Influence of interleukin-6 G-174C gene polymorphism on coronary artery disease, cardiovascular complications and mortality in dialysis patients

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Abstract

Background. Inflammation is a well recognized central component of atherosclerotic processes in chronic kidney disease. Interleukin-6 (IL-6) levels are a strong determinant of cardiovascular mortality in dialysis patients. We evaluated the impact of IL-6 gene G-174C polymorphism associated with modified IL-6 production on the development of coronary artery disease (CAD), cardiovascular events and mortality in chronic dialysis patients.

Methods. We studied n = 463 patients on chronic dialysis with angiographically confirmed (n = 218) or excluded (n = 245) CAD followed up for 65 months after initiation of dialysis. Monitored were arterial hypertension, diabetes mellitus, hyperlipidemia, smoking, CRP and fibrinogen. IL-6 gene G-174C polymorphism was determined by PCR amplification.

Results. The CC genotype was associated with an impaired patient survival (p < 0.05) remaining an independent risk factor for death in multivariate analysis (HR for CC genotype: 3.58, CI: 1.41-9.07, p < 0.01). CC genotype carrying CAD patients suffered significant frequently cardiovascular events (revascularization, myocardial infarction, death) compared to GG/GC genotype carriers (85.2% vs. 66.5, p < 0.05). However, the IL-6 gene G-174C polymorphism was not related to the onset and development of CAD itself (ns) and the inflammation parameters CRP and fibrinogen did not differ between the genotypes under investigation (ns).

Conclusions. Our results suggest that IL-6 gene G-174C polymorphism is associated with the incidence of cardiovascular events and mortality in chronic dialysis patients.

Keywords: cardiovascular; dialysis; gene polymorphism; interleukin-6; kidney

Introduction

Patients with end-stage renal disease (ESRD) have a dramatically higher risk of morbidity and mortality due to cardiovascular diseases (CVD) compared to the general population, as a result of accelerated atherosclerotic processes. The high prevalence of traditional cardiovascular risk factors contributes to the excessive risk of CVD. Nevertheless, recent studies focused on non-traditional risk factors, such as oxidative stress and inflammation in ESRD [1,2]. Inflammation is recognized as a central component of atherosclerotic processes, although the underlying regulation and molecular mechanisms are not clearly understood. Many uraemic patients show serological evidence of an activated inflammatory response, as indicated by increased circulating levels of non-specific markers of inflammation such as C-reactive protein (CRP) and proinflammatory cytokines such as interleukin-6 (IL-6) [2]. Recently, a stronger predictivity of IL-6 on total and cardiovascular mortality than CRP has been demonstrated in haemodialysis patients [3]. The causes of chronic inflammation in ESRD remain unknown. It seems conceivable that both dialysis- and non-dialysis-related factors may contribute. Since circulating cytokine levels vary considerably interindividually, one may speculate that genetic factors, such as polymorphisms in genes encoding them, may be involved in determining the individual inflammatory reaction in response to a given insult. Genetic variations of the IL-6 gene may be key regulators of IL-6 production and therefore may predispose an individual to cardiovascular events. G-174C polymorphism in the promoter region of the IL-6 gene has been shown to be functional and influence IL-6 production both in vitro and in vivo [4].

The aim of the present study was to evaluate the impact of IL-6 gene G-174C polymorphism on the development of coronary artery disease (CAD), cardiovascular events and mortality in patients with ESRD.
as the presence of one or more stenoses of >50% in at least one major artery or a main stem stenosis of >30%.

For all individuals enrolled in the study, a complete medical history was collected. Blood specimens for analysis of lipids, inflammation parameters and genotyping were taken at the time of coronary angiography. Patients were followed up for a mean of 32 months after coronary angiography (65 months after initiation of dialysis). Coronary interventions [percutaneous coronary angioplasty with or without stenting, coronary artery bypass grafting (CABG)], myocardial infarction (MI) and death were defined as the endpoints of the study. Patients were censored at the time of renal transplantation. Informed consent was obtained from all patients. The study protocol was approved by the local ethics committee.

**Determination of IL-6 G-174C polymorphism**

Genomic DNA was extracted from peripheral leukocytes from whole blood samples using the QIAmp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. IL-6 gene G-174C polymorphism was determined by PCR amplification followed by restriction digestion with the endonuclease Lwe I. The following primers were used: sense, 5′-CAG AAG AAC TCA GAT GAC TGG-3′, antisense, 5′-GCT GGG CTC CTG GAG GGG-3′ (MWG Biotech AG, Ebersberg, Germany). The PCR products were incubated with the endonuclease Lwe I (MBI Fermentas GmbH, St. Leon-Rot, Germany). The polymorphism created a recognition site for the enzyme so that the initial PCR product (610 bp) was cut into two fragments (367 and 243 bp) in the presence of the -174G allele. Internal and external controls were used in order to prohibit misclassifications during genotyping.

**Statistical analysis**

Data management and statistical analysis were performed using the SPSS 11.5 program (Statistical Package for Social Sciences, SSPS GmbH, Munich, Germany). All data were expressed as percentages or mean ± standard deviation (SD). Comparisons of non-numerical variables were made with Pearson’s chi-square test. The expected allele frequencies under the assumption of the Hardy–Weinberg equilibrium were compared with the observed ones in each study population. Both genotype and allele frequencies were compared in the different groups in order to reduce the possibility of spurious associations. Continuous variables were tested in each group for normal distribution using the Kolmogorov–Smirnov test for one variable. Comparisons of continuous variables between two groups were performed using Student’s t-test; the non-parametric Mann–Whitney U-test was used when normal distribution was skewed. Survival curves were calculated by the Kaplan–Meier method and compared by the log-rank test.

To adjust confounding risk factors, a Cox regression model was used for multivariate analysis with death as a dependent variable. Following covariates were included in the model: IL-6 genotype, diabetes mellitus, CAD, gender, fibrinogen, CRP, age at the end of follow-up period, history of previous coronary intervention or MI and smoking habits. A P-value of <0.05 was considered to be statistically significant.

**Results**

**Characteristics of the study population**

The study population consisted of \( n = 463 \) Caucasian dialysis patients (87.2% on haemodialysis and 12.8% on peritoneal dialysis) with a mean dialysis time of 65 months at the end of follow-up period (Table 1). There was a high prevalence of hypertension (94%), whereas 23.3% of the patients were diabetics. Mean baseline CRP and fibrinogen levels were above normal values. CAD was angiographically excluded in \( n = 245 \) patients and documented in \( n = 218 \) patients. The mean age at the time of coronary angiography was 55.3 years. Fifty-one patients experienced MI during the follow-up period. In 39.9% of the patients one-vessel, in 25.2% two-vessel and in 34.9% three-vessel CAD was diagnosed. CAD was treated in \( n = 66 \) patients with percutaneous transluminal coronary angioplasty (PTCA with or without stent implantation), while \( n = 60 \) patients underwent CABG. The patients who underwent both PTCA and subsequently CABG were summarized under CABG. In the mean follow-up period of 32 months after coronary angiography, \( n = 45 \) patients (9.7%) died.

**Table 1. Characteristics of the study population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>463</td>
</tr>
<tr>
<td>Men/women (%)</td>
<td>67.2/32.8</td>
</tr>
<tr>
<td>Haemodialysis/peritoneal dialysis (%)</td>
<td>87.2/12.8</td>
</tr>
<tr>
<td>Time on dialysis in months (mean ± SD)</td>
<td>65 ± 48</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>23.3</td>
</tr>
<tr>
<td>Arterial hypertension (%)</td>
<td>94.0</td>
</tr>
<tr>
<td>ACE inhibitors or AT1 receptor antagonists (%)</td>
<td>47.2</td>
</tr>
<tr>
<td>Cholesterol synthesis inhibitors (%)</td>
<td>33.9</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>38.3</td>
</tr>
<tr>
<td>Coronary artery disease (CAD) (n)</td>
<td>218</td>
</tr>
<tr>
<td>Mean age at coronary angiography (mean ± SD)</td>
<td>55.3 ± 10.3</td>
</tr>
<tr>
<td>One-vessel CAD (n)</td>
<td>87</td>
</tr>
<tr>
<td>Two-vessel CAD (n)</td>
<td>55</td>
</tr>
<tr>
<td>Three-vessel CAD (n)</td>
<td>76</td>
</tr>
<tr>
<td>Myocardial infarction (n)</td>
<td>51</td>
</tr>
<tr>
<td>PTCA ± stenting (n)</td>
<td>66</td>
</tr>
<tr>
<td>CABG (n) (including PTCA before CABG)</td>
<td>60</td>
</tr>
<tr>
<td>Deceased (n)</td>
<td>45</td>
</tr>
<tr>
<td>Follow-up after coronary angiography (months)</td>
<td>32 ± 27 (1–139)</td>
</tr>
</tbody>
</table>

PTCA ± stenting = percutaneous transluminal coronary angioplasty with or without stenting, CABG = coronary artery bypass grafting.

**CAD and baseline characteristics**

There was no difference in terms of age, hypertension, smoking habits, total LDL or HDL cholesterol (under treatment), body mass index, duration of dialysis and treatment with angiotensin-converting enzyme inhibitors/angiotensin receptor blockers between patients with and without CAD. There was a higher frequency of males (73.9 versus 61.2%, \( P < 0.01 \)) and diabetics (29.8 versus 17.6%, \( P < 0.01 \)) among patients with CAD. Furthermore, patients with CAD had higher CRP (1.15 versus 0.94 mg/dl, \( P < 0.05 \)) and fibrinogen levels (453 versus 417 mg/dl, \( P < 0.05 \)) and were treated more often with statins (44.5 versus 24.2%, \( P < 0.0001 \)).

**Mortality and baseline characteristics**

Deceased patients had significantly more often CAD (75.6 versus 44%, \( P < 0.001 \)) and coronary interventions (PTCA ± stent and/or CABG, 44.4 versus 27.8%, \( P < 0.05 \)) and were more frequently smokers (53.5 versus 36.7%, \( P < 0.05 \)). Fibrinogen levels were also significantly higher in the deceased patients (502 versus 427 mg/dl, \( P < 0.01 \)), whereas no difference was found in the CRP levels (ns).
IL-6 genotyping

The genotype distribution and the allele frequencies of G-174C polymorphism were similar in healthy controls (GG/GC: 83%, CC: 17%; G-allele: 0.58, C-allele: 0.42) and dialysis patients (GG/GC: 86%, CC: 14%; G-allele: 0.61, C-allele: 0.39; ns). In all groups, the observed genotype frequencies corresponded to the expected values according to the Hardy–Weinberg equilibrium.

IL-6 genotypes and baseline characteristics

Patients with different genotypes were comparable for age, gender, prevalence of diabetes mellitus, hypertension, time on dialysis, smoking, lipids, BMI and treatment with ACE inhibitors, angiotensin receptor blockers or statins (ns). CRP and fibrinogen levels did not differ between patients with the GG/GC and the CC genotypes (ns) (Table 2).  

### Relationship between IL-6 genotypes and CAD, coronary interventions, MI and mortality

The distribution of IL-6 genotypes did not differ between patients with and without CAD (ns, Table 3). IL-6 gene G-174C polymorphism did not correlate with the number of stenosed coronary vessels (ns, data not shown). However, IL-6 gene G-174C polymorphism was associated with the prognosis of patients with CAD: CC genotype carriers had significantly more frequent cardiovascular events (revascularization, MI, death) compared to GG/GC genotypes carriers (85.2% versus 66.5%, P < 0.05). There was no significant difference between patients with coronary revascularization (PTCA with or without stent implantation and/or CABG) and patients without need for revascularization regarding the G-174C genotypes. An association between IL-6 gene G-174C polymorphism and MI was detectable only in men: male CC genotype carriers suffered significantly more often from MI compared to GG/GC carriers (P < 0.05). Survival was significantly related to the IL-6 gene G-174C polymorphism. Kaplan–Meier survival analysis for the overall population showed a significantly higher (P < 0.05) mortality rate for patients carrying the IL-6 CC genotype compared to GG/GC carriers in the mean dialysis time of 65 months (Figure 1). Analysing after splitting of the population by age showed that the risk of mortality was significantly associated with the CC genotype only in patients of age 55 years and younger and not in patients older than 55 years (Table 3). Also, smokers with the CC genotype had a higher mortality risk compared to GG/GC carriers (contrary to non-smokers; P = 0.06).

### Multivariate analysis

After adjusting for confounding variables, such as diabetes mellitus, CAD, gender, fibrinogen, CRP, age at the end of follow-up period, coronary revascularization procedures,
MI and smoking, the IL-6 gene polymorphism remained an independent risk factor for death in multivariate analysis [HR for the CC genotype: 3.58, CI: 1.41–9.07, $P < 0.01$ (Cox regression model)]

**Discussion**

Chronic dialysis patients are in a chronic activated inflammatory state as demonstrated by elevated inflammation markers and proinflammatory cytokines like IL-6. Recently, Danesh et al. evaluated prospectively in two large population-based cohorts and in a systematic review the association of long-term circulating IL-6 levels with fatal and non-fatal coronary heart disease (CHD) risk. They concluded that long-term IL-6 levels are associated with a CHD risk about as strongly as are some major established risk factors [5]. It has been shown that IL-6 has a stronger predictivity on total and cardiovascular mortality than CRP in non-renal [6] and in haemodialysis patients [3] and that IL-6 gene G-174C polymorphism modifies IL-6 production in non-renals [4,7] and in renal populations [8].

In the present study, we demonstrate that IL-6 gene G-174C polymorphism is associated with mortality and cardiovascular complications in chronic dialysis patients. Patients with a CC genotype had a higher risk for mortality, independently of other risk factors in multivariate analysis. Furthermore, the CC genotype was associated with worse prognosis of CAD. Patients with CAD carrying the CC genotype suffered significantly more frequent cardiovascular events like revascularization, MI and death compared to GG/GC genotype carriers. Bittar et al. found in a non-renal population an association of the C-allele with higher post-operative IL-6 levels and a less favourable outcome after coronary revascularization [9]. As in our population, in a dialysis cohort a higher risk for CVD in C-allele carriers has been demonstrated [10]. In non-renal populations, it has been shown that the IL-6 G-174C allele was associated with higher IL-6 levels [7], increased risk for CAD [11,12] and mortality [7]. However, other investigators did not confirm these results [13,14]. Our results showed no association between the IL-6 gene G-174C polymorphism and development of CAD. The fact that IL-6 gene G-174C polymorphism was not associated with the development of CAD itself, but with complications of CAD (revascularization, MI, death) in our dialysis population, suggests that the effect of IL-6 gene G-174C polymorphism on the on-set and development of atherosclerosis in dialysis patients is relatively marginal at the initial stages. Supporting this hypothesis, IL-6 serum levels were increased in patients with unstable angina [15] and were associated with a worse in-hospital outcome [16]. Furthermore, patients with MI carrying the CC genotype had higher IL-6 plasma levels than GG genotype carriers [17].

The impact of IL-6 gene G-174C polymorphism seems to depend on or be modified by gender, age and smoking habits. In our population, the effect of IL-6 gene G-174C polymorphism was more pronounced in male, younger patients and smokers. The association with mortality was not detectable in patients older than 55 years. Supporting the importance of age, an additive effect of the chemokine receptor 2, vitamin D receptor and GC/CC genotype on the prevalence of MI has been shown in patients below 65 years [18]. An association between IL-6 gene G-174C polymorphism and MI in our patients was detectable in men only. The greatest risk for CAD in general population has been described in men who smoked and carried the C-allele [12]. In a case–control study in non-renal patients, the C-allele was associated with an increased risk of developing MI in men [17]. In the non-renal population, the C-allele has been shown to be associated with the unfavourable profile of early predictors of atherosclerosis such as carotid artery compliance, lipoproteins and blood pressure in men only [19]. Carotid artery plaques obtained from women have a more stable, less inflammatory phenotype compared to men [20]. The expression of matrix metalloproteinases (MMPs) is induced in atherosclerotic plaques prone to rupture. A correlation has been shown between admission levels of MMP-9, CRP and IL-6 in patients with acute coronary syndromes [21]. Samnegard et al. demonstrated different patterns of association between inflammatory markers (including IL-6) and MMPs in men (MMP-9) and women (MMP-3), strengthening the hypothesis of gender-specific differences in the pathophysiological mechanism of MI [22]. Furthermore, the transcriptional control of IL-6 expression is sex hormone regulated as it was shown that oestrogens inhibit IL-6 promoter activity [23,24]. These results may partly explain to our observation that MI was associated with IL-6 gene polymorphism in men only.

After splitting our population into smokers and non-smokers, we found a tendency towards a higher mortality rate in smokers with a CC genotype contrary to non-smokers. Smoking triggers inflammation and has a significant effect on IL-6 levels [25]. In young healthy smokers and C-allele carriers, elevated leukocyte, lymphocyte and monocyte counts have been documented, but not in non-smokers [26]. An effect of smoking on endothelial function has been seen most clearly in men carrying the CC genotype [27]. These results suggest that the impact of IL-6 gene G-174C polymorphism may depend on or be modified by gender, age and smoking habits.

Although the C-allele was associated with higher IL-6 levels in peritoneal dialysis population [8] and with higher CRP levels in a non-renal population [28], we did not find significantly different levels of the inflammation markers CRP and fibrinogen in the different genotypes of the IL-6 gene G-174C. It has been proposed that drugs influence the phenotypic relevance of IL-6 gene G-174C polymorphism. Patients receiving statins had significantly lower IL-6 levels than those without statins [13], and CC genotype carriers under statin therapy had a significantly lower risk for CAD [29]. It has also been demonstrated that ACE inhibitors and angiotensin receptor blockers decrease cytokines in patients undergoing cardiac surgery carrying the GG genotype [30]. Because a high proportion of our patients were treated with statins, ACE inhibitors and angiotensin receptor blockers, the results may be partly influenced by drugs. As the impact of IL-6 gene polymorphism on cardiovascular events was influenced by age, gender, smoking and medication in our study population, it is assumed that the association between the gene polymorphism and IL-6 as well as inflammatory markers is also influenced by them. These contradictory
results also suggest that multiple levels of regulation mechanisms of the IL-6 system exist.

In conclusion, our results demonstrate that the IL-6 gene G-174C polymorphism is associated with the incidence of cardiovascular events and mortality in chronic dialysis patients. This effect of the IL-6 gene polymorphism is modified by gender, age and smoking habits, suggesting an interaction with these risk factors.

Conflict of interest statement. None declared.

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