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

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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 adenylecyclase following isoprorenaline 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, LCRRed 640 or LCRRed705) 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 LCRRed 640 of the sensor (acceptor) and FRET occurs. This fluorescence signal from LCRRed 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), 0.2 mM of each deoxynucleotide triphosphate (Roht GmbH, Karlsruhe, Germany), 10 mg bovine serum albumin (Sigma-Aldrich GmbH, Hamburg, Germany), 0.2 mM of each deoxyribonucleotide triphosphate (Roht 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 H2O. At least 15–30 ng isolated genomic DNA was added. The primers
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.

### Table 1. Patients characteristics of the whole study group.

| Categorical Variable                  | Frequency |
|--------------------------------------|-----------|
| Number of patients, n=116            | 116       |
| Age (years)                          | 63±1.2    |
| Females (%)                          | 34%       |
| Body weight (kg)                     | 80±1      |
| height (cm)                          | 171±0.7   |
| BMI (kg/m²)                          | 27.0±0.4  |
| Diabetes mellitus (%)                | 25        |
| Hypertension (%)                     | 69.8      |
| Atrial fibrillation (%)              | 22.4      |
| Chronic obstructive pulmonary 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      |

### Coronary bypass (%)

- Aortic valve replacement/-repair (%) 28.8
- With ascendent aorta replacement (%) 12.6
- Mitral valve replacement/-repair (%) 36.9
- Pulmonary valve replacement/-repair (%) 1.8
- Re-operation (%) 3.6

IABP implantation (%) 2.7

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.
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.

### Results

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Table 3. FRET-RT-PCR protocol.

| Gene product | Denaturation | Amplification | Melting curve analysis | Cooling |
|--------------|-------------|---------------|------------------------|---------|
| Arg389Gly    | Temperature (°C) | 95 | 95 | 57 | 72 | 95 | 55/40 | 85 | 40 |
|              | Incubation time (min/s) | 10 | 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 | 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 | 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 | Heterozygous | Homozygous for the mutated allele |
|--------------|-------------------------------|--------------|----------------------------------|
| Ser49Gly-β1-adrenoceptor | Ser49Ser | Ser49Gly | Gly49Gly |
| Arg389Gly-β1-adrenoceptor | Arg389Gly | Gly389Gly | Gly389Gly |
| Val158Met-COMT | Val158Val | Val158Met | Met158Met |

Table 5. Effects of the homozygous and heterozygous genotypes of the β1-adrenoceptor (Ser49Gly-polymorphism and Arg389Gly polymorphism) and of the Cathecol-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.

| Gene product | 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 |
| Gly49Gly     | /                                | /                             | /                                  | /                        | 0  |
| Arg389Arg    | /                                | /                             | /                                  | /                        | 63 |
| 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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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
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
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 accord-ance 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 accor-dance with those found by Ulucan et al. [20] in normal sub-jects and nearly unchanged in cardiac patients. The homozy-gous 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, Pelka-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 pop-ulation, 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% heterozy-gous, 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,
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-β1AR         | p<0.001*       | p=0.013*               |
| A389G-β1AR        | p=0.020*       | p=0.253*               |
| V158M-COMT        | p<0.001*       | p=0.204                |
| S49G*A389G        | p<0.001*       | p=0.018                |
| S49G*V158M        | p<0.001*       | p=0.264                |
| A389G*V158M       | p=0.018*       | 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            |
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-Polyorphism 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.
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