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No positive association between adrenergic receptor variants of α_{2c}Del322–325, β_1Ser49, β_1Arg389 and the risk for heart failure in the Japanese population

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Aims
We investigated the correlation of adrenergic receptor polymorphisms, α_{2c}Del322–325, β_1Ser49Gly and β_1Arg389Gly, with the risk of heart failure in the Japanese population.

Methods
These polymorphisms were analysed by polymerase chain reaction-restriction fragment length polymorphism in patients with chronic heart failure due to idiopathic dilated cardiomyopathy (DCM) and compared with the control group.

Results
There were no differences or any trends in the allele and genotype frequencies of the β_1Ser49Gly and β_1Arg389Gly polymorphisms. The allele frequency of the α_{2c}Del322–325 variant was lower in patients than in controls (0.11 *vs.* 0.04, $P = 0.011 < 0.017$, by Bonferroni correction), while the genotype frequency just failed to reach significance ($P = 0.022 > 0.017$, by Bonferroni correction).

Conclusions
In this population, the variants β_1Ser49, β_1Arg389, and α_{2c}Del322–325 do not appear to be risk factors for chronic heart failure due to DCM. The α_{2c}Del322–325 variant may in fact confer some protection.

Introduction

Neurohumoral factors play important roles in cardiac remodelling, determining the prognosis of heart failure. In particular, the sympathetic nervous system is activated in patients with chronic heart failure (CHF) [1] and sustained stimulation of the adrenergic system exerts direct adverse effects on cardiac function [2]. In spite of the importance of the adrenergic system, the effects of polymorphic mutation of adrenergic receptors on CHF remain to be fully elucidated.

In the present study, we focus on the presynaptic α_{2c} adrenergic receptor (AR) polymorphism with the deletion of four consecutive amino acids, α_{2c}Del322–325, and polymorphic amino acid variants of β_1AR, Ser49Gly and Arg389Gly. These polymorphic changes result in alteration of AR function. The presynaptic

α_2AR negatively regulates the release of norepinephrine from cardiac sympathetic nerves [3] and α_{2c}Del322–325 polymorphism shows a 'loss-of-function' phenotype [4]. The postsynaptic β_1AR polymorphism, β_1Ser49Gly, affects receptor sensitivity and promotes the downregulation of the receptor to agonists *in vitro* [5]. The change of β_1AR from Arg to Gly at the 389 amino acid residue leads to the decrease in G-protein coupling [6]. Considering the importance of the adrenergic system as a modulator of cardiac remodelling, it could be proposed that polymorphisms of adrenergic receptor genes may be closely related to the risk of heart failure.

Recently, Small *et al.* proposed that the polymorphisms of β_1Arg389Gly and α_{2c}Del322–325 are synergistically related to the risk of CHF in a black population [7]. However several concerns, including the aetiology of heart failure and the absence of analysis of β_1Ser49Gly frequency, have been raised against this study [8]. We have investigated the clinical significance of α_{2c}Del322–325, β_1Ser49Gly, and β_1Arg389Gly for the risk of heart failure due to dilated cardiomyopathy (DCM) in the Japanese.

Methods

Subjects

The study subjects consisted of 91 unrelated consecutive patients with CHF due to idiopathic DCM (males 79.5%, age 58.4 ± 13.7 years, ejection fraction 34.6 ± 15.8%) who attended or were admitted to Hokkaido University Hospital, Kyoto Katsura Hospital, Osaka Prefectural Medical Centre for Respiratory and Allergic Diseases, Aizenbashi Hospital or Osaka City University Medical School Hospital. The ratio of the patients, classified as NYHA class I, II, III or IV, was 13.9, 46.8, 15.1, 24.1%, respectively. Patients with ischaemic cardiomyopathy were excluded. One hundred and nineteen subjects (all males, aged from 20 to 40 years) who had no history or symptoms of cardiovascular disease were chosen as controls. This study was approved by the institutional review committee. All subjects gave their informed consent to participate.

Genotyping

Genomic DNA was extracted from samples of peripheral blood leucocytes using the QIAamp DNA Blood Maxi Kit (Qiagen K.K., Tokyo, Japan) according to the manufacturer's protocol. Genotyping of α_{2c}Del322–325, β_1Ser49Gly and β_1Arg389Gly polymorphisms was performed as described previously [4–6] with minor modifications.

Statistical analysis

Values were expressed as means ± SD. χ^2 test of independence was used to test for associations between heart failure and allele. The 2×3 exact probability test was used to evaluate associations between heart failure and genotype. All of the analyses were corrected by Bonferroni correction. $P < 0.017$ was considered to be significant. Statistical analysis was performed with StatView Version 5.0 software (SAS Institute, Cary, NC, USA).

Results

The allele and genotype frequencies of α_{2c}Del322–325, β_1Ser49Gly and β_1Arg389Gly polymorphisms in the patients and controls are shown in Table 1. The allele

Table 1
Distribution of α_{2c} and β_1 adrenergic receptor (AR) variants in controls and patients with heart failure

Alleles and subjects	Allele Frequency	*P*-value	Frequency		Genotype	*P*-value
α_{2c}Del322–325			WT/WT	WT/Del	Del/Del	
Controls	0.11	0.011	95/119 (79.8%)	23/119 (19.3%)	1/119 (0.8%)	0.022
Patients with heart failure	0.04		84/91 (92.3%)	7/91 (7.7%)	0/91 (0%)	
β_1Arg389			Gly/Gly	Gly/Arg	Arg/Arg	
Controls	0.81	0.82	5/119 (4.2%)	35/119 (29.4%)	79/ 119 (66.4%)	0.94
Patients with heart failure	0.80		5/91 (5.5%)	26/91 (28.6%)	60/91 (65.9%)	
β_1Ser49			Gly/Gly	Gly/Ser	Ser/ Ser	
Controls	0.84	0.90	3/119 (2.5%)	33/119 (27.7%)	83/119 (69.7%)	0.58
Patients with heart failure	0.84		4/91 (4.4%)	21/91 (23.1%)	66/91 (72.5%)	

P-values for comparisons of allele frequency or genotype frequency between controls and patients with heart failure were determined by 2×2 χ^2 or by 2×3 exact probability test, respectively. P-value < 0.017 (0.05/3) was considered to be significant.

Table 2
Combined genotypes of β_1AR and the risk for heart failure

β_1Ser49Gly	β_1Arg389Gly	Controls	Patients with heart failure	Odds ratio for heart failure (95%CI)	*P*-value
		No. of subjects			
		119	91		
≧ 1Gly	≧ 1Gly	6	6	1.00	–
≧ 1Gly	Arg/Arg	30	19	0.63 (0.18–2.25)	0.35
Ser/Ser	≧ 1Gly	34	25	0.74 (0.21–2.55)	0.43
Ser/Ser	Arg/Arg	49	41	0.84 (0.25–2.79)	0.50

Subjects with at least one β_1Gly49 allele and at least one β_1Gly389 allele served as the reference group. Odds ratios and P-values between the reference group and each other group were determined by 2 × 2 χ^2 test. P-value < 0.017 (0.05/3) was considered to be significant.

frequency of the α_{2c}Del322–325 variant was lower in patients with CHF than in controls (0.04 *vs.* 0.11, $P = 0.011 < 0.017$, by Bonferroni correction) and the genotype frequency was not significant but showed the *P*-value nearly equal to the borderline of significance ($P = 0.022 > 0.017$, by Bonferroni correction). The allele and genotype frequencies of the β_1Arg389 and β_1Ser49 variants in the patients with heart failure were consistent with those of controls.

Combined genotypes of β_1Arg389 and β_1Ser49 variants were not associated with the risk of heart failure (Table 2).

Discussion

The frequency of β_1Arg389Gly and β_1Ser49Gly polymorphisms did not differ from those in the control group, nor were there any trends, suggesting that these polymorphisms are not associated with susceptibility to CHF. Similarly, the combined genotype of β_1Arg389Gly and β_1Ser49Gly was not associated with the risk of CHF. However, we cannot definitely exclude the possibility that lack of association is derived from a β error problem, although the number of samples in our study was more than that in the previous study [7].

The allele frequency of the α_{2c}Del322–325 variant was statistically lower in CHF than in the controls. However, it is uncertain that the α_{2c}Del322–325 variant is a negative risk factor clinically, because the genotype frequency of this variant was of borderline significance, probably due to the low frequency of the homozygous genotype for α_{2c}Del322–325 variant in the Japanese population. Thus, considering the limitation of low genotype frequency of this polymorphism, a reasonable interpretation of our results is that α_{2c}Del322–325 variant is not a positive risk factor for CHF due to DCM in the Japanese population.

Previously, it was reported that allele frequency of α_{2c}Del322–325 positively correlated with heart failure in both the white and black populations [7]. It remains to be clarified why our data are not consistent with the previous study [7]. One possibility is that the inconsistency might be derived from the difference in the cause of heart failure. The previous study included patients with ischaemic cardiomyopathy, while we excluded ischaemia because some adrenoceptor polymorphisms are related to hypertension, a risk factor for ischaemic heart disease [9, 10]. Another possibility is that the pathophysiological significance of the adrenoceptor polymorphism is closely related to the severity of heart failure. Importantly, the ratio of the patients classified as NYHA III or IV is lower in the present study than that in the previous report [7]. It may be hypothesized that there is a racial difference in the severity of CHF. Indeed, the previous study reported that Japanese patients with congestive heart failure show low mortality [11].

In addition to healthy controls, who are all males aged between 20 and 40 years, we analysed 189 diabetes patients (58% male with an age 60.4 ± 9.8 years) who did not suffer from CHF, as an age-matched control. It was found that there were no differences in the allele and genotype frequencies of α_{2c}Del322–325 among healthy controls, male diabetes patients, and female diabetes patients (data not shown). Moreover, the allele frequency of α_{2c}Del322–325 was lower in the patients with CHF than in those with diabetes (0.04 and 0.10, respectively). Thus it is unlikely that α_{2c}Del322–325 polymorphism affected survival through other causes, resulting in the influence on allele frequency of samples.

Recent studies demonstrated that a genetic variability of β_2AR, Thr164Ile, is closely related to heart failure. In patients with congestive cardiac failure, patients with homozygous genotype Ile/Ile show high mortality compared with those with other genotypes [12]. However, we could not statistically confirm the previous findings, probably because of low frequency of the mutation.

In summary, the α_{2c}Del322–325, β_1Ser49 and β_1Arg389 variants do not appear to be risk factors for CHF due to DCM in a Japanese population, and the α_{2c}Del322–325 variant may be protective. Considering the contradiction with the previous report, it is proposed that there may be a racial difference in the clinical importance of this polymorphism. Further efforts should be made to address any possible racial differences in the responsiveness of heart failure from different causes to β-blockers.

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