

# A novel polymorphism in the gene coding for the beta<sub>1</sub>-adrenergic receptor associated with survival in patients with heart failure

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**Aims** The adrenergic nervous system is of major importance in congestive heart failure. No genetic polymorphism has previously been identified in the beta<sub>1</sub>-adrenergic receptor gene. The aim of this study was to find possible mutations in this gene and to relate such findings to morbidity and prognosis in heart failure.

**Methods and Results** Genomic DNA was extracted from blood leukocytes from patients with congestive heart failure (n=184) and from age-matched controls (n=77). The part of the beta<sub>1</sub>-adrenergic receptor gene corresponding to nucleotide 1–255 was amplified by polymerase chain reaction and analysed by automated sequencing. The patients were investigated by echocardiography and followed regarding symptoms and survival for 5 years. A missense mutation was identified at nucleotide position 145 in the beta<sub>1</sub>-adrenergic receptor gene, which predicted an amino acid substitution at position 49 (*Ser49Gly*). The allele frequency of the *Gly49* variant was 0.13 in controls and 0.18 in patients ( $P=0.19$ ). At the time of the 5-years follow-up, 62% of the patients with the wild type gene and 39% of the patients with the *Ser49Gly* variant had died or had

experienced hospitalization ( $P=0.005$ ). Patients without the mutation had significantly poorer survival compared to those with the mutation, risk ratio 2.34 (95% CI 1.30–4.20),  $P=0.003$ . In a multivariate analysis, the risk ratio was 2.03 (95% CI 0.99–4.16)  $P=0.05$ .

**Conclusion** A novel missense mutation in the beta<sub>1</sub>-adrenergic receptor gene was associated with a decreased mortality risk in patients with congestive heart failure. These data suggest that the beta<sub>1</sub>-receptor *Ser49Gly* variant might be associated with altered receptor function, resulting in myocardial protection in patients with heart failure. (Eur Heart J 2000; 21: 1853–1858, doi:10.1053/euhj.1999.1994)

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**Key Words:** Polymorphism (genetics), receptors, adrenergic, beta<sub>1</sub>, prognosis, congestive heart failure, congestive cardiomyopathy, hospitalization.

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## Introduction

Activation of the sympathetic nervous system is part of the pathophysiological adaptation of the circulatory system in congestive heart failure. Whereas previous attempts to improve cardiac symptomatology and outcome by facilitating sympathetic activity using inotropic drugs have failed, beta-adrenergic blockers have become increasingly promising as a treatment

alternative in congestive heart failure. The first test with a beta-blocker in heart failure was done at our department in 1974<sup>[1]</sup>, and a 3-year follow-up of the first major controlled study (the Metoprolol in Dilated Cardiomyopathy trial) has been presented<sup>[2]</sup>. Two large survival studies (CIBIS-II and MERIT-HF) were both stopped early due to significant survival benefit in the active treated groups<sup>[3,4]</sup>.

Studies of genetic polymorphisms have shown two different point mutations in the beta<sub>2</sub>-adrenergic receptor gene associated with asthma and obesity and with an altered adipocyte receptor function<sup>[5–7]</sup>. It has also been suggested that beta<sub>2</sub>-receptor polymorphisms might predict mortality in congestive heart failure<sup>[8]</sup>. The gene coding the beta<sub>1</sub>-adrenergic receptor has been cloned and sequenced<sup>[9]</sup>. It is a gene without introns localized to

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the chromosome 10q24-26<sup>[10]</sup>. Two new polymorphisms in the  $\beta_1$ -adrenergic receptor have been discovered. The mutation in the N-terminal part of the receptor was first reported by us at the American College of Cardiology Scientific Sessions 1999 and is presented in this paper<sup>[11]</sup>. We and others have also found a mutation in the C-terminal, but as yet without clinical associations<sup>[12,13]</sup>.

The aim of the present study was to find possible genetic polymorphisms in the  $\beta_1$ -adrenergic receptor by automated sequencing, and to relate such findings to clinical prognosis and morbidity in patients with idiopathic dilated cardiomyopathy.

## Patients and methods

### *Patients and controls*

Patients with idiopathic congestive heart failure were recruited from an epidemiological survey performed in the five western counties in Sweden, between 1985 and 1988. This study aimed primarily at studying the frequency of idiopathic heart failure and dilated cardiomyopathy. At the time of investigation there were 1.64 million inhabitants in the region, being served by 19 hospitals. A survey of all patients, aged 16-65, hospitalized with a registered diagnosis of congestive heart failure from 1980-1987 was performed (n=2711). Besides a diagnosis of heart failure made by the responsible doctor, one of the following six findings was required for inclusion in the study: pulmonary rales; pulmonary congestion on chest X-ray; peripheral oedema of probable cardiac origin; significant weight loss following diuretic treatment; cardiogenic shock; or autopsy findings of congestion and heart failure. From the hospital records all possible causes of congestive heart failure were identified, including ischaemic heart disease, hypertension, valvular heart disease, alcoholism, insulin-treated diabetes mellitus, systemic diseases, serious infections and myocarditis, hypertrophic cardiomyopathy, cancer treatment, and pericardial diseases<sup>[14]</sup>. Patients with no obvious cause of heart failure were invited to a clinical investigation. Out of 584 eligible patients with apparently idiopathic congestive heart failure, 411 of whom were alive, 293 accepted the investigation. The present investigations were performed at seven of the regional hospitals and blood samples for central analysis at the coordinating centre were obtained at four of these seven hospitals, comprising the present study population (n=184). A random sample from the general population of individuals free from cardiovascular disorders and age-matched to the patient group was used as controls (n=77). The selection procedure of the control group has been described previously<sup>[15]</sup>.

All patients and control subjects were investigated by echocardiography and had a clinical examination including ECG, chest radiographs and routine laboratory tests<sup>[15,16]</sup>. Peripheral blood was drawn from a

venous cannula. Plasma and blood cells were separated by centrifugation, and stored in a  $-70^\circ\text{C}$  freezer until further analysis. Coronary angiography or autopsy could be evaluated in 46 patients, 39 of whom had normal coronary angiograms.

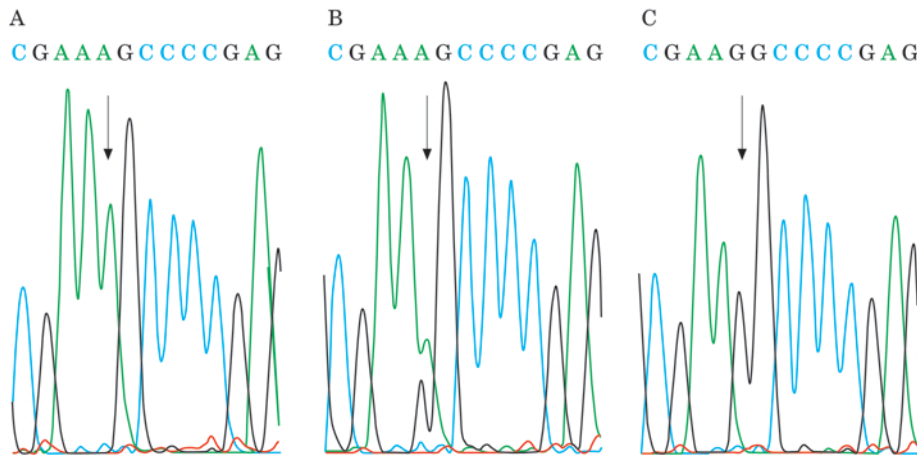
Two years and 5 years after the echocardiographic investigation all living patients were asked through a questionnaire about symptoms, treatment, episodes of hospitalization or cardiac surgery. Survival status was checked repeatedly during the follow-up period with the population registry, in which all individuals and deaths are registered. Death or cardiac transplantation was considered as an end-point.

### *Genetic analyses*

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp Blood Kit (QIAGEN Inc.). Polymerase chain reactions were performed to amplify the fragment corresponding to the nucleotide position 1-794 of the  $\beta_1$ -adrenergic receptor gene, using the forward primer 5' ATG GGC GCG GGG GTG CTC GTC 3' and the reverse primer 5' GAA ACG GCG CTC GCA GCT GTC G 3'. Each 100  $\mu\text{l}$  polymerase chain reaction contained 250 ng genomic DNA, 150 nM of each primer, 200  $\mu\text{M}$  dNTPs, 2 mM  $\text{MgCl}_2$ , 20 mM  $(\text{NH}_4)_2\text{SO}_4$ , 75 mM Tris-HCl pH 9, 0.01% (w/v) Tween, 2.5 units Taq Polymerase (Life Technologies) and 10% DMSO. The polymerase chain reaction was accomplished in 35 cycles ( $94^\circ\text{C}$ , 1 min;  $62^\circ\text{C}$ , 1 min;  $71^\circ\text{C}$ , 1 min) preceded by 2 min at  $94^\circ\text{C}$  and followed by 7 min at  $71^\circ\text{C}$ . The polymerase chain reaction fragments were purified using Centricon-100 columns. Cycle sequencing was performed using the reverse primer 5' CTT GGC GAT GGC CAC GAT CAC 3' (nucleotide 234-255) and the ABI PRISM Big Dye Terminator Ready Reaction Mix according to the manufacturer's protocol. The extension products were purified by ethanol/sodium acetate precipitation and the pellet was resuspended in 6  $\mu\text{l}$  loading buffer, containing deionized formamide and 25 mM EDTA (pH 8.0) with blue dextran ( $50\text{ mg} \cdot \text{ml}^{-1}$ ) in a 5:1 ratio. Sequence analysis of dye-labelled products was performed using the Applied Biosystems Model 377 DNA Sequencer. Individual lane files of interest were transferred to the Factura software analysis package (ABI), which processes files to identify likely heterozygous positions.

### *Statistical analysis*

A possible difference in allele frequency between patients and controls was tested by the chi-square test. Survival curves were constructed using the Kaplan-Meier method. The log rank test was used to correlate mortality to the following variables: presence of the  $\beta_1$ -adrenergic receptor mutation (heterozygous and homozygous patients analysed together), gender, age,



**Figure 1** Automated sequence analysis of the human  $\beta_1$ -adrenergic receptor gene. The electropherogram shows the nucleotide sequences 141-153 of the wild type (panel A) and the *Ser49Gly* variant in its heterozygote (panel B) and homozygote forms (panel C). The mutation site at nucleotide 145 is indicated by an arrow.

**Table 1** Baseline characteristics of 184 patients with idiopathic congestive heart failure, with the wild type and the *Ser49Gly* variant in its heterozygote and homozygote forms of the  $\beta_1$ -adrenergic receptor gene

	Wild type n=123	<i>Ser49Gly</i> variant n=61	P
Female gender	25%	27%	0.86
Age (years)	58 ± 11	56 ± 11	0.38
Disease duration (months)	84 ± 86	78 ± 72	0.58
Systolic blood pressure (mmHg)	133 ± 26	133 ± 23	0.93
Heart rate	77 ± 17	75 ± 17	0.41
Ejection fraction	0.40 ± 0.18	0.45 ± 0.16	0.052
Serum sodium (mmol . l <sup>-1</sup> )	138 ± 3.6	139 ± 2.7	0.09
Serum creatinine (μmol . l <sup>-1</sup> )	100 ± 25	97 ± 22	0.51
ACE-inhibitor treatment	31%	20%	0.16
Beta-blocker treatment	39%	38%	1.00

disease duration, systolic blood pressure, heart rate, serum sodium, serum creatinine, ejection fraction, and drug treatment with ACE inhibitors and beta-blockers. These variables were used (if  $P < 0.10$  in the univariate analysis) in a multivariate stepwise Cox proportional hazards model to evaluate the influence on mortality. A test for interaction between the effects on survival of the mutation and the other tested variables was performed. The statistical analyses were performed on SAS statistical software (SAS Inc, Cary, NC). Mean values are expressed as mean ± SD. Risk evaluation is expressed as odds ratio (OR) with 95% confidence intervals (CI).  $P < 0.05$  was considered statistically significant.

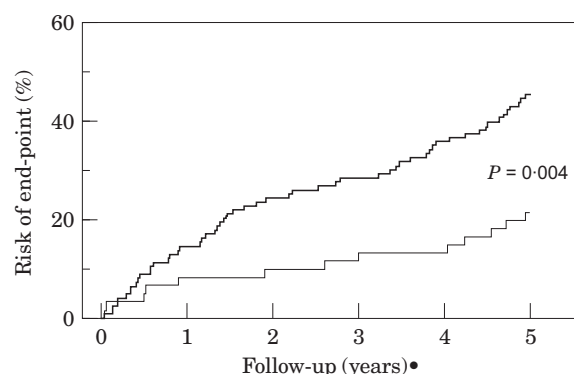
## Results

Complete sequence analysis of nucleotides 1 to 255 of the  $\beta_1$ -adrenergic receptor gene was performed on DNA from 261 patients and controls. A polymorphism was detected at nucleotide position 145, resulting in an

amino acid substitution of serine (AGC) by glycine (GGC) at amino acid position 49 (*Ser49Gly*) (Fig. 1). To verify the mutation, the opposite strand was sequenced. Repeated polymerase chain reactions followed by automated sequencing provided reproducible results. The allele frequency of the missense mutation was 0.13 in healthy controls (n=77) and 0.18 in patients with idiopathic heart failure (n=184) ( $P=0.19$ ). Among the patients there were 61 with the *Ser49Gly* variant (five homozygous and 56 heterozygous), and 19 cases among the controls (one homozygous and 18 heterozygous). In the 39 patients with normal coronary angiograms, the allele frequency was 0.13.

## Follow-up

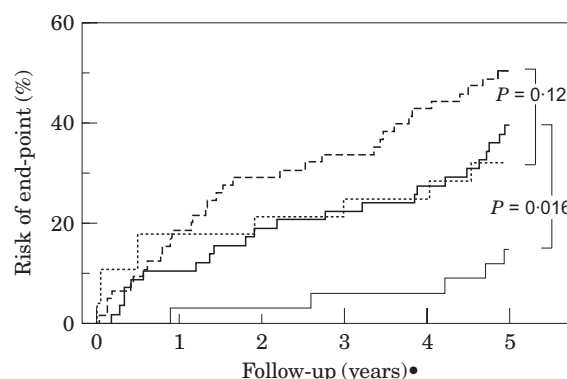
The baseline characteristics of the patients with and without the *Ser49Gly*  $\beta_1$ -adrenergic receptor variant (heterozygous or homozygous) are shown in Table 1. According to the inquiry at the 2-year follow-up, 29%



**Figure 2** Survival curves expressing the risk of end-point (death or cardiac transplantation) for 184 patients with congestive heart failure. The bold line represents patients with the wild type gene ( $n=123$ ), and the medium line represents patients with the  $\beta_1$ -adrenergic receptor *Ser49Gly* variant ( $n=61$ ). The two curves were significantly different according to the log rank test ( $P=0.004$ ).

(22 of 76) of the patients alive and with the wild type  $\beta_1$ -adrenergic receptor had experienced at least one occasion of hospitalization due to a cardiac disorder, as compared with 12% (five of 42) of the patients with the  $\beta_1$ -adrenergic receptor *Ser49Gly* variant ( $P=0.060$ ). After 5 years, 62% (77 of 123) and 39% (24 of 61) of the patients had experienced death or hospitalization ( $P=0.005$ ), with the wild type and  $\beta_1$ -adrenergic receptor *Ser49Gly* variant, respectively. During the follow-up there were 46% (56 of 123) deaths in the group of patients with the wild type  $\beta_1$ -adrenergic receptor gene, 23% (13 of 56) deaths in the group with the heterozygous *Ser49Gly* variant, and 20% (one of five) deaths among the homozygous patients. In the univariate Cox regression analysis, several variables were significantly correlated to 5-year mortality, including the  $\beta_1$ -adrenergic receptor gene polymorphism. The wild type gene was associated with an increased risk of death as compared with the mutated gene (OR 2.34 [95% CI 1.30–4.20],  $P=0.003$ ) (Fig. 2). In a multivariate stepwise Cox regression analysis, ejection fraction (OR 0.97 [95% CI 0.95–0.99],  $P=0.006$ ), systolic blood pressure (OR 0.98 [95% CI 0.97–0.99],  $P=0.006$ ), and serum creatinine (OR 1.02 [95% CI 1.01–1.02],  $P=0.001$ ) were independently associated with long-term survival. When the wild type genotype was tested separately with correction for all other variables with  $P<0.10$ , the OR for the genotype was 2.03 (95% CI 0.99–4.16),  $P=0.05$ . There was no significant interaction between the two genotypes and any other prognostic variable regarding effects on survival. Further, the influence on survival was also checked in the 39 patients with confirmed normal coronary anatomy. There was a similar trend regarding the risk of end-points as compared with the total investigated group ( $P=0.059$ ).

At baseline, 38% of the patients used beta-blockers, 42% at the 2-year follow-up, and 49% at the 5-year follow-up. The influence of the *Ser49Gly* genotype on survival in patients with beta-blocker treatment at any



**Figure 3** Survival curves expressing the risk of end-point (death or cardiac transplantation) for patients with congestive heart failure according to the use of beta-blockers and the presence of the *Ser49Gly* polymorphism. The broken line represents patients without beta-blockers and possessing the wild type gene ( $n=63$ ); the dotted line represents patients without beta-blockers and with the *Ser49Gly* variant ( $n=28$ ); the bold line represents wild type patients with beta-blockade treatment any time during follow-up ( $n=59$ ); the medium line represents patients with beta-blockade treatment and possessing the *Ser49Gly* variant gene ( $n=33$ ). Differences between curves were tested with the log rank test. In patients without beta-blockers, the curves between the wild type and *Ser49Gly* groups were not statistically different ( $P=0.12$ ). In patients using beta-blockers, there was a statistically significant difference between the survival curves, wild type and *Ser49Gly* respectively ( $P=0.016$ ).

time during follow-up (50%, 92 of 184) was compared to patients without treatment ( $n=92$ ). OR for the wild type genotype in patients on beta-blocker treatment was 3.08 (95% CI 1.17–8.10),  $P=0.02$ , and in patients without beta-blocker treatment 1.79 (95% CI 0.86–3.75),  $P=0.12$  (Fig. 3).

## Discussion

We have discovered a naturally occurring polymorphism in the  $\beta_1$ -adrenergic receptor gene and found an association between the presence of the *Ser49Gly* variant and improved long-term survival.

### Patient selection

In the present study we used a patient group that was sampled for other purposes. The patients in this epidemiological investigation were considered to have idiopathic heart failure and comprised patients with idiopathic dilated cardiomyopathy. The possibility of concealed coronary artery disease in these patients cannot be excluded without the performance of angiography. In a subset of the presently studied patients we performed coronary angiography and found that



15% of these had coronary artery disease<sup>[15]</sup>. Therefore, a proportion of the cases were of ischaemic aetiology.

A non-significant trend towards higher ejection fraction was noted in the *Ser49Gly* group, which might represent a more favourable disease progression as a consequence of altered receptor function. There was no significant correlation between ejection fraction and *Ser49Gly* polymorphism, either among controls or among patients.

### *Possible consequences for beta-receptor function*

The reported missense gene variant at nucleotide position 145 corresponds to a replacement of serine by glycine at position 49 in the extracellular N-terminal region of the beta<sub>1</sub>-adrenergic receptor. Experimental deletion studies have shown that the N-terminal region may be important to fold the receptor within the membrane, while not being the target for ligand binding or receptor activation<sup>[17]</sup>. Nevertheless, a polymorphism in the N-terminal region might have conformational consequences in transmembrane and intracellular regions essential for catecholamine binding and down-regulation. Indeed, recent experimental findings of polymorphisms in the beta<sub>2</sub>-adrenergic receptor gene — corresponding to differences in the N-terminal region — were associated with altered receptor function in human adipocytes and with altered receptor processing and down-regulation<sup>[7,18]</sup>.

There are several plausible clinical consequences of an aberrant receptor protein, such as alterations in catecholamine sensitivity, receptor signalling, changes in down-regulation<sup>[19]</sup>, and differences in autoimmunity. In contrast to normals and patients with ischaemic heart disease, patients with idiopathic dilated cardiomyopathy have been found to have autoantibodies toward the beta<sub>1</sub>-adrenergic receptor<sup>[20,21]</sup>. They also display differences in beta<sub>1</sub>-adrenergic receptor properties as compared to patients with ischaemic cardiomyopathy<sup>[22,23]</sup>.

### *Risk of death or cardiac transplantation*

The association with long-term survival was an unexpected finding. The wild type gene was associated with a significant two-fold increased risk of end-point (death or cardiac transplantation) during the 5-year follow-up. Furthermore, the association was also of borderline significance in the multivariate analysis ( $P=0.05$ ). It would have been anticipated that a gene defect might be associated with a dysfunction in the receptor protein and subsequently with an increased risk of disease. Other genetic polymorphisms, such as in the beta<sub>2</sub>-adrenergic receptor and the beta<sub>3</sub>-adrenergic receptor genes have been associated with an increased risk of morbidity. However, in accordance with the concept that beta-blockade is beneficial to the failing heart, dysfunction in

the beta<sub>1</sub>-adrenergic receptor protein might have similar effects. It was therefore hypothesized that the beta<sub>1</sub>-adrenergic receptor mutation might cause myocardial protection and a more favourable course of the disease. Another possibility is that the receptor might be resistant to down-regulation, and thereby maintain normal receptor function. The majority of beta-receptor studies have been conducted on beta<sub>2</sub>-adrenergic receptors, and beta<sub>1</sub>-adrenergic receptor function is not well understood. Further investigations are required to establish a relationship between the beta<sub>1</sub>-adrenergic receptor mutation and possible changes in receptor function and autoimmunity, and such studies are presently in progress.

It has long been known by cardiologists who have used beta-blockers in heart failure, that there are responders and non-responders to this therapy. Many attempts have been made to identify responders, but the association with different markers is only slight. It was therefore intriguing that the difference between genotypes appeared to be more pronounced in patients on beta-blocker treatment, suggesting that beta-blockade might be most effective in patients with the *Ser49Gly* variant. The survival curve for the *Ser49Gly* patients without beta-blockade was almost identical to the curve of the wild type group treated with beta-blockade. As this was a retrospective analysis, the hypothesis that beta-blockade treatment would be more effective in patients with the *Ser49Gly* genotype needs to be prospectively tested.

## **Conclusion**

A novel naturally occurring polymorphism in the beta<sub>1</sub>-adrenergic receptor gene was discovered. Although the pathophysiological importance of this mutation has not yet been clarified, our data suggest that the *Ser49Gly* variant in the beta<sub>1</sub>-adrenergic receptor gene might be associated with a decreased risk of morbidity and mortality in patients with congestive heart failure.

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