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Effects of β-Adrenoceptor and Catechol-O-Methyl-Transferase (COMT) Polymorphism on Postoperative Outcome in Cardiac Surgery Patients

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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* This article is dedicated to the honour of my deceased academic teacher Prof. Dr. Otto-Erich Brodde (1942–2007), who made me interested in the possible implications of beta-adrenoceptor polymorphisms

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Background: There is a long-standing debate about the role of beta-adrenoceptor polymorphisms in the cardiovascular system. We wanted to elucidate whether β1-adrenoceptor-polymorphisms affects the postoperative catecholamine consumption and the length of intermediate care unit stay in patients undergoing cardiac surgery, and whether this might be enhanced or attenuated by catechol-O-methyl-transferase (COMT) polymorphism.

Material/Methods: We included 116 patients (63±1.2 years; 34% females; 81±1 kg) undergoing cardiac surgery. We assessed Arg389Gly and Ser49Gly-β1-adrenoceptor (B1AR) polymorphism together with Val158Met-COMT polymorphism by real-time PCR using fluorescence resonance energy transfer (PCR-FRET). The preoperative risk was assessed by EuroSCORE. In addition, we measured the endogenous preoperative epinephrine and norepinephrine plasma concentrations using an electrochemical HPLC method.

Results: 84.6% were homozygous for Ser49Ser, 52.1% homozygous for Arg389Arg B1AR, and 32.5% for Val158Val-COMT, while 15.4% showed Ser49Gly B1AR, 38.5% Arg389Gly-B1AR, and 35.6% Val158Met-COMT. We found that the Gly49-variant, the Gly389-variant, and the Val158-COMT-variant were associated with higher postoperative norepinephrine consumption. All patients carrying the Val158-COMT allele exhibited higher preoperative norepinephrine concentrations. Moreover, we found that both β1-adrenoceptor polymorphisms were associated with a longer stay in hospital, which was modulated by the COMT polymorphism.

Conclusions: These data show that the β1-adrenoceptor polymorphisms, together with the COMT polymorphism, affect norepinephrine consumption and stay in hospital in a situation of enhanced cardiovascular stress, reflected here by the postoperative period after cardiac surgery. Moreover, we conclude that patients with the Val158-COMT genotype exhibit higher endogenous resting plasma norepinephrine levels.

MeSH Keywords: Postoperative Care • Receptors, Adrenergic, beta-1 • Thoracic Surgery

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Background

β1-adrenoceptors play an essential role in the regulation of the cardiovascular system, particularly heart rate and inotropy [1]. In recent years, it was shown that these receptors are polymorphic with 2 polymorphisms of potential physiological or pathophysiological relevance [2–8]. At position 49, a serine can be replaced by a glycine, which leads to a faster down-regulation of the β1-adrenoceptors [9,10]. At position 389, the arginine can be replaced by a glycine, which leads to a loss of function [6]. Regarding the latter, the Gly389-variant was first considered to resemble the wild type, but in between it was shown that the Arg389 is more frequent and is considered as the wild type now. However, although a higher activation of adenylcyclase following isoprenaline stimulation was found in Arg389Arg-β1-adrenoceptor genotype [6,8,11], in healthy humans under *in vivo* conditions the functional role of the β1-adrenoceptor polymorphism is questionable [12]. Thus, in heart failure patients, the β1-adrenoceptor polymorphism did not influence the time to death or time to transplant [13]. However, a loss or gain of function or an altered receptor down-regulation may be modulated *in vivo* by alterations in the norepinephrine metabolizing enzyme catechol-O-methyl-transferase (COMT), which also is polymorphic; at position 158 the exchange of a valine against a methionine leads to a less active variant of the enzyme [14,15]. The Val158-variant was suggested to be associated with elevated blood pressure [16]. Since patients undergoing cardiac surgery are postoperatively exposed to a situation with enhanced cardiovascular stress, we wanted to know whether under these conditions the β1-adrenoceptor polymorphisms alone or in combination might show effects on postoperative outcome, and whether this might be modulated by the Val158Met-COMT polymorphism.

Material and Methods

Patients

This prospective study included 116 white patients undergoing cardiac surgery (for characteristics, see Table 1). The study was approved by the local ethics board and performed according to the Declaration of Helsinki. All patients gave written informed consent. For each patient, the EuroSCORE was determined preoperatively. Postoperatively, we determined the total catecholamine consumption (including norepinephrine, epinephrine, dopamine, and dobutamine), the norepinephrine consumption, the time in the intensive care unit, time in the intermediate care unit, total stay in hospital, the preoperative catecholamine levels, and the genotype regarding β1-adrenoceptor and COMT. Staff of the hospital and the investigators were blinded to the genotypes.

Samples and DNA isolation

For assessment of the genotypes of the ADRB1 at codon 389, 49 and of the COMT at codon 158, we obtained blood samples (2.5 ml in EDTA tubes) from 116 white patients. The genomic DNA was extracted from the blood samples using a commercially available isolation Kit utilizing spin columns (peqGOLD Blood DNA Mini Kit, Peqlab GmbH, Erlangen, Germany).

Genotyping by real-time fluorescence PCR using FRET

The methods for genotyping using FRET-RT-PCR have been described previously by our group and were tested by PCR followed by restriction fragment length polymorphism and DNA sequencing [17]. In principle, we used a single-step FRET-RT-PCR approach with sensor and anchor hybridization probes labelled with different fluorescent dyes. The sensor probe, which is complementary to the sequence of either the wild type or the polymorphic variant, is labelled at its 5'-end (with, for example, LCRed 640 or LCRED705) and binds across the mutation site. The anchor labelled with fluorescein (FL) at its 3'-end binds on the DNA close to the sensor (less than 5 nucleotides (nt) apart). When the annealing temperature during the cycling process is reached, sensor and anchor hybridize to the specific PCR fragment. Then, the FL of the anchor (donor) transfers energy to the LCRed 640 of the sensor (acceptor) and FRET occurs. This fluorescence signal from LCRed 640 is monitored. Following the last cycle of PCR, DNA samples are cooled (45°C) and then slowly heated. The measurement of the melting temperature ranges from 45°C to 85°C.

Fluorescence is continuously monitored and results in different melting curves, which represent melting peaks for the different alleles (Figure 1). If the sensor sequence is completely complementary to the wild type sequence, the melting curve will indicate a higher melting point than that which is achieved in the presence of a sequence mismatch caused by the presence of polymorphic alleles. Such a difference in the melting temperature (Tm) of various genotypes was first described in 1979 [18].

The 129nt DNA sequence including the mutation site was amplified in a final volume of 20 µl using glass capillaries. The PCR-reaction mix consisted of 1x PCR Buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, Invitrogen GmbH, Karlsruhe, Germany), 3 mM MgCl2 (Invitrogen), 5% dimethyl sulfoxide (Roth GmbH, Karlsruhe, Germany), 10 mg bovine serum albumin (Sigma-Aldrich GmbH, Hamburg, Germany), 0.2 mM of each deoxynucleotide triphosphate (Roth GmbH, Karlsruhe, Germany), 1 U of Platinum Taq DNA Polymerase (Invitrogen), 0.5 µM (10 pmol) of each primer (sense and antisense), 0.25 µM (3 pmol) of each hybridization probe (sensor and anchor), and distilled H_2O. At least 15–30 ng isolated genomic DNA was added. The primers

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Table 1. Patients characteristics of the whole study group.

Number of patients, n=116	
Age (years)	63±1.2
Females (%)	34%
Body weight (kg)	80±1
height (cm)	171±0.7
BMI (kg/m^2)	27.0±0.4
Diabetes mellitus (%)	25
Hypertension (%)	69.8
Atrial fibrillation (%)	22.4
Chronic obstructive pulmon. disease (%)	6.9
Cardiomyopathy (%)	7.8
Smoker (%)	32.8
Renal failure (%)	10.3
EuroSCORE	5.5±0.5
CCS-State	
0 (%)	50.9
1 (%)	10.3
2 (%)	26.7
3 (%)	9.5
4 (%)	1.7
NYHA-State	
0 (%)	17.2
1 (%)	9.5
2 (%)	40.5
3 (%)	31.9
4 (%)	0.9
Ejection fraction (%)	58.7±0.9
Aortic valve disease (%)	42.2
Mitral valve disease (%)	38.8
Trikuspid valve disease (%)	17.2
Pulmonary valve disease (%)	2.6
Coronary heart disease (%)	45.7
Operation relative frequency	
Coronary bypass (%)	36
Aortic valve replacement/ -repair (%)	28.8
With ascendent aorta replacement (%)	12.6
Trikuspid valve replacement/ -repair (%)	12.6
Mitral valve replacement/ -repair (%)	36.9
Pulmon. valve replacement/ -repair (%)	1.8
Re-operation (%)	3.6
IABP implantation (%)	2.7

and hybridization probes (Table 2) were designed and synthesized by TIB MOLBIOL (Berlin, Germany). The FRET-RT-PCR conditions for the light cycler™ were established and optimized after the pre-denaturation step at 95°C for 10 min (Table 3).

After PCR amplification, the melting curve was obtained. During this final step, the samples were denatured (95°C, incubation time 20 s) first. The subsequent cooling of the samples was performed in 2 steps (first step: 55°C, incubation time 20 s, transition rate 20°C/s; second step: 40°C, incubation time 120 s, transition rate 0.05°C/s). Finally, a slow heating step to 85°C with an incubation time of 1 s and a transition rate of 0.5°C/s terminated the program.

High-Performance liquid chromatography (HPLC)

Plasma catecholamines (epinephrine, norepinephrine, and dopamine) were determined pre-operatively by HPLC according to Nette et al. [19]. Blood was taken intravenously from the patient after the patient was at rest >30 min in a supine position. Briefly, for preparation of the blood samples, we used a commercial kit from Chromsystems (Martinsried, Germany). For detection of the catecholamines, we used the detector Recipe EC3000 (Munich, Germany) and an HPLC apparatus from Knauer (Berlin, Germany). Each probe was injected twice and the mean was used to determine catecholamine concentrations.

Statistics

All data are expressed as means ± SEM. Data were analyzed using multivariate analysis of variance (MANOVA), and if MANOVA indicated significance, with a post hoc Tukey HSD. A p value of <0.05 was considered to indicate significance.

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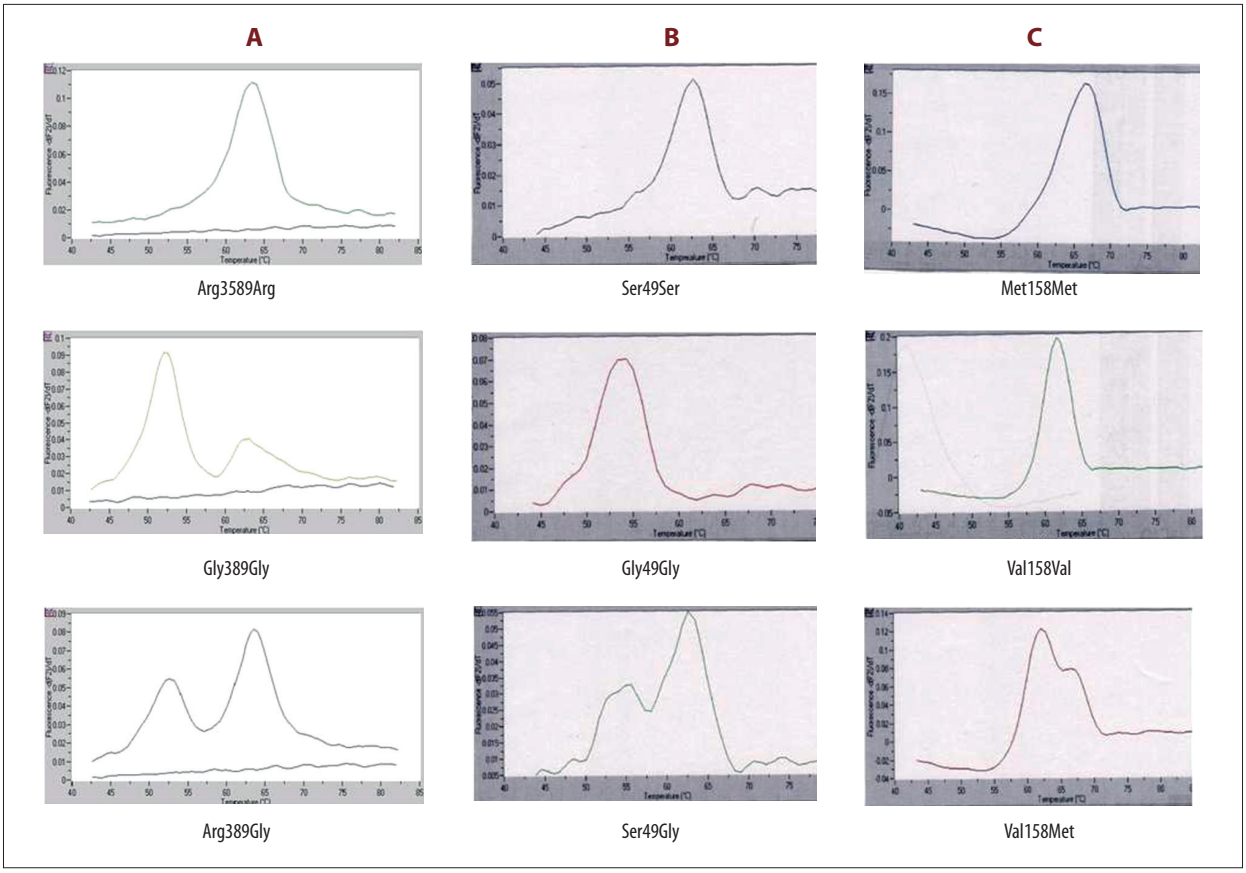

Figure 1. Original melting curves for Arg389Gly-polymorphism (**A**), Ser49Gly-polymorphism (**B**) and Val158Met-polymorphism (**C**).

Table 2. Primers used for detection of the polymorphism.

Name	Sequence
Arg389Gly-β1-AR	
Forward primer	5'-GCTGGGCTACGCCAACTC-3'
Backward primer	5'-GTGCGCTGGGTGCCTCT-3'
Anchor	5'-CGCAGCCCCGACTTCCGCAA-FL-3'
Sensor	5'-LC640-GCCTTCCAG**C**GACTGCTCT-3'
Ser49Gly-β1-AR	
Forward primer	5'-GGGCTTCTGGGGTGTTCC-3'
Backward primer	5'-CCGAGCCCGGTAACCTG-3'
Anchor	5'-CCGCCTCGTTGCTGCCTCCC-FL-3'
Sensor	5'-Cy5-CCAGCGAA**A**GCCCCGAGC-3'
Val158Met-COMT	
Forward primer	5'-GGGCCTACTGTGGCTACTCA-3'
Backward primer	5'-GGCCCTTTTTCCAGGTCTG-3'
Anchor	5'-LC640-TGTGCATGCCTGACCCGTTGTCA-3'
Sensor	5'-ATTTCGCTGGCATGAAGGACAAG-3'

Results

The Arg389-variant of the β1-adrenoceptor exhibited a melting point at 63.6°C, while the Gly-variant melted at 52.5°C (Figure 1A), and heterozygosity showed both melting points. Regarding Ser49Gly polymorphism, we found the melting point for the Ser49Ser-variant at 62°C and the Gly49Gly variant at 52°C (Figure 1B). For the variations of the COMT gene, we found a melting point at 67°C for the Met158Met variant and at 61°C for the Val158Val variant (Figure 1C).

In our population, we found the most frequent Arg389Arg-isoform (52.1%) was the Ser49Ser variant (84.6%), while for COMT158 polymorphism, the Val158Val, the Val158Met and the Met158Met-isoform all occurred with comparable frequency in about one-third of our patients (Table 4). As could be expected, homozygous Gly49Gly patients are very rare, and in our population only heterozygous Gly49 genotypes were found. Thus, for statistical reasons, in the graphs we show the homozygous Ser49Ser, Arg389Arg, and Val158Val versus the mutated isoforms (the sum of both heterozygous and homozygous mutants). For information, however, the data for the homozygous and heterozygous genotypes are given in Table 5.

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Table 3. FRET-RT-PCR protocol.

	Denaturation	Amplification			Melting curve analysis			Cooling
Arg389Gly								
Temperature (°C)	95	95	57	72	95	55/40	85	40
Incubation time (min/s)	10 min	10s	10s	10s	20s	20s/120s	1s	30 min
Transition rate (°C/s)	20	20	20	20	20	20/0.05	0.5	20
Ser49Gly								
Temperature (°C)	95	95	56	72	95	40	85	40
Incubation time (min/s)	10 min	10s	10s	10s	30s	20s	0s	30 min
Transition rate (°C/s)	20	20	1	20	20	0.5	0.2	20
COMT								
Temperature (°C)	95	95	57	72	95	40	90	40
Incubation time (min/s)	10 min	5s	10s	15s	20s	20s	0s	30 min
Transition rate (°C/s)	20	20	20	20	30	20	0.2	20

Table 4. Distribution of the polymorphisms in the study population (n=116 patients).

Gene product	Homozygous for wildtype-allele	Heterozugous	Homozygous for the mutated allele
Ser49Gly-β1-adrenoceptor	Ser49Ser	Ser49Gly	Gly49Gly
	84.6*	15.4%	0%
Arg389Gly-β1-adrenoceptor	Arg389Gly	Gly389Gly	Gly389Gly
	52.1%	38.5%	9.4%
Val158Met-COMT	Val158Val	Val158Met	Met158Met
	32.5%	35.9%	31.6%

Table 5. Effects of the homozygous and heterozygous genotypes of the β1-adrenoceptor (Ser49Gly-polymorphism and Arg389Gly polymorphism) and of the Catechol-O-Methyl-Transferase (COMT) Val158Met-polymorphism on the time of stay on intermediate care, on norepinephrine consumption and on pre-operative plasma norepinephrine concentration. For comparison the Euroscore is also given.

	Norepinephrine consumption (ml*h)	Time on intermediate care (h)	Pre-operative norepinephrine (ng/l)	Euroscore (score values)	N
Ser49Ser	579±235	38.8±6.5	323±25	5.1±0.6	98
Gly49Ser	5605±3525	65.9±22.4	356±67	7.4±1.9	18
Gyl49Gly	/	/	/	/	0
Arg389Arg	252±127	31.4±5.5	315±34	5.5±0.8	61
Gly389Arg	3233±1525*	61.7±15.0	338±35	5.9±0.9	44
Gly389Gly	7±7	32.4±8.5	398±84	4.0±0.9	11
Val158Val	3246±1791*	50.4±14.2	416±45*	5.1±0.6	37
Met158Met	457±242	33.2±7.9	328±38	6.4±1.3	37
Met158Met	493±308	45.0±11.2	239±27	5.1±0.9	42

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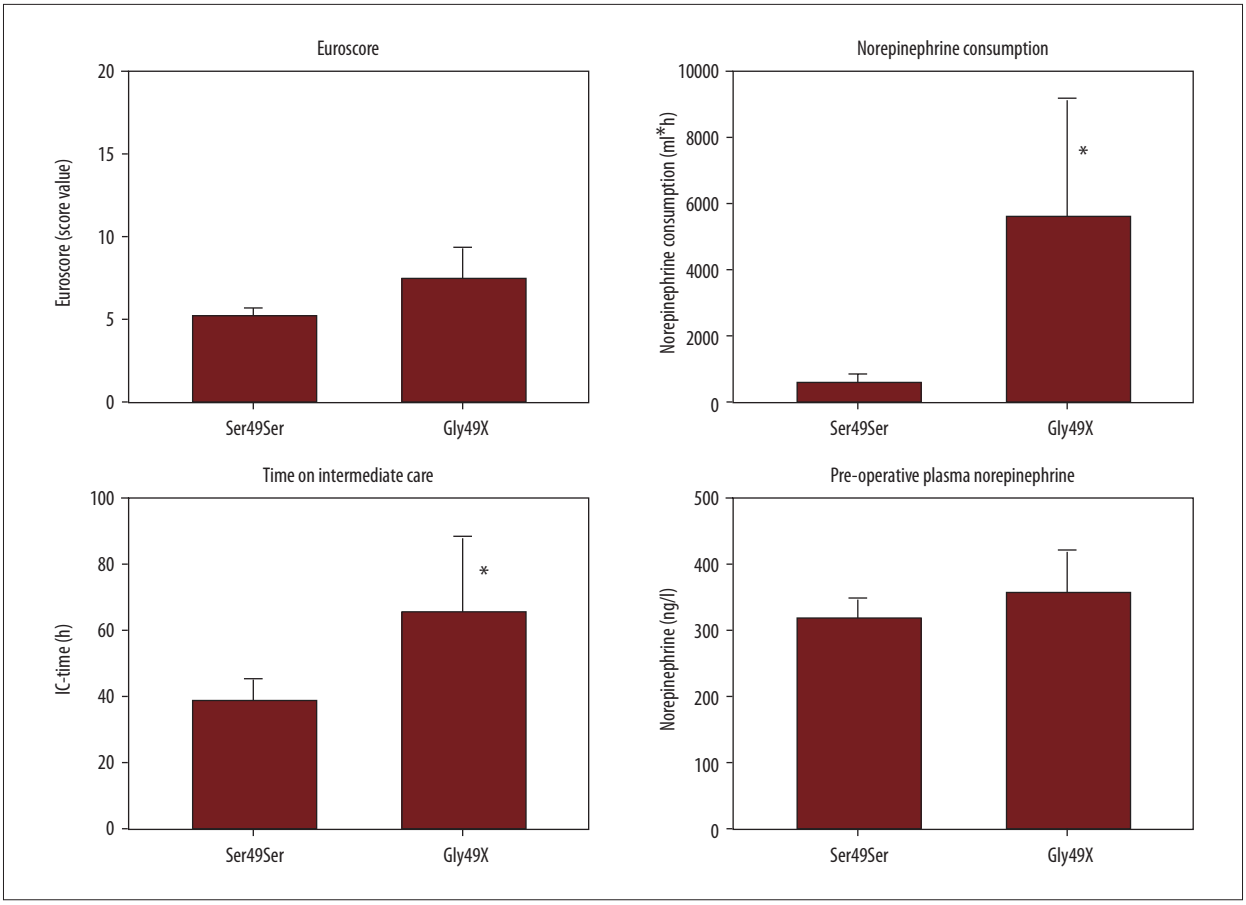

Figure 2. Post-operative norepinephrine consumption, stay in intermediate care unit, pre-operative norepinephrine plasma concentration, and Euroscore in dependence of the Ser49Gly polymorphism. All values are given as means ±SEM. Significance is indicated by an asterisk ($p<0.05$).

Intensive care unit stay was needed by 54.3% of the patients (mean duration: 45.9±17.2 h), and 73.3% of the patients needed a stay on the intermediate care unit (mean duration of 43±6.5 h). During the postoperative period (mean total stay in hospital, 14.4±1.1 days), 0.9% (n=1 patient) of patients developed lung failure and 4.3% (n=5) exhibited renal failure. This latter complication was not clearly associated with a certain genotype.

Patients carrying the Gly49 allele stayed about 27 h longer in the intermediate care unit than did the Ser49Ser-patients. However, the carriers of the Gly49 allele had a slightly higher EuroSCORE value (Figure 2). The patients carrying the Gly49 allele needed about 10-fold more norepinephrine than the Ser49Ser population (Figure 2). The total stay in hospital tended to be longer in patients carrying the Gly49 allele (20.0±4.0 *vs.* 13.5±1.1 days).

Regarding the Arg389Gly polymorphism, we found that although the EuroSCORE was identical for carriers of the Arg389 allele and of the Gly389 allele, patients carrying the 389Gly allele stayed 24 h longer in the intermediate care unit and needed significantly more norepinephrine support (Figure 3). Regarding the total hospital stay time, there was no significant difference (Arg389Arg: 15.5±1.9 days *vs.* patients carrying the Gly389 allele: 13.4±1.2 days).

Concerning the polymorphisms of COMT, we found differences between patients carrying the Val158 allele (which is the isoform with higher metabolic activity) as compared to the Met158Met population. Thus, although the EuroSCORE was identical among carriers of the Met158 allele and the Val158Val group, the carriers of the Met158 allele had a shorter stay (by 24 h) in the intermediate care unit than did the Val158Val population. However, the total time in hospital was nearly identical for both groups (Val158Val: 15.5±2.0 days *vs.* patients carrying the Met158 allele: 14.0±1.4 days). The norepinephrine consumption was significantly higher (by about 10-fold) in patients carrying the Val158 allele (Figure 4).

Next, we investigated the combination of polymorphisms. It became obvious that patients carrying a combination of the Gly49 allele, the Gly389 allele, and the Val158 allele had the

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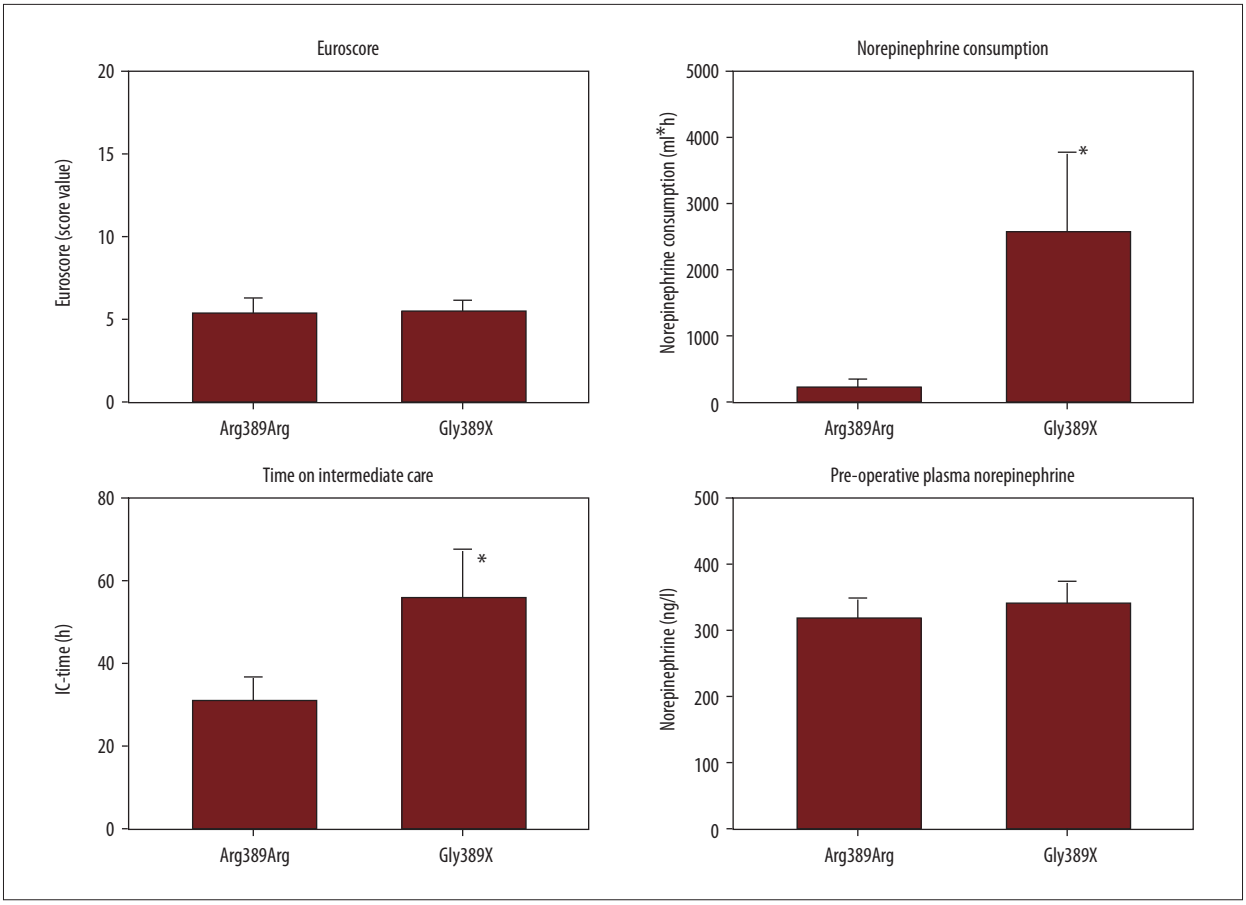

Figure 3. Post-operative norepinephrine consumption, stay in intermediate care unit, pre-operative norepinephrine plasma concentration, and Euroscore in dependence of the Arg389Gly polymorphism. All values are given as means ±SEM. Significance is indicated by an asterisk ($p<0.05$).

highest norepinephrine consumption, which was significantly higher than that of all other groups (Figure 5), although the EuroSCORE of this group (5±0.9) was not significantly different from the mean EuroSCORE. Moreover, this group had the highest catecholamine consumption of all haplotypes (Figure 5). However, the mean intermediate care unit stay was only slightly higher (and not significantly different) than in other haplotypes. The mean total length of hospital stay was 16±2 days in the whole patient population.

Although within the groups with Ser49Gly-polymorphism, Arg389Gly-polymorphism, or Val18Met-polymorphism, the EuroSCORE did not significantly differ (Figures 2–4). There was slight variation among the haplotypes (Figure 5A–5D), with the highest EuroSCOREs found in the Gly49X-Arg389Arg-Val158Val and the Gly49X-Gly389X-Met158X-haplotypes. As could be expected, the EuroSCORE alone also affected the parameters, and a high EuroSCORE was associated with high catecholamine consumption ($p=0.012$) and long intermediate care unit stay ($p=0.004$), but not with enhanced norepinephrine consumption ($p=0.087$), prolonged intensive care unit stay ($p=0.437$), or prolonged total stay in hospital ($p=0.255$). This indicated that the EuroSCORE is an important factor but that other factors also might contribute.

Thus, we investigated the complex effect of all polymorphisms with the EuroSCORE as a covariate for MANOVA. Taking EuroSCORE into account, MANOVA revealed that the norepinephrine consumption still depended on both β1-adrenoceptor polymorphisms and on the COMT polymorphism. Moreover, we found a positive interaction, so that the effect of a β1-polymorphism is significantly modulated by the simultaneous presence of a mutated COMT (Table 6). Interestingly, the length of total hospital stay also was affected by these complex interactions, in particular the simultaneous presence of both β1-adrenoceptor polymorphisms (Table 6).

Finally, we also investigated the preoperative endogenous catecholamine level, which did not differ between the Ser49Gly polymorphism and the Arg389Gly polymorphism (Table 7). However, patients carrying the Val158 allele of COMT (which is the variant with the higher metabolic activity) exhibited

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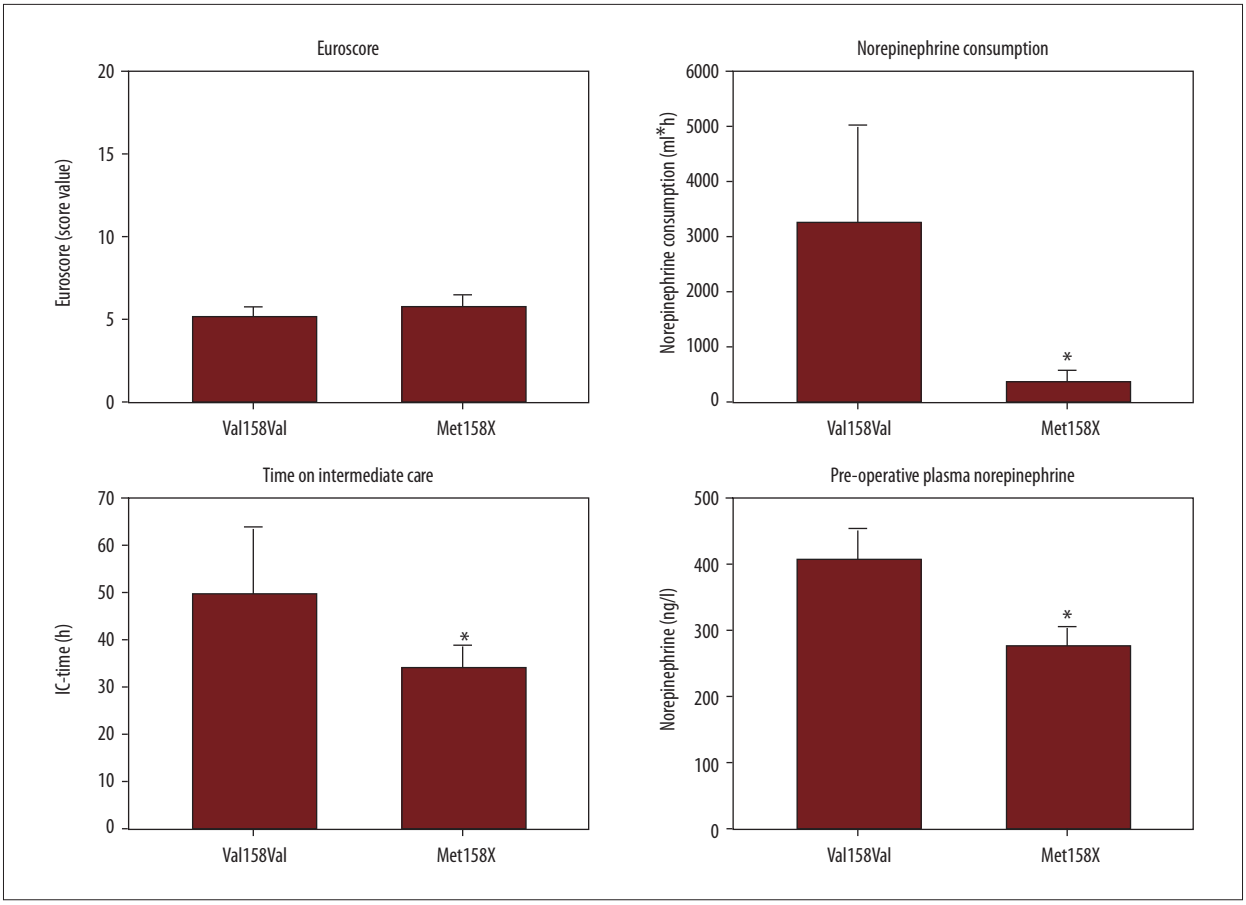

Figure 4. Post-operative norepinephrine consumption, stay in intermediate care unit, pre-operative norepinephrine plasma concentration, and Euroscore in dependence of the Val158Met polymorphism. All values are given as means ±SEM. Significance is indicated by an asterisk ($p<0.05$).

significantly higher endogenous norepinephrine and slightly higher epinephrine levels (Table 7). This can also be seen from Figure 5D, where all haplotypes carrying the Val158-COMT-allele exhibit higher preoperative norepinephrine levels.

Discussion

Until now, the effect of a combination of the Ser49Gly, Arg389Gly-β1-adrenoceptor, and the Val158Met-COMT polymorphism on postoperative outcome in patients undergoing cardiac surgery has not been evaluated.

Regarding the genotypes found in our population, we found 52.1% were carrying Arg389Arg-β1-AR, which is higher than the reported frequency in healthy white populations [20]. However, it is known that patients carrying the Arg389Arg variant more often suffer from cardiac infarction [21], so it might be reasonable to assume that this genotype is more frequently seen in cardiac care centers. Also, we found a lower frequency of the Gly389Gly genotype, which is less than was reported by these authors for a normal population. However, in good accordance with our data, Ulucan et al. [20] also reported higher frequency for the Arg389Arg genotype and lower frequency for the Gly389Gly genotype in cardiology patients suffering from ventricular arrhythmia. The frequencies for the Ser49Gly β1-AR-polymorphisms found in our study are also in good accordance with those found by Ulucan et al. [20] in normal subjects and nearly unchanged in cardiac patients. The homozygous Gly49Gly genotype is extremely rare [22], and was not present in our population. Regarding the Val158Met-COMT polymorphism, there are sparse data on the normal frequency in healthy white subjects; however, Pełka-wysiecka et al. [23] found the Val158Val genotype in 14.2%, the heterozygous in 60.5%, and the Met158Met genotype in 25.3% in a Polish population, which would mean that in our patients, heterozygous are less frequent but Val158Val genotype is more frequent. Similarly, in Swedish men, 19.6% Val158Val, 54.6% heterozygous, and 25.7% Met158Met genotype carriers were found [24], while others found a more even distribution among whites [25]. Although this seems to suggest that at least the Arg389Arg genotype might be associated somehow with cardiac diseases,

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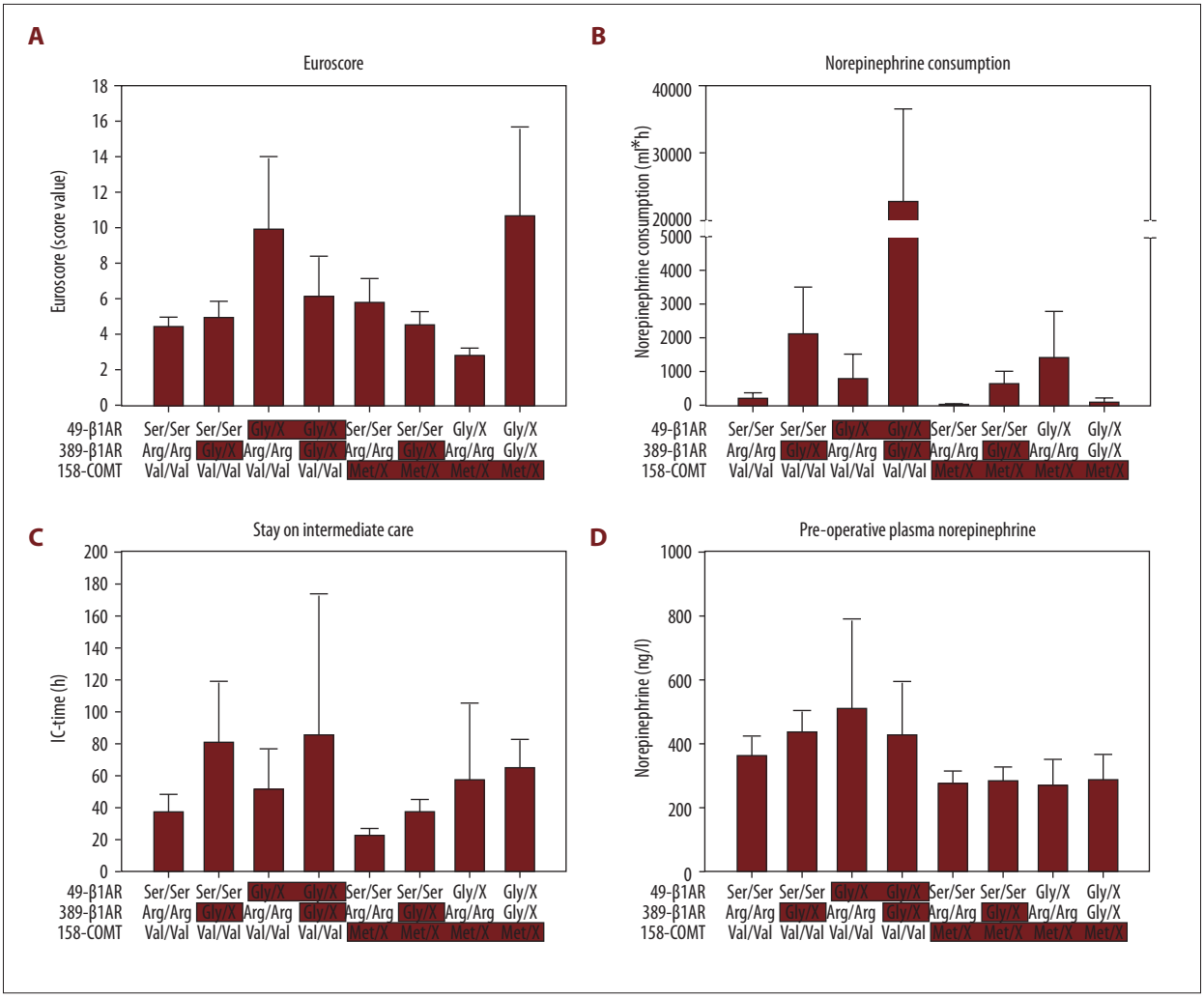

Figure 5. (**A**) Variation of the Euroscore in dependence on the haplotypes. (**B**) Effect of haplotypes on post-operative norepinephrine consumption; (**C**) Effect of haplotypes on the time of stay on the intermediate care; (**D**) effect of haplotypes on pre-operative norepinephrine plasma concentrations. All values are given as means ±SEM. For statistical analysis and significance see text and Tables 5–7.

Table 6. Results (p-values) of MANOVA on interaction of the β1-adrenoceptor polymorphisms, the COMT polymorphism and the Euroscore.

Independent	NE-consumption	Total time in hospital
S49G-β1-AR	p<0.001*	p=0.013*
A389G-β1-AR	p<0.001*	p=0.024*
V158M-COMT	p<0.001*	p=0.204
S49G*A389G	p<0.001*	p=0.018
S49G*V158M	p<0.001*	p=0.261
A389G*V158M	p<0.001*	p=0.247
S48G*A389G*V158M	p<0.001*	p=0.046*
Euroscore	p=0.181	p=0.910

Table 7. Endogenous preoperative catecholamine levels of the patients. (* p<0.05: Val158 *vs.* Met158).

	Norepinephrine (ng/l)	Epinephrine (ng/l)
Ser49Ser	323.1±24.8	3.1±1.2
Gly49 allele carriers	356.1±66.8	4.1±2.7
Arg389Arg	315.2±33.9	2.5±1.5
Gly389 allele carriers	342.5±33.9	4.0±1.5
Val158Val	412.2±44.9*	4.0±2.2
Met158 allele carriers	280.0±24.6	2.8±1.1

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from our data it is not possible to conclude that a genotype increases risk of cardiac disease.

Our aim was to assess a possible influence of the genotypes or haplotypes on postoperative norepinephrine consumption, length of intermediate care unit stay, and total time in hospital.

Interestingly, we observed that in Gly49X, Gly389X, and Val158Val carriers, norepinephrine consumption was significantly enhanced (Figures 2–4). This polymorphism effect was independent of EuroSCORE. According to the statistical analysis, the effect of the 2 β1-adrenoceptor polymorphisms was also relevant for the length of hospital stay (Table 6).

Regarding the Ser49Gly-polymorphism, it is known that the genotypes carrying a Gly49-allele are associated with a faster down-regulation of the β1-adrenoceptors [9,10,26,27] and a lower heart rate under resting conditions [7], which might result in a higher norepinephrine consumption as observed in our study. With regard to the Arg389Gly polymorphism, the Gly389-variant was found to be a "loss of function" mutation which exhibits a 3–4-fold lower activation of adenylcyclase to isoprenaline stimulation [6,8,11]. Accordingly, these patients carrying the Gly389 allele in our study needed more norepinephrine than the others. This is in good accordance to the findings of Leineweber et al. [5], who also found higher catecholamine consumption in patients carrying the Gly389 variant. Next, the effect of the COMT polymorphism needs to be considered. For the Met158Met-COMT variant, a lower thermostability was described [28] with lower activity [14,15]. In consequence, those patients carrying the variants with the higher COMT activity (the Val158 variant) should metabolize norepinephrine faster, so that it can be expected that they need more norepinephrine. Accordingly, our data showed higher norepinephrine consumption for this group. This seems also to be reflected in higher basal preoperative resting norepinephrine levels in these patients. Based on the considerations above, it seems reasonable that patients with a combination of the Gly49-allele, the Gly389-allele, and the Val158-COMT allele needed the highest amount of norepinephrine.

Although it was described that humans with the Ser49-Gly389 genotype had a lower oxygen consumption than those with the Gly49-Arg389 genotype [29,30], in young healthy adults no influence of the Arg389Gly-Polymorphism on the effects of epinephrine and norepinephrine was found [12,31,32]. Similarly, in the isolated right atrium from patients undergoing aortocoronary bypass, there was no influence of Ser49Gly or Arg389Gly-polymorphism on the effect of norepinephrine [33]. Thus, the relevance of the polymorphisms to the *in vivo* situation was controversial when we started our study. However, these authors did not investigate the COMT polymorphism as a possible modulator in their study. We assumed that under at least some of the situations described in the literature, in particular when dealing with healthy subjects, the effects of the polymorphisms might be masked by compensatory mechanisms. Our hypothesis was that in a situation of enhanced cardiovascular stress, such as the postoperative period after cardiac surgery, the effects of the polymorphisms, in particular when also taking the COMT polymorphism into account, might become unmasked.

Thus, we found that the effects of the polymorphisms and the effects of the β1-adrenoceptor polymorphism were modulated by the COMT genotype.

Conclusions

We conclude from our data that the β1-adrenoceptor polymorphisms together with the COMT polymorphism affect norepinephrine consumption and length of hospital stay in a situation of enhanced cardiovascular stress, reflected here by the postoperative period after cardiac surgery. Moreover, we conclude that patients with the Val158-COMT genotype exhibit higher endogenous resting plasma norepinephrine levels.

These data might suggest further prospective studies investigating whether a preoperative determination of haplotype and use of catecholamine therapy according to the haplotype may help to optimize postoperative outcomes.

References:

1. Brodde OE, Michel MC: Adrenergic and muscarinic receptors in the human heart. Pharmacol Rev, 1999; 51(4): 651–90
2. Liggett SB, Mialet-Perez J, Thaneemit-Chen S et al: A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. Proc Natl Acad Sci USA, 2006; 103(30): 11288–93
3. Leineweber K, Büscher R, Bruck H, Brodde OE: Beta-adrenoceptor polymorphisms. Naunyn Schmiedebergs Arch Pharmacol, 2004; 369(1): 1–22
4. Leineweber K, Bruck H, Temme T et al: The Arg389Gly beta1-adrenoceptor polymorphism does not affect cardiac effects of exercise after parasympathetic inhibition by atropine. Pharmacogenet Genomics, 2006; 16(1): 9–13
5. Leineweber K, Bogedain P, Wolf C et al: In patients chronically treated with metoprolol, the demand of inotropic catecholamine support after coronary artery bypass grafting is determined by the Arg389Gly-beta 1-adrenoceptor polymorphism. Naunyn Schmiedebergs Arch Pharmacol, 2007; 375(5): 303–9
6. Mason DA, Moore JD, Green SA, Liggett SB: A gain-of-function polymorphism in a G-protein coupling domain of the human beta1-adrenergic receptor. J Biol Chem, 1999; 274(18): 12670–74
7. Ranade K, Jorgenson E, Sheu WH et al: A polymorphism in the beta1 adrenergic receptor is associated with resting heart rate. Am J Hum Genet, 2002; 70(4): 935–42

Indexed in: [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

8. Bruck H, Leineweber K, Temme T et al: The Arg389Gly beta1-adrenoceptor polymorphism and catecholamine effects on plasma-renin activity. J Am Coll Cardiol, 2005; 46(11): 2111–15
9. Levin MC, Marullo S, Muntaner O et al: The myocardium-protective Gly-49 variant of the beta 1-adrenergic receptor exhibits constitutive activity and increased desensitization and down-regulation. J Biol Chem, 2002; 277(34): 30429–35
10. 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(2): 155–60
11. La Rosée K, Huntgeburth M, Rosenkranz S et al: The Arg389Gly beta1-adrenoceptor gene polymorphism determines contractile response to catecholamines. Pharmacogenetics, 2004; 14(11): 711–16
12. Büscher R, Belger H, Eilmes KJ et al: *In vivo* studies do not support a major functional role for the Gly389Arg beta 1-adrenoceptor polymorphism in humans. Pharmacogenetics, 2001; 11(3): 199–205
13. Leineweber K, Frey UH, Tenderich G et al: The Arg16Gly-β(2)-adrenoceptor single nucleotide polymorphism: Exercise capacity and survival in patients with end-stage heart failure. Naunyn Schmiedebergs Arch Pharmacol, 2010; 382(4): 357–65
14. Lotta T, Vidgren J, Tilgmann C et al: Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry, 1995; 34(13): 4202–10
15. Lachman HM, Papolos DF, Saito T et al: Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics, 1996; 6(3): 243–50
16. Hagen K, Stovner LJ, Skorpen F et al: The impact of the catechol-O-methyltransferase Val158Met polymorphism on survival in the general population – theHUNT study. BMC Med Genet, 2007; 8: 34
17. Sauer M, Tews J, Schwarzer M et al: Rapid detection of the R389G polymorphism of ADRB1 by real time PCR using fluorescence resonance energy transfer. Clin Lab, 2009; 55: 128–36
18. Wallace RB, Shaffer J, Murphy RF et al: Hybridization of synthetic oligodeoxyribonucleotides to phi chi 174 DNA: the effect of single base pair mismatch. Nucleic Acids Res, 1979; 6(11): 3543–57
19. Nette AF, Abraham G, Ungemach FR et al: Interaction between simvastatin and metoprolol with respect to cardiac beta-adrenoceptor density, catecholamine levels and perioperative catecholamine requirements in cardiac surgery patients. Naunyn Schmiedebergs Arch Pharmacol, 2005; 372: 115–24
20. Ulucan C, Cetintas V, Tetik A et al: Beta1 and beta2-adrenergic receptor polymorphisms and idiopathic ventricular arrhythmias. J Cardiovasc Electrophysiol, 2008; 19(10): 1053–58
21. Iwai C, Akita H, Kanazawa K et al: Arg389Gly polymorphism of the human beta1-adrenergic receptor in patients with nonfatal acute myocardial infarction. Am Heart J, 2003; 146(1): 106–9
22. Maqbool A, Hall AS, Ball SG, Balmforth AJ: Common polymorphisms of beta1-adrenoceptor: Identification and rapid screening assay. Lancet, 1999; 353(9156): 897
23. Pełka-Wysiecka J, Ziętek J, Grzywacz A et al: Association of genetic polymorphisms with personality profile in individuals without psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry, 2012; 39(1): 40–46
24. Annerbrink K, Westberg L, Nilsson S et al: Catechol-O-methyltransferase val158-met polymorphism is associated with abdominal obesity and blood pressure in men. Metabolism, 2008; 57(5): 708–11
25. Weinshilboum RM, Otterness DM, Szumlanski CL: Methylation pharmacogenetics: Catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. Annu Rev Pharmacol Toxicol, 1999; 39: 19–52
26. Sandilands AJ, O'Shaughnessy KM: The functional significance of genetic variation within the beta-adrenoceptor. Br J Clin Pharmacol, 2005; 60(3): 235–43
27. Sandilands A, Yeo G, Brown MJ, O'Shaughnessy KM: Functional responses of human beta1 adrenoceptors with defined haplotypes for the common 389R>G and 49S>G polymorphisms. Pharmacogenetics, 2004; 14(6): 343–49
28. Chen J, Lipska BK, Halim N et al: Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet, 2004; 75(5): 807–21
29. Wagoner LE, Craft LL, Zengel P et al: Polymorphisms of the beta1-adrenergic receptor predict exercise capacity in heart failure. Am Heart J, 2002; 144(5): 840–46
30. Defoor J, Martens K, Zielinska D et al: The CAREGENE study: polymorphisms of the beta1-adrenoceptor gene and aerobic power in coronary artery disease. Eur Heart J, 2006; 27(7): 808–16
31. Snapir A, Koskenvuo J, Toikka J et al: Effects of common polymorphisms in the alpha1A-, alpha2B-, beta1- and beta2-adrenoreceptors on haemodynamic responses to adrenaline. Clin Sci (Lond), 2003; 104(5): 509–20
32. Xie HG, Dishy V, Sofowora G et al: Arg389Gly beta 1-adrenoceptor polymorphism varies in frequency among different ethnic groups but does not alter response *in vivo*. Pharmacogenetics, 2001; 11(3): 191–97
33. Molenaar P, Rabnott G, Yang I et al: 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, 2002; 40(7): 1275–82

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