.092	163.0 (3.8)	164.5 (3.5)	0.78
DBP (mmHg)	104.1 (1.4)	100.9 (1.7)	0.17	103.7 (1.5)	105.1 (1.4)	0.51
HR (beats/min)	67.6 (1.5)	63.6 (2.0)	0.12	71.5 (2.7)	66.9 (1.7)	0.14
LVMI (g/m^2)	142.0 (5.0)	145.9 (4.6)	0.61	149.3 (5.9)	151.2 (7.7)	0.85
Dose (mg)	74.2 (4.9)	62.5 (6.1)	0.15	272.4 (13.1)	236.1 (15.4)	0.097

Mean values ± standard deviation.
Abbreviations: BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, LVMI = left ventricular mass index.

TABLE II Changes in blood pressure and heart rate at 12 weeks stratified by treatment and genotype

	Atenolol			Irbesartan		
Ser49Gly	Ser/Ser (n = 35)	Ser/Gly and Gly/Gly (n = 17)	p Value	Ser/Ser (n = 33)	Ser/Gly (n = 16)	p Value
SBP change (mmHg)	−11.6 (2.6)	−10.5 (3.8)	0.81	−14.1 (3.4)	−18.9 (4.9)	0.42
DBP change (mmHg)	−11.9 (1.3)	−11.6 (1.9)	0.91	−9.7 (1.8)	−7.9 (2.6)	0.56
HR change (beats/min)	−5.9 (1.7)	−11.7 (2.5)	0.063	−0.1 (1.4)	−0.6 (2.0)	0.86
Arg389Gly	Arg/Arg (n = 34)	Arg/Gly and Gly/Gly (n = 18)	p Value	Arg/Arg (n = 21)	Arg/Gly and Gly/Gly (n = 28)	p Value
SBP change (mmHg)	−13.7 (2.6)	−6.7 (3.6)	0.12	−12.5 (4.2)	−18.0 (3.7)	0.33
DBP change (mmHg)	−12.8 (1.3)	−9.9 (1.8)	0.19	−7.3 (2.3)	−10.5 (2.0)	0.29
HR change (beats/min)	−8.7 (1.8)	−6.2 (2.5)	0.41	−2.8 (1.7)	1.7 (1.5)	0.057

Mean values ± standard error.
Abbreviations as in Table I.

Studies of the functional importance of the Ser49Gly polymorphism have shown somewhat divergent results. In vitro studies have shown that the 49Gly variant of the receptor had a more profound adenylylcyclase activity on agonist stimulation, was more sensitive to the inhibitory effect of the antagonist metoprolol,[13] and showed a greater downregulation on long-term agonist stimulation.[13, 14] Börjesson *et al.*[9] showed that patients suffering from heart failure and who were homozygous for the wild type allele (Ser49Ser) had a significantly worse long-term prognosis, which was suggested to represent a more favorable disease progression among carriers of the Gly49 allele as a consequence of altered receptor function. In this study, a nonsignificant trend toward higher ejection fraction among patients with the Gly49 allele was noted, suggesting a function-

al importance of the Ser49Gly polymorphism. Furthermore, Ranade *et al.*[15] recently demonstrated an association between polymorphism and resting HR, with Ser homozygoutes having the highest mean HR and Gly homozygoutes having the lowest. We could not observe this in our group of hypertensive patients, but found a trend toward greater HR reduction among atenolol-treated patients carrying the Gly49 allele. Another case-control study, however, showed no association between polymorphism and hypertension.[16] Combining these data suggests a possible association between the Gly49 variant of the β_1-AR and an inherent cardioprotective effect.

Concerning the Arg389Gly polymorphism, in vitro studies have demonstrated that this polymorphism alters the function of the receptor so that the Gly389-variant exhibits lower basal levels of adenylyl cyclase activity and reduced responsiveness upon agonist-induced stimulation.[7] This finding suggests that the polymorphism could influence blood pressure level and HR, as well as an individual's response to β_1-AR blockade. A large case-control association study by Bengtsson *et al.* in Scandinavians identified individuals homozygous for the Arg389 allele as being at increased risk of developing hypertension.[16] In the same study, a genotype-discordant sibling analysis also showed that Finnish subjects with the Arg389Arg genotype had higher HR and DBP than carriers of the Gly389 allele. Bengtsson *et al.* suggested that the increased activity of the Arg389 variant of the β_1-adrenoceptor shown in vitro could be expected to lead to increased activity in vivo and thus to a higher cardiac output, which would explain the association between the Arg389 allele and hypertension.[16] Recently, however, two studies found no difference in exercise-induced HR and SBP response among healthy subjects who were homozygous for the Arg389 or Gly389 alleles, suggesting a limited functional importance of this polymorphism in vivo.[17, 18]

In our study, we observed no Arg389Gly genotype-dependent difference in baseline blood pressure or HR, nor did we find any significant differences between reduction of blood pressure and HR during treatment with the β_1-AR blocker atenolol. This suggests that this polymorphism is of at least no major importance in determining the response to this antihypertensive drug.

The strength of the present study is that the subjects represent a clinically well-characterized group, randomized to treatment in a prospective, double-blind trial; the limitation, on the other hand, is the small number of patients.

Conclusions

The Ser49Gly and Arg389Gly β_1-adrenergic receptor polymorphisms do not seem to exert a major effect on the changes in HR and blood pressure during 12 weeks of treatment with atenolol in patients with essential hypertension and LV hypertrophy. Nevertheless, we observed a tendency toward an association between the Ser49Gly polymorphism and the change in HR, which prevents us from rejecting completely the possibility of a functional role of the Ser49Gly polymorphism in determining the response to β_1-AR blockade. Most likely, a possible effect of the polymorphism would be a minor one which, in a clinical setting, may be irrelevant. Larger studies will be needed to explore the role of this polymorphism during β_1-AR blocker treatment further.

References

1. Engelhardt S, Hein L, Wiesmann F, Lohse MJ: Progressive hypertrophy and heart failure in beta$_1$-adrenergic receptor transgenic mice. *Proc Natl Acad Sci USA* 1999;96:7059–7064
2. Rohrer DK, Desai KH, Jasper JR, Stevens ME, Regula DP Jr, Barsh GS, Bernstein D, Kobilka BK: Targeted disruption of the mouse beta$_1$-adrenergic receptor gene: Developmental and cardiovascular effects. *Proc Natl Acad Sci USA* 1996;93:7375–7380
3. Rohrer DK, Chruscinski A, Schauble EH, Bernstein D, Kobilka BK: Cardiovascular and metabolic alterations in mice lacking both beta$_1$- and beta$_2$-adrenergic receptors. *J Biol Chem* 1999;274:16701–16708
4. Strader CD, Fong TM, Tota MR, Underwood D, Dixon RA: Structure and function of G protein-coupled receptors. *Ann Rev Biochem* 1994;63: 101–132
5. Tesson F, Charron P, Peuchmaurd M, Nicaud V, Cambien F, Tiret L, Poirier O, Desnos M, Jullières Y, Amouyel P, Roizes G, Dorent R, Schwartz K, Komajda M: Characterization of a unique genetic variant in the beta$_1$-adrenoceptor gene and evaluation of its role in idiopathic dilated cardiomyopathy. CARDIGENE Group. *J Mol Cell Cardiol* 1999;31:1025–1032
6. Maqbool A, Hall AS, Ball SG, Balmforth AJ: Common polymorphisms of beta$_1$-adrenoceptor: Identification and rapid screening assay (letter). *Lancet* 1999;353:897
7. Mason DA, Moore JD, Green SA, Liggett SB: A gain-of-function polymorphism in a G-protein coupling domain of the human beta$_1$-adrenergic receptor. *J Biol Chem* 1999;274:12670–12674
8. Moore JD, Mason DA, Green SA, Hsu J, Liggett SB: Racial differences in the frequencies of cardiac beta(1)-adrenergic receptor polymorphisms: Analysis of c145A>G and c1165G>C (letter). *Hum Mutat* 1999;14:271
9. Börjesson M, Magnusson Y, Hjalmarson A, Andersson B: A novel polymorphism in the gene coding for the beta(1)-adrenergic receptor associated with survival in patients with heart failure. *Eur Heart J* 2000;21:1853–1858
10. Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Öhman KP, Nyström F, Hagg A, Lind L: Angiotensin-converting enzyme gene polymorphism predicts blood pressure response to angiotensin II receptor type 1 antagonist treatment in hypertensive patients. *J Hypertens* 2001;19:1783–1787
11. Kurland L, Melhus H, Karlsson J, Kahan T, Malmqvist K, Öhman P, Nyström F, Hagg A, Lind L: Aldosterone synthase (CYP11B2) -344 C/T polymorphism is related to antihypertensive response: Result from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. *Am J Hypertens* 2002;15:389–393
12. Malmqvist K, Kahan T, Edner M, Held C, Hagg A, Lind L, Muller-Brunotte R, Nyström F, Öhman KP, Osbakken MD, Ostergern J: Regression of left ventricular hypertrophy in human hypertension with irbesartan. *J Hypertens* 2001;19:1167–1176
13. Levin MC, Marullo S, Muntaner O, Andersson B, Magnusson Y: The myocardium-protective Gly49 variant of the beta 1-adrenergic receptor exhibits constitutive activity and increased desensitization and downregulation. *J Biol Chem* 2002;277:30429–30435
14. Rathz DA, Brown KM, Kramer LA, Liggett SB: Amino acid 49 polymorphisms of the human beta1-adrenergic receptor affect agonist-promoted trafficking. *J Cardiovasc Pharmacol* 2002;39:155–160
15. Ranade K, Jorgenson E, Sheu WH, Pei D, Hsiung CA, Chiang FT, Chen YD, Pratt R, Olshen RA, Curb D, Cox DR, Botstein D, Risch N: A polymorphism in the beta1 adrenergic receptor is associated with resting heart rate. *Am J Hum Genet* 2002;70:935–942
16. Bengtsson K, Melander O, Orho-Melander M, Lindblad U, Ranstam J, Rastam L, Groop L: Polymorphism in the beta(1)-adrenergic receptor gene and hypertension. *Circulation* 2001;104:187–190
17. Xie HG, Dishy V, Sofowora G, Kim RB, Landau R, Smiley RM, Zhou HH, Wood AJ, Harris P, Stein CM: Arg389Gly beta 1-adrenoceptor polymorphism varies in frequency among different ethnic groups but does not alter response in vivo. *Pharmacogenetics* 2001;11:191–197
18. Buscher R, Belger H, Eilmes KJ, Tellkamp R, Radke J, Dhein S, Hoyer PF, Michel MC, Insel PA, Brodde OE: In-vivo studies do not support a major functional role for the Gly389Arg beta 1-adrenoceptor polymorphism in humans. *Pharmacogenetics* 2001;11:199–205