The eNOS 786C/T polymorphism in cardiac surgical patients with cardiopulmonary bypass is associated with renal dysfunction

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Abstract

Objective: Renal dysfunction is one of the most serious complications following cardiac surgery with cardiopulmonary bypass. The causes of renal dysfunction following cardiac surgery are poorly understood. We hypothesised that T-786C endothelial NO synthase (eNOS) polymorphism may lead to an increase in the occurrence of postoperative renal dysfunction following cardiac surgery with cardiopulmonary bypass. Methods: A total of 497 patients undergoing cardiac surgery with cardiopulmonary bypass were included in the study. The T-786C eNOS polymorphism was detected by a polymerase chain reaction. The patients were grouped on the basis of whether they were homozygous or heterozygous for the C allele (TC + CC; n = 289) or only homozygous for the T allele (TT; n = 208). Results: No significance was demonstrated in the preoperative risk factors, with the exclusion of smoking habits (p = 0.04) for the C-allele carrier. The administration of anti-lipid agents (p = 0.01) and anti-arrhythmics (p = 0.01) was significantly lower in the TC/CC group. The TC + CC genotype group had a significantly greater decrease in creatine clearance (p = 0.024), the lowest creatine clearance (p = 0.004) and more C-allele carriers received acute renal replacement therapy (p = 0.04). The usage of norepinephrine (p = 0.02) and dobutamine (p = 0.02) was significantly higher in C-allele carriers. In the TC + CC genotype group, cross-clamp time (p = 0.02) and administration of red cell transfusion (p = 0.04) achieved statistically significant difference. The overall inhospital mortality rate was 8.2% for all patients and was not significant between genotypes. Conclusions: The present findings support the hypothesis that the T-786C eNOS polymorphism may play a role in the development of renal dysfunction and increase the occurrence of renal replacement therapy following cardiac surgery with cardiopulmonary bypass. This polymorphism may be useful in stratifying the risk for the development of postoperative renal dysfunction.

Keywords: Cardiac surgery; Cardiopulmonary bypass; eNOS 786C/T polymorphism; Renal dysfunction

1. Introduction

Despite improvements in the surgical techniques, cardiopulmonary bypass equipment and anaesthesia management, postoperative renal dysfunction is still a challenging problem in cardiac surgical patients [1]. It is a frequent complication in the postoperative period of cardiac surgery with cardiopulmonary bypass, with a reported incidence of between 1% and 31% [2—6]. When renal failure develops following cardiac surgery, and is served and associated with the need for haemodialysis support, it is associated with an increased mortality and hospital stay [7,2]. Despite steady improvements in the results of cardiac surgery, there has been a trend in operating on higher risk patients, which leads to increased morbidity and mortality. The causes of renal dysfunction following cardiac surgery with cardiopulmonary bypass are poorly understood, but it is believed that they are multifactorial and usually attributed to the use of cardiopulmonary bypass, injury by exo- and endotoxins and pre-existing renal impairment [8—10]. Although many variables have been described that can identify the cardiac surgical patients who are at risk for renal dysfunction [2,5,7], significant unexplained variability in renal outcome still exists.

Some genetic polymorphism has been identified as factor in the occurrence and progression of renal dysfunction following cardiac surgery with cardiopulmonary bypass [11—14]. It has been hypothesised that impairments in endothelial nitrous oxide (NO) generation may be influenced by gene polymorphisms, which ultimately may result in impaired renal haemodynamics [15]. The endothelial NO synthase (eNOS) gene is located on chromosome 7 (7q35—36), which consists of 26 exons with an entire length of 21 kb and is constitutively expressed in the vascular endothelial cells. eNOS is the key enzyme respon-
sible for basal vascular production of NO [16] and, therefore, polymorphism in eNOS is considered one of the major predisposing factors for endothelial dysfunction. The T-786C eNOS polymorphism is strongly linked to the intron 4 polymorphisms [17], and is the one most important for the regulation of the transcription rate of the eNOS gene [18]. Moreover, this T-786C polymorphism in the human eNOS gene had been identified as being associated with various cardiovascular diseases, including coronary spasm, myocardial infarction and coronary artery disease [18–20].

Nakayama et al. [18] have shown the C allele to have significantly lower promoter activity compared to the T allele. The C allele influences eNOS transcription, which is consistent with reduced NO production. Recently, Dengel et al. [21] have demonstrated an association between the eNOS T-786C polymorphism in the eNOS gene promoter and changes in renal haemodynamics and mean arterial blood pressure in response to dietary Na+ loading. Therefore, it is possible that the eNOS gene is involved in the regulation of NO in the kidney during cardiac surgery with cardiopulmonary bypass and may play a role in the occurrence of postoperative renal dysfunction.

The purpose of the present study was to elucidate the eNOS 786C/T polymorphism in patients who undergo cardiac surgery with cardiopulmonary bypass, to determine its role as a predisposing factor for a postoperative renal dysfunction.

2. Methods

2.1. Study population

The study protocol was approved by the local ethics committee of the Medical Faculty, University of Göttingen, and all patients gave informed consent for participation in the study. We studied 497 adult, white patients requiring cardiac surgery with cardiopulmonary bypass. The exclusion criteria were patients aged >80 and with known neoplasms. Patients with chronic kidney disease on dialysis, and renal transplant patients, were excluded from the analysis. The preoperative characteristics of the patients are depicted in Table 1.

2.2. Clinical data collection and definition

The variables of this study were collected prospectively. The preoperative, intra-operative variables and postoperative complications were observed and documented consecutively.

The preoperative patient characteristics included age, gender, body mass index (BMI) score, smoking habits, hypertension, history of diabetes, hypercholesterolaemia, positive family history of cardiovascular disorders, left ventricular ejection fraction, peripheral disease, history of neurocerebral events, pulmonary hypertension and chronic obstructive pulmonary disease. The serum creatinine was determined prior to surgery and the estimated creatinine clearance was calculated using the Cockcroft–Gault formula: creatine clearance = (140 – age) \times \text{weight (kg)}/(\text{serum creatinine} \times 72 [/0.85 for women]). Additionally, the haematocrit was determined, and the preoperative medications, urgency of surgery, associated cardiac surgical procedures and additive Euroscore were recorded. A non-elective operation was defined as the necessity to operate on the patient in the next available operating list within the same week of referral (urgent case) or the necessity to take the patient to theatre out of normal working hours and ahead of the next morning’s operating list (emergency cases) (Table 1).

The perioperative patient characteristics within 24 h of surgery were studied for renal function. At our institution, serum creatinine determinations are available daily as part of standard post-cardiac-surgery-care protocols. The highest postoperative creatinine level was defined as the greatest value of serum creatinine (creatinine_{\text{max}}) determined in the postoperative period. The difference in the percentage of increased creatinine is defined as the difference between the pre- and postoperative creatinine serum levels expressed as a percentage. The lowest creatinine clearance is defined as

<table>
<thead>
<tr>
<th>Variable</th>
<th>TT, n = 208 (%)</th>
<th>TC + CC, n = 289 (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.7 ± 10.9</td>
<td>67.9 ± 10.24</td>
<td>0.30</td>
</tr>
<tr>
<td>Male/female</td>
<td>141 (28.2)/</td>
<td>181 (36)/</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>67 (13.4)</td>
<td>108 (22)</td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.04 ± 4.5</td>
<td>27.9 ± 4.7</td>
<td>0.48</td>
</tr>
<tr>
<td>Smoking</td>
<td>80 (38.5)</td>
<td>87 (30.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypertension</td>
<td>151 (72.6)</td>
<td>211 (73)</td>
<td>0.93</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>105 (50.5)</td>
<td>136 (47.1)</td>
<td>0.39</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>59 (28.4)</td>
<td>95 (32.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>Positive family history</td>
<td>37 (17.8)</td>
<td>42 (14.5)</td>
<td>0.30</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>54.03 ± 14.09</td>
<td>52.66 ± 14.88</td>
<td>0.25</td>
</tr>
<tr>
<td>Peripheral disease</td>
<td>16 (7.7)</td>
<td>19 (6.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Neurocerebral events</td>
<td>24 (11.5)</td>
<td>42 (14.5)</td>
<td>0.35</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>14 (6.7)</td>
<td>26 (9)</td>
<td>0.38</td>
</tr>
<tr>
<td>COPD</td>
<td>18 (8.7)</td>
<td>22 (7.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>Renal function variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/d)</td>
<td>1.21 ± 0.89</td>
<td>1.13 ± 0.60</td>
<td>0.28</td>
</tr>
<tr>
<td>Estimated creatinine clearance (ml/min)</td>
<td>74.8 ± 32.3</td>
<td>78.0 ± 33.1</td>
<td>0.28</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>39.7 ± 5.9</td>
<td>39.7 ± 5.8</td>
<td>0.97</td>
</tr>
<tr>
<td>Preoperative medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>123 (59.1)</td>
<td>193 (55.7)</td>
<td>0.11</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>99 (47.6)</td>
<td>164 (56.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Oral nitrates</td>
<td>40 (19.2)</td>
<td>53 (18.3)</td>
<td>0.76</td>
</tr>
<tr>
<td>Anti-arrhythmics</td>
<td>14 (6.7)</td>
<td>5 (1.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diuretics</td>
<td>86 (41.3)</td>
<td>113 (39.1)</td>
<td>0.55</td>
</tr>
<tr>
<td>Anti-lipid agent</td>
<td>113 (54.3)</td>
<td>125 (43.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>37 (17.8)</td>
<td>57 (19.7)</td>
<td>0.63</td>
</tr>
<tr>
<td>Antihypertensive agents</td>
<td>51 (24.5)</td>
<td>60 (20.7)</td>
<td>0.92</td>
</tr>
<tr>
<td>Bronchodilator</td>
<td>9 (4.3)</td>
<td>6 (2.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>Anticoagulation</td>
<td>145 (69.7)</td>
<td>192 (66.4)</td>
<td>0.35</td>
</tr>
<tr>
<td>Urgency of surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elective (n = 394)</td>
<td>162</td>
<td>232</td>
<td>0.54</td>
</tr>
<tr>
<td>Urgent (n = 41)</td>
<td>17</td>
<td>24</td>
<td>0.98</td>
</tr>
<tr>
<td>Emergency (n = 62)</td>
<td>29</td>
<td>33</td>
<td>0.44</td>
</tr>
<tr>
<td>Associated surgical procedures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG (n = 249)</td>
<td>108</td>
<td>141</td>
<td>0.49</td>
</tr>
<tr>
<td>Valve (n = 106)</td>
<td>43</td>
<td>63</td>
<td>0.99</td>
</tr>
<tr>
<td>Combined procedures (n = 100)</td>
<td>38</td>
<td>62</td>
<td>0.38</td>
</tr>
<tr>
<td>Other procedures (n = 42)</td>
<td>19</td>
<td>23</td>
<td>0.53</td>
</tr>
<tr>
<td>Euroscore additive</td>
<td>5 ± 4</td>
<td>6 ± 4</td>
<td>0.80</td>
</tr>
</tbody>
</table>
creatinine clearance. The decrease in creatinine clearance was defined as the difference between the pre- and postoperative creatinine clearance. A reduced creatinine clearance or raised plasma creatinine concentration were defined as renal dysfunction.

Furthermore, the lowest haematocrit, administration of furosemide and the need of renal replacement therapy during the hospital stay were determined. Acute renal failure was defined as patients with a postoperative requirement for dialysis support. The Acute Physiology and Chronic Health Evaluation Score (APACHE II), Simplified Acute Physiology Score (SAPS II), RIFLE-classification (Risk, Injury, Failure, Loss of Kidney-function) and ARF score [22] were studied. The haemodynamic measurements comprised the heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), mean pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure (PCWP). The cardiac index (CI), systemic (SVRI) and pulmonary vascular resistance (PVRI) indices were calculated from standard formulae. Extended haemodynamic measurements were carried out if the patient was in poor condition and if inotropic drug support was needed. In addition, catecholamine support, administration of amiodarone, cortisone and vasopressin were recorded. Intraoperative cross-clamp time and cardiopulmonary bypass time were recorded. The details are summarised in Table 2.

The postoperative details recorded the red blood cells suspension used, fresh frozen plasma transfusion required, intra-aortic balloon pump (IABP) usage, extracorporeal membranoxgenation (ECMO) required, the length of hospital and intensive care unit (ICU) stay and in-hospital mortality (Table 3). The in-hospital mortality was defined as death within the same hospital admission regardless of cause.

2.3. Genotyping

The genotyping of all patients was performed using the polymerase chain reaction (PCR) amplification in accordance with the procedures described earlier [23]. Following the genotyping, the patients were divided into the following

<table>
<thead>
<tr>
<th>Variable</th>
<th>TT, n = 208 (%)</th>
<th>TC + CC, n = 289 (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine serum max</td>
<td>1.95 ± 3.77</td>
<td>1.83 ± 1.18</td>
<td>0.63</td>
</tr>
<tr>
<td>Increased in serum creatinine (%)</td>
<td>69.8 ± 196.3</td>
<td>72.9 ± 106.7</td>
<td>0.82</td>
</tr>
<tr>
<td>Creatinine clearance max (ml/min)</td>
<td>62.08 ± 35.8</td>
<td>55.8 ± 31.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Decrease creatinine clearance (ml/min)</td>
<td>13.0 ± 38.0</td>
<td>21.05 ± 39.9</td>
<td>0.024</td>
</tr>
<tr>
<td>Haematocrit, min (%)</td>
<td>26.9 ± 4.1</td>
<td>26.8 ± 3.8</td>
<td>0.91</td>
</tr>
<tr>
<td>Furosemide mean (mg)</td>
<td>82.3 ± 136.3</td>
<td>92.8 ± 137.5</td>
<td>0.42</td>
</tr>
<tr>
<td>Renal replacement therapy during hospital stay</td>
<td>27 (12.9)</td>
<td>56 (19.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARF Score</td>
<td>3.31 ± 1.83</td>
<td>3.09 ± 1.63</td>
<td>0.07</td>
</tr>
<tr>
<td>RIFLE Score (1st POD) (n = 287)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R (risk) (n = 61)</td>
<td>25</td>
<td>36</td>
<td>0.88</td>
</tr>
<tr>
<td>I (injury) (n = 65)</td>
<td>29</td>
<td>36</td>
<td>0.63</td>
</tr>
<tr>
<td>F (failure) (n = 161)</td>
<td>73</td>
<td>88</td>
<td>0.27</td>
</tr>
<tr>
<td>APACHE II Score</td>
<td>15 ± 6</td>
<td>15 ± 7</td>
<td>0.15</td>
</tr>
<tr>
<td>SAPS II Score</td>
<td>25 ± 7</td>
<td>25 ± 7</td>
<td>0.58</td>
</tr>
<tr>
<td>Haemodynamic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>81.7 ± 12.2</td>
<td>82.3 ± 12.8</td>
<td>0.57</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>80.5 ± 8.9</td>
<td>80.0 ± 8.8</td>
<td>0.49</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>11 ± 3</td>
<td>11.5 ± 3.5</td>
<td>0.09</td>
</tr>
<tr>
<td>PCWP(mmHg)</td>
<td>15 ± 4</td>
<td>14 ± 4</td>
<td>0.28</td>
</tr>
<tr>
<td>PAP mean (mmHg)</td>
<td>25 ± 10</td>
<td>26 ± 4.7</td>
<td>0.75</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>2.9 ± 0.9</td>
<td>3.0 ± 2.68</td>
<td>0.71</td>
</tr>
<tr>
<td>SVRI (dyne s · m² · cm⁻⁵)</td>
<td>977 ± 471</td>
<td>963 ± 350</td>
<td>0.86</td>
</tr>
<tr>
<td>PVRI (dyne s · m² · cm⁻⁵)</td>
<td>210 ± 184</td>
<td>210 ± 148</td>
<td>0.99</td>
</tr>
<tr>
<td>Inotropes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine (mg/d)</td>
<td>1.35 ± 5.4</td>
<td>9.7 ± 120</td>
<td>0.31</td>
</tr>
<tr>
<td>Norepinephrine (mg/d)</td>
<td>1.11 ± 4.3</td>
<td>3.74 ± 16.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Levosimendan (mg/d)</td>
<td>0.06 ± 0.61</td>
<td>0.04 ± 0.67</td>
<td>0.73</td>
</tr>
<tr>
<td>Eonoximone (mg/d)</td>
<td>10.53 ± 66.7</td>
<td>27.7 ± 188</td>
<td>0.28</td>
</tr>
<tr>
<td>Other agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTG (mg/d)</td>
<td>8 ± 22</td>
<td>13 ± 31</td>
<td>0.05</td>
</tr>
<tr>
<td>Amiodarone (mg/d)</td>
<td>74 ± 315</td>
<td>55 ± 230</td>
<td>0.41</td>
</tr>
<tr>
<td>Cortisone (mg/d)</td>
<td>18 ± 181</td>
<td>53 ± 360</td>
<td>0.20</td>
</tr>
<tr>
<td>Operative characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-clamp time (min)</td>
<td>89 ± 34</td>
<td>97 ± 41</td>
<td>0.02</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (min)</td>
<td>137 ± 59</td>
<td>148 ± 74</td>
<td>0.09</td>
</tr>
</tbody>
</table>
three groups based on their genotypes: TT, CT and CC. In the next step, the patients were classified into two different groups based on whether they were homozygous or heterozygous for the C allele (TC + CC; n = 292; dominant model of inheritance) or only homozygous for the T allele (TT; n = 208).

2.4. Statistical analysis

The Statistical Package for the Social Sciences Version 15 (SPSS Inc., Chicago, USA) for Windows was used to perform all statistical analyses. The data are expressed as mean ± standard deviation (SD). The data were checked for normality before the statistical analysis. The comparison of the means of two continuous variables was done using an independent t-test. The Mann–Whitney test was used in cases which did not have a normal distribution. The chi-square analysis was used to compare the categorical variables. The allele frequencies in the study population were counted and compared to an expected distribution in the normal population by the Hardy–Weinberg’s equilibrium, and checked by the \( \chi^2 \)-test. A multivariate logistic regression analysis was used to assess the independent effect of the traditional risk factors on the occurrence of postoperative renal replacement therapy, if the variables in the univariate analysis achieved significances. The significance was accepted at the \( p < 0.05 \) level.

3. Results

3.1. eNOS genotype distribution and demographic characteristics

The eNOS genotypes were available in all of the patients. When the patients were divided into groups based upon the investigated polymorphism (TT, TC and CC), it was found that 208 subjects were TT and 253 were TC. Thirty-six individuals were homozygous for the C allele (CC), which concurs with the reported frequency [19,20,23] and was consistent with the Hardy–Weinberg equilibrium. The frequency of the CC genotype in the population is between 0.02 and 0.10. Hence, we have combined all C-allele carriers in the TC + CC genotype group to get an adequate statistical power.

Moreover, the C allele is considered to have associations with cardiovascular events, with the CC and TC genotype group demonstrating similar responses that are different from the genotype TT [17–21]. Thus, the patients were grouped into a combined TC + CC genotype group (dominant model of inheritance) and compared to the TT genotype group. Of all the subjects studied, 208 patients were homozygous for the T allele (TT), and 289 patients were C-allele carriers (TC + CC).

There were no significant differences in gender, age, urgency of surgery, associated surgical procedures and additive Euroscore between the two groups. The risk factors did not reach a level of statistical significance except for smoking habits. In the TC + TT group, fewer patients have the risk factor of smoking (\( p = 0.04 \)). The administration of anti-arrhythmics (\( p = 0.03 \)) and anti-lipid agent (\( p = 0.01 \)) was significantly less in the C-allele carriers. The other preoperative medications were similar for all the groups. The preoperative characteristics of the patients are shown in Table 1.

3.2. Perioperative patient characteristics

With regards to renal function, statistical analysis revealed significant differences among the groups, concerning creatinine clearance \( c_{\text{min}} \) (TT vs TC + CC; \( p = 0.004 \)), decreased creatinine clearance (TT vs TC + CC; \( p = 0.024 \)), and the need for renal replacement therapy (TT vs TC + CC; \( p = 0.04 \)). The Rifle, APACHE II, SAPS II and ARF scores, and haemodynamic measurements conducted within 24 h revealed no significant differences between the genotypes. There was no difference in epinephrine support and the administration of levosimendan or eonoximone. However, there was an interaction between the genotypes and the administration of nitroglycerin, amiodarone, cortisone and cardiopulmonary bypass time achieved no statistically significant difference. However, the cross-clamp time was significantly \( (p = 0.02) \) higher in the TC + CC genotype group. All results are summarised in Table 2.

3.3. Postoperative outcomes of patients according their genotypes

There was no relation between the administration of fresh frozen plasma and the genotype. The transfusion of red blood cells revealed a statistical difference between two groups. The C-allele carrier (TC + CC) required a larger transfusion of red blood cells (\( p = 0.04 \)). Using a multiple logistic regression after the univariate analysis, the transfusion of red blood cells had (odds ratio (OR): 1.0007; 95% confidence interval (CI): 1.0005–1.0010; \( p < 0.0001 \)) a higher adjusted odds for the occurrence of postoperative renal impairment followed with renal replacement therapy.

Furthermore, similar results were observed concerning IABP and ECMO usage, mean postoperative stay in the
renal physiology and function. This pathophysiology is explained based on extracorporeal circulation, it is conceivable that the investigated polymorphism interacts with renal dysfunction following cardiac surgery with cardiopulmonary bypass and investigated for potential interactions between this genetic variation and the risk of renal dysfunction. The study identified the fact that the eNOS T-786C polymorphism represents a risk for the development of a renal dysfunction and renal replacement therapy after cardiac surgery with cardiopulmonary bypass, in individuals who were C-allele carriers for the eNOS T-786C polymorphism. This finding highlights the importance of studying gene polymorphisms for their biological pathways in understanding factors associated with renal dysfunction following cardiac surgery with cardiopulmonary bypass. However, to date, our knowledge on the importance of genetic polymorphisms in influencing the susceptibility to, and severity of, renal dysfunction remains limited.

A number of endogenous substances have been shown to produce changes in the renal haemodynamics. Many of these substances exert their vasodilatory action through endothelium-derived NO. The eNOS T-786C polymorphism was investigated in several studies for its associations with cardiovascular diseases, including coronary spasm, myocardial infarction and coronary artery disease [18—20], but, to our knowledge, it has never been investigated in patients in view of the postoperative renal dysfunction following cardiac surgery with cardiopulmonary bypass. The distribution of the genotypes in our study was compatible with the Hardy—Weinberg equilibrium, which indicated that the screening method was appropriate. Furthermore, the frequencies of the genotypes are in accordance with previously reported results in other European populations [19,21,23].

In the present study, we observed a significant postoperative reduction of creatinine clearance (p = 0.024) with the lowest creatinine clearance of C-allele carriers. Furthermore, postoperative renal replacement therapies were more frequent in patients carrying the C allele (p = 0.04). In contrast to Liakopoulos et al. [23], who did not detect clinical differences in patients with the eNOS T-786C polymorphism after cardiac surgery, our results are supported by the findings of Dengel et al. who investigated the influence of eNOS T-786C polymorphism on renal haemodynamics due to changes in the NO metabolism [21]. Furthermore, it has been hypothesised that impairments in the endothelial NO generation may be influenced by gene polymorphisms, which ultimately may result in impaired renal haemodynamics [15]. Based on these pathophysiological backgrounds and the knowledge that impaired renal haemodynamics can be explained based on extracorporeal circulation, it is conceivable that the investigated polymorphism interacts with renal function following cardiac surgery.

Otherwise, it is well known that the extracorporeal circulation is associated with multiple perturbations in the renal physiology and function. This pathophysiology is attributed to non-pulsatile blood flow, macro- and microembolic insults to the kidney, increases in circulating catecholamines and inflammatory mediators and the release of free haemoglobin from the traumatised red blood cells. Therefore, during cardiopulmonary bypass, there are substantial decreases (25—75%) in the renal blood flow and the glomerular filtration rate, with corresponding increases in the renal vascular resistance. The influence of these phenomena on the clinical outcome is considerable [8—10]. Thus, it is possible that this observed renal dysfunction is induced through the eNOS T-786C polymorphism and/or cardiopulmonary bypass in consequence to a decreased transcription rate and, presumably, with a reduction in NO production in the C-allele carrier.

Interestingly, an increased vasopressor support was observed (norepinephrine; p = 0.02) in the C-allele carriers. These findings are not in accordance with the findings of Cattaruzza et al. [19]. In their study, they demonstrated that the CC carriers have a decreased capacity in the endothelium for generating NO. Thus, in our study, the consumption of norepinephrine in the C-allele carriers should be lower than that in the homozygous T-allele carriers. But otherwise, Ruel et al. [24] demonstrated that the altered release of NO following cardiac surgery with cardiopulmonary bypass may lead to a reduced peripheral and pulmonary resistance and abnormal vascular permeability. It could be conceivable that more homozygous C-allele carriers received catecholamine support. However, in the present study, the subjects were categorised into a combined TC + CC genotype group and compared to the TT genotype group, because it has been shown that the C allele is considered to have deleterious cardiovascular effects — with the CC and TC genotype group demonstrating similar responses that are different from the group with the TT genotype [18]. Therefore, we cannot exclude the possibility that this may have influenced the results of catecholamine support in the present study. In addition, the cause of renal dysfunction in patients with cardiac surgery is multifactorial. Cardiopulmonary bypass per se has often been considered to be responsible for renal damage [8—10]. Furthermore, Boldt et al. [25] could demonstrate that the patients undergoing cardiopulmonary bypass for longer than 90 min always demonstrated significantly higher urinary levels of kidney-specific proteins, indicating considerable kidney damage. In the present study, C-allele carriers have an increased duration of cardiopulmonary bypass and a significantly increased cross-clamp time (TC + CC, p = 0.02). In addition, the multivariate analysis revealed that the duration of the cross-clamp time has a potential effect on renal replacement therapy (p = 0.001). However, all our patients had a prolonged duration of cardiopulmonary bypass; thus, it is unlikely that the cross-clamp time is responsible for the differences in renal dysfunction and renal replacement therapy between the two groups.

In the present study, we have also observed a significant difference in the transfusion of red blood cells in C-allele carriers (TT vs TC + CC; p = 0.04). Our findings, with respect to the effect of the transfusion of red blood cells on renal dysfunction and the use of renal replacement therapy, are consistent with prior research. This observation potentially also had a clinical impact on the postoperative outcome with regards to renal function. It is well known that patients...
undergoing cardiac surgery with the transfusion of red blood cells are exposed to an increased mortality and postoperative morbidity [26]. However, it is still unexplained if the C-allele carriers of eNOS T-786C polymorphism undergoing cardiac surgery with cardiopulmonary bypass potentially need a larger transfusion of red blood cells. Thus, we assume, for the present, that the T-786C eNOS polymorphism has an impact on the renal function following cardiac surgery. It is important to note that this study suffered from important limitations. Furthermore, although our patients were distributed according to the Hardy—Weinberg equilibrium, our study is limited by the relatively small number of patients in the groups, which may have affected the data concerning the renal function and the requirement for postoperative renal replacement therapy. In addition, the patients were not divided into separate groups based on the associated surgical procedures. Moreover, the eNOS gene is located near other genes; therefore, it is possible that the observed association is the result of linkage disequilibrium with other gene mutations, and we cannot exclude the possibility that this may have influenced the results of the present study.

In conclusion, the results of the present study demonstrate that the C-allele carriers have a significantly higher risk of renal dysfunction and renal replacement therapy following cardiac surgery with cardiopulmonary bypass. These data may contribute to the renal risk stratification for cardiac surgery and may raise questions with regards to the T-786C eNOS polymorphism and the pathophysiology of acute renal injury.

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