Genetic impact of TNF-β on risk factors for coronary atherosclerosis

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ABSTRACT. ***Background.*** **Inflammatory processes are considered to play an important role in the development of coronary atherosclerosis. The proinflammatory cytokine, tumor necrosis factor β (TNF-β), is thought to contribute to the pathogenesis of atherosclerosis.** ***Study design.*** **In this clinical study, the influence of genetic variants of TNF-β (c.7G>A, IVS1+90G>A, C13R, T60N) on major coronary risk factors, including gender, smoking, history of cardiovascular diseases, biochemical data (inflammatory markers, factors of lipid metabolism, coagulation/fibrinolysis balance), and angiographically-proven coronary state, was investigated in 176 European Caucasian probands (130 males, mean age: 51.9 ± 8.9 y).** ***Results.*** **The most frequent combinations of the polymorphisms investigated were significantly associated with four of the coronary risk factors evaluated: hypertension, body mass index, the common inflammatory marker TNF-α (mRNA expression), and fibrinogen ($p < 0.05$). However, on testing the impact of the genetic background on the incidence of coronary stenosis in this sample of European Caucasians, no significant influence of these polymorphisms (stepwise binary logistic regression analysis) could be proven. These findings emphasise a distinct influence of TNF-β polymorphisms on important modulators of the development of coronary atherosclerosis, but exclude its genetic background, investigated in this study as an independent coronary risk factor.**

Keywords: TNF-β, gene polymorphism, genetic risk, coronary stenosis, coronary risk factors

Coronary atherosclerosis is regarded as a complex disease mediated by a variety of biological processes. The major role of inflammation concerning the development of atherosclerotic lesion has been emphasized in different clinical and epidemiological studies (for review see [1]). In genome-wide association studies, significant links between the development of cardiovascular diseases and the proinflammatory cytokine TNF-β were evident [2]. The fatty streaks, as the earliest signs of atherosclerosis, consist mainly of T-lymphocytes and monocyte-derived macrophages, major sources of cytokine release. Furthermore, proinflammatory stimuli, including infectious agents, can lead to the manifestation of endothelial dysfunction. The attraction and migration of inflammatory cells into the vascular wall is mediated by proinflammatory cytokines [3]. The further atherosclerotic development that results finally in a stable or unstable fibrous plaque is also driven by proinflammatory cytokines [4]. Cytokines trigger the induction of cellular adhesion molecules, which play an important role in the adhesion of leukocytes to the vascular endothelium [5].

In previous studies, it was demonstrated that, in patients with high coronary risk, the level of circulating inflammatory markers may be dramatically increased [6]. Accordingly, reduction in TNF-β could be shown to contribute to a tremendous reduction in lesion size [7]. Polymorphisms in the TNF-β gene were shown to be associated with TNF expression [8, 9]. In different clinical studies, the relevance of polymorphic variants of TNF-β gene for coronary atherosclerosis has been investigated [10-13].

Although it has been shown that genomic variants of TNF-β influence both TNF-α gene and protein expression, and might therefore change the susceptibility to the development of atherosclerosis, hardly anything is known about the complex interaction between the genetic background for TNF-β, and the clinical outcome when considering classical coronary risk factors.

Therefore, the aim of the present study was first to investigate the possible effects of TNF-β (its genetic background represented by polymorphisms c.7G>A, IVS1+90A>G: corresponds to +252A>G (intron 1), C13R: corresponds to +492T>C (exon 2), T60N: corresponds to +720C>A (exon 3)) on coronary risk factors, either separately or in combinations.

The second purpose was to find out, by binary logistic regression analysis adjusted for classical coronary risk factors such as gender, smoking, hypertension, diabetes mellitus, low HDL-cholesterol and hypercholesterolemia, if the TNF-β variants investigated could have an impact on the incidence of significant coronary stenosis.

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PATIENTS AND METHODS

Study population

One hundred and seventy six clinically and biochemically characterized Caucasians from Central Germany were included in this clinical study. All of the 176 individuals underwent quantitative coronary angiography (Integris H 5000S; Phillips, Germany) for clinical indications such as suspected coronary artery disease, planned heart surgery or further diagnosis. Two skilled cardiologists carried out the procedure - both were blind to the inclusion criteria and results of this study. Significant stenosis was defined as an observed narrowing of the luminal diameter of > 50% in at least one major coronary vessel, equivalent to a 70% reduction in the luminal diameter *in vivo* [14]. An age-matched, control patient without stenosis was included for every patient with significant stenosis and 31.8% of the coronary patients underwent invasive interventions such as ACVB (aorto-coronary venous bypass) and PCI (percutaneous coronary intervention). None of the patients exhibited septic symptoms as complications of the procedures. Furthermore, none of the patients suffered from any autoimmune diseases or cancer. All clinical and biochemical details of the entire patient group and the two subgroups are given in *table 1*.

All participants were recruited irrespective of concomitant risk factors. At the time of the investigations they were hospitalized, clinically stable and on standard medical therapy. The clinical chemistry variables were measured using standard hospital techniques.

For the genetic and biochemical investigation, fresh blood was obtained from the patients. The biochemical markers, including inflammatory markers, factors of the lipid metabolism and of the coagulation/fibrinolysis balance were determined according specific laboratory protocols (detailed information available from the authors, [15]).

All individuals gave their written consent. The study was approved by the local ethics committee, and investigations were carried out in accordance with the ethical guidelines

Table 1
Clinical and biochemical characteristics of the patient groups. Values are displayed as means ± standard deviation

Variable	All	Significant stenosis		
	Patients (n = 176)	**With** (n = 88)	**Without** (n = 88)	**p**
Age (years) *4	51.9 ± 8.9	51.9 ± 8.6	51.9 ± 9.3	n.s.
Gender (% males) *1	73.9	87.5	60.2	**< 0.001**
Smoking (%) *1	69.9	85.2	54.5	**< 0.001**
Body mass index (kg/m^2) *3	27.21 ± 3.9	27.31 ± 3.6	27.11 ± 4.2	n.s.
History of				
Myocardial infarction (%) *2	17	34.1	0	**< 0.001**
Hypertension (%) *1	42.4	44	40.9	n.s.
Diabetes mellitus (%)*1	15.5	17	14	n.s.
Hypercholesterolemia (total-cholesterol > 5.2mmol/L) (%) *1	34.7	29.5	39.8	n.s.
Invasive intervention (ACVB% + PCI %) ***2**	17.6 + 14.2	35.2 & 28.4	0 & 0	**< 0.001**
Biochemical data				
Immunological/inflammatory markers				
Uric acid male (µmol/L) *3	361.4 ± 82.3	355.8 ± 80.1	371.3 ± 87.9	n.s.
Uric acid female (µmol/L) *3	293.7 ± 92.3	323.1 ± 60.6	284.2 ± 99.4	n.s.
α2-macroglobulin (g/L) *4	1.52 ± 0.5	1.46 ± 0.4	1.59 ± 0.6	n.s.
Leucocytes (Gpt/L) *3	6.99 ± 1.8	7.08 ± 1.9	6.82 ± 1.6	n.s.
TNF-α gene expression in monocytes (ag/cell) *4	6.48 ± 5.99	6.59 ± 5.67	6.36 ± 6.33	n.s.
TNF-α plasma protein expression (pg/ml) *3	2.47 ± 0.72	2.43 ± 0.62	2.52 ± 0.8	n.s.
Factors of the lipid metabolism				
Apo AI male (g/L) *3	1.37 ± 0.25	1.4 ± 0.28	1.35 ± 0.22	n.s.
Apo AI female (g/L) *3	1.48 ± 0.2	1.37 ± 0.2	1.52 ± 0.2	n.s.
Apo B (g/L) *3	1.22 ± 0.3	1.31 ± 0.3	1.13 ± 0.2	**< 0.001**
Total-cholesterol (mmol/L) *3	5.73 ± 1.2	6.04 ± 1.3	5.42 ± 1.1	**0.001**
HDL-cholesterol male (mmol/L) *3	1.31 ± 0.4	1.35 ± 0.4	1.26 ± 0.4	n.s.
HDL-cholesterol female (mmol/L) *3	1.45 ± 0.4	1.28 ± 0.3	1.5 ± 0.4	n.s.
LDL-cholesterol (mmol/L) ***3**	3.58 ± 1.1	3.93 ± 1.2	3.25 ± 0.9	**< 0.001**
Lipoprotein (a) (g/L) *4	0.24 ± 0.3	0.27 ± 0.4	0.22 ± 0.3	n.s.
Triglycerides (mmol/L) *4	2.05 ± 1.8	2.1 ± 1.8	1.99 ± 1.7	n.s.
Factors of the coagulation/fibrinolysis balance				
Plasminogen (%) *3	118.7 ± 25.7	122.9 ± 29.9	114.1 ± 19.2	n.s.
Plasminogen activator inhibitor (U/ml) *4	3.23 ± 2.7	2.84 ± 2.8	3.62 ± 2.5	**0.034**
Fibrinogen (g/L) *4	3.37 ± 0.9	3.36 ± 0.8	3.38 ± 0.99	n.s.
Glucose (mmol/L) *4	5.44 ± 1.52	5.42 ± 1.7	5.44 ± 1.3	n.s.

Significances: *1 X^2-test (*2 exact test), for data distributed normally: *3 Student's T-Test, for data that were not normally distributed: *4 Mann-Whitney-U-Test.

of the 1975 "Declaration of Helsinki" and its amendment in "Tokyo and Venice".

Genomic studies on TNF-β polymorphisms

The genomic DNA was prepared from leucocytes from human venous blood (DNA Blood Kit, Qiagen). For the detection of the genotype distribution of the genomic variants, SSCP- (c.7G>A, C13R; T60N) or RFLP-analyses (IVS1+90A>G) were used. The PCR primers are given in *table 2*.

For SSCP analyses, PCR products were precipitated and re-dissolved in 6 µL of a loading dye (95% formamide). The single stranded DNA fragments were separated in a polyacrylamide gel (PAA gel: C = 10.4%, T = 3.7%) and visualized by silver staining.

For restriction digestion, 10U of *Nco*I (Promega) were added to PCR-tubes with reaction buffer supplied. The G-allele resulted in 97bp and 456bp fragments and the A-allele produced a 556bp band. After an incubation of at least 4 h at 37°C, the bands were visualized after agarose gel electrophoresis and ethidium bromide staining (0.5 µg/mL).

The sequence changes, detected by an aberrant SSCP- or RFLP-pattern, were confirmed by cycle sequencing of a mixture of four independent PCR-samples (ABI Prism™ system 373A). For sequencing, a Thermo Sequenase II dye terminator cycle sequencing premix kit (Amersham Pharmacia Biotech) was used in accordance with the supplier's recommendations.

Statistical evaluation

Statistical analyses were carried out using SPSS 12. Values of $p < 0.05$ were considered to be significant. The genotype distributions of the polymorphisms were tested according to the Hardy-Weinberg equilibrium. Categorical variables were plotted in contingency tables and evaluated using Pearson's Chi square analysis and Fisher's exact test. Metric parameters are presented as mean ± standard deviation (SD). These data were analysed using the Kolmogorov-Smirnov-Test (test of normal distribution). For the statistical evaluation, Student's t test or one-way Anova (normally distributed values) and Mann-Whitney U-Test or Kruskal-Wallis-Test (values not distributed normally) were used. Binary logistic regression analysis was applied for investigating the effects of classical risk markers and polymorphic variants on the development of a significant stenosis.

Table 2
Primer design for genotyping of TNF-β polymorphisms

Polymorphism		Primer sequence
c.7G>A	upper	5' gct gct caa ctg cct cct c 3'
	lower	5' agt cca aag cac gaa gca cg 3'
IVS1+90A>G	upper	5' gcc tgg gcc ttg gtg ggt tt 3'
	lower	5' cat ctt ggg gtg ctg acg gg 3'
C13R	upper	5' tgc agg ttc tcc cca tga cac 3'
	lower	5' ctc ctg ctg cct cac ctg gg 3'
T60N	upper	5' cac ctt cag ctg ccc aga ct 3'
	lower	5' gag agc tgg tgg gga cat gt 3'

*[1] All PCR were carried out in a total volume of 25 µL with 50 ng of genomic DNA using PCR ingredients of Invitek (predenaturation for 5 min at 95 °C, 30 cycles: 20 sec at 92 °C, 20 sec at 53°C, 30 sec at 72 °C, last extension 10 min at 72 °C, ending with cooling up to 4°C in a Mastercycler gradient (Eppendorf)).

GeneBank accession number

All sequence data were derived from the sequence of the human TNF-β gene (GeneBank accession no. **Z15026**).

RESULTS

Clinical evaluation of the patient subgroups

A causative analysis of the outcome of significant coronary stenosis was carried out in two, age-matched subgroups of the 176 probands investigated, 88 individuals with, versus 88 without stenosis. According to the guidelines of the American Heart Association and the American College of Cardiology [16], different risk factors for coronary atherosclerosis were included. The statistical analysis revealed significant associations of coronary status with gender, smoking, Apo B, total cholesterol, LDL-cholesterol and plasminogen activator inhibitor, all being established risk markers of coronary atherosclerosis *(table 1)*.

Influence of TNF-β polymorphisms on coronary risk markers

All patients included in this study were genotyped for TNF-β polymorphisms: c.7G>A, IVS1+90A>G, C13R, T60N. Investigating the possible genotype combinations of the five polymorphisms, it was shown that only 6 genotype combinations occurred more frequently (> 2%) in the patient sample.

The evaluation of the impact of the genetic background on the incidence of angiographically proven stenosis revealed no significant associations *(table 3)*.

In a second step of analysis the interdependencies of TNF-β polymorphisms and established coronary risk markers (including gender, smoking status, diabetes mellitus, hypercholesterolemia, hypertension, body mass index, uric acid, α2-macroglobulin, leucocytes, TNF-α protein and mRNA expression, Apo A, Apo B, total cholesterol, HDL-cholesterol, LDL-choleserol, lp(a), triglycerides, plasminogen, plasminogen activator inhibitor, fibrinogen, glucose) was tested in a bivariate evaluation. The significant findings are shown in *table 4*. Hypertension, body mass index, the transcriptional expression of the inflammatory marker TNF-α, as well as fibrinogen, turned out to be significantly associated with the distribution of genetic variants in TNF-β. When evaluating associations in the two patient subgroups, was be demonstrated that these associations were not randomly distributed, but were either caused mainly by changes in the subgroup of individuals without significant stenosis (hypertension, body mass index), or by differences within the subgroup with coronary stenosis (TNF-α mRNA expression, fibrinogen).

Multivariate evaluation of the importance of the genetic background on the incidence of coronary stenosis

The investigation of possible interdependencies of polymorphisms in TNF-β gene on the development of coronary

Table 3
Genotype distributions of polymorphisms in TNF-β c.7G>A, IVS1+90A>G, C13R, T60N and the 6 most frequent combinations in association with the occurrence of angiographically-proven significant stenosis

	All patients		Coronary stenosis				p
			With		Without		
	n	%	n	%	n	%	
TNF-β: c.7G>A							
aa	17	9.7	11	12.5	6	6.8	
ag	81	46	38	43.2	43	48.9	
gg	78	44.3	39	44.3	39	44.3	0.411
TNF-β: IVS1+90A>G							
gg	18	10.2	12	13.6	6	6.8	
ag	80	45.5	38	43.2	42	47.7	
aa	78	44.3	38	43.2	40	45.5	0.342
TNF-β: C13R							
RR	12	6.8	8	9.1	4	4.5	
CR	69	39.2	31	35.2	38	43.2	
CC	95	54	49	55.7	46	52.3	0.343
TNF-β: T60N							
NN	17	9.7	11	12.5	6	6.8	
TN	79	44.9	37	42	42	47.7	
TT	80	45.5	40	45.5	40	45.5	0.409
Combinations (c.7G>A)-(IVS1+90A>G)-(C13R)-(T60N)							
ag-ag-CC-TN	42	25.6	20	25	22	26.2	
ag-ag-CR-TN	34	20.7	15	18.8	19	22.6	
gg-aa-CR-TT	32	19.5	15	18.8	17	20.2	
gg-aa-CC-TT	31	18.9	14	17.5	17	20.2	
aa-gg-CC-NN	14	8.5	9	11.3	5	6	
gg-aa-RR-TT	11	6.7	7	8.8	4	4.8	0.724

stenosis was carried out using a multivariate model including coronary risk factors proven to be associated with the genetic background of TNF-β.
In logistic regression analysis, possible interactions of TNF-β polymorphisms on the development of coronary atherosclerosis were evaluated. Even though significant associations between the TNF-β polymorphisms and coronary risk factors could be proven in bivariate analysis, in the multivariate model, the genetic background of TNF-β could not be proven as an independent predictor of coronary stenosis.

DISCUSSION

Coronary atherosclerosis is considered to be influenced by a variety of different factors.
However, recent research has established that inflammation plays an important role in its development [17]. The present clinical study is the first study conducted to evaluate the impact of 4 polymorphisms in TNF-β on the occurrence of significant stenosis and on coronary risk factors.

Clinical evaluation of the patient subgroups

In order to evaluate the importance of classical risk factors on the development of significant coronary stenosis, and to exclude age as an important coronary risk factor, two, age-matched proband subgroups (with and without angiographically proven stenosis) were included.

In this clinical study, established coronary risk factors could be proven to account for the incidence of significant coronary stenosis. In agreement with other clinical studies, significant, angiographically-proven stenosis was shown to be influenced by gender, smoking, factors of the lipid metabolism and the coagulation/fibrinolysis balance *(table 1)* [16, 18].

Influence of TNF-β polymorphisms on stenosis and on coronary risk markers

In a previous clinical study, the influence of polymorphisms in TNF-β gene on the incidence of coronary stenosis was evaluated. Bearing in mind that controversial data exist about the impact of the genetic background of TNF-β on the incidence of coronary events [10-13], we could not confirm any association between TNF-β polymorphisms and the incidence of coronary stenosis, in bivariate analyses. These controversial results may have been arrived at as a function of the various clinical hypotheses tested and/or the recruitment of particular coronary patients, as well as possible ethnic variations that have been demonstrated in other studies [19-22].

Hypertension

Since the vascular changes seen in hypertension are thought to be associated with mechanical and humoral factors that modulate signalling events, resulting in for example inflammation [23], the influence of TNF-β polymorphisms on hypertension was investigated in this study.

Table 4
Genotype-phenotype correlations: significant interdependencies between the 6 most frequent genotype combinations (c.7G>A)-(IVS1+90A>G)-(C13R)-(T60N) and coronary risk markers investigated. Values are displayed as means ± standard deviation

	All patients		**Coronary stenosis**			
			With		**Without**	
	Yes (n)	**No (n)**	**Yes (n)**	**No (n)**	**Yes (n)**	**No (n)**
Hypertension Fisher's exact test						
ag-ag-CC-TN	16	26	10	10	6	16
ag-ag-CR-TN	20	14	7	8	13	6
gg-aa-CR-TT	14	17	7	7	7	10
gg-aa-CC-TT	14	17	6	8	8	9
aa-gg-CC-NN	3	9	3	4	0	5
gg-aa-RR-TT	2	6	2	4	0	4
significances	0.185		0.987		**0.014**	
	Means ± SD	**n**	**Means ± SD**	**n**	**Means ± SD**	**n**
Body mass index (kg/m^2) data distributed normally One-way ANOVA						
ag-ag-CC-TN	28.6 ± 4.2	40	29.2 ± 4.4	19	28 ± 4	21
ag-ag-CR-TN	28.1 ± 4.2	34	28.3 ± 3.9	15	28 ± 4.6	19
gg-aa-CR-TT	27 ± 3.2	30	26 ± 3.2	15	28 ± 3	15
gg-aa-CC-TT	25.9 ± 3.3	30	26.3 ± 2.1	14	25.5 ± 4.2	16
aa-gg-CC-NN	25.3 ± 3.4	14	26.8 ± 2.9	9	22.6 ± 2.5	5
gg-aa-RR-TT	26.6 ± 4	11	27.2 ± 4	7	25.4 ± 4.3	4
significances	**0.015**		0.103		**0.041**	
TNF-α mRNA-expression (ng/cell) data that were not normally distributed: Kruskal-Wallis						
ag-ag-CC-TN	3.4 ± 0.9	40	3.7 ± 0.8	19	3.2 ± 0.9	21
ag-ag-CR-TN	3.5 ± 1	33	3.5 ± 0.8	14	3.5 ± 1.1	19
gg-aa-CR-TT	3.2 ± 0.8	30	3.1 ± 0.9	14	3.2 ± 0.8	16
gg-aa-CC-TT	3.2 ± 0.9	28	2.8 ± 0.4	13	3.6 ± 1.1	15
aa-gg-CC-NN	3.6 ± 0.8	14	3.6 ± 1	9	2.7 ± 0.5	5
gg-aa-RR-TT	3.5 ± 1.1	8	3.2 ± 0.5	5	4 ± 1.8	3
significances	**0.002**		**0.022**		0.062	
Fibrinogen (g/L) data that were not normally distributed: Kruskal-Wallis						
ag-ag-CC-TN	6.7 ± 6.1	42	7.1 ± 7	20	6.4 ± 5.3	22
ag-ag-CR-TN	9 ± 7.5	34	10.1 ± 8.2	15	8.1 ± 7	19
gg-aa-CR-TT	4.8 ± 3.4	32	5.4 ± 4	15	4.3 ± 2.7	17
gg-aa-CC-TT	4.6 ± 3.6	31	4.6 ± 2.2	14	4.6 ± 4.5	17
aa-gg-CC-NN	4.5 ± 4.5	14	3.3 ± 2.1	9	6.5 ± 6.9	5
gg-aa-RR-TT	11.6 ± 10	11	9.4 ± 3.1	7	15.5 ± 16.9	4
significances	0.223		**0.017**		0.367	

In line with this assumption, a significant influence of genetic background of TNF-β on the incidence of hypertension could be considered. However, these data are in contrast to previous findings of Nakayama *et al*., who showed no association between the polymorphic variants of TNF-β and the occurrence of essential hypertension in a group of Japanese individuals [24]. This discrepancy could, at least partly, be explained by possible ethnic differences in genetic background. Furthermore, the significant association between the genetic TNF-β variants shown here was evident in the group of patients without stenosis, but not in the whole patient group nor in the stenosis group. Because of the complex pathogenesis of coronary atherosclerosis, patient selection criteria may also account for the evidence of possible associations of the genetic background and clinical factors investigated.

Body mass index

In the present study, a significant association between TNF-β polymorphisms and body mass index was found. In contrast to this finding, the study of Kankova *et al*. [25] revealed no association with the IVS1+90A>G polymorphism and body mass index. This discrepancy may be due to the fact that obesity was not a selection criteriion in the present study. The relation between inflammation and pathological body weight has recently come to the researcher's attention [15, 26-28]. It was shown that inflammatory markers, including TNF-α, CRP, and Il-6 are significantly associated with body mass index [15, 27]. Since it could be shown, that the genetic background of TNF-β influences the expression of inflammatory factors such as TNF-α [29], an impact of TNF-β polymorphisms on body weight via the expression of inflammatory markers may be presumed.

Inflammatory marker: TNF-α

For the first time, significant effects of all TNF-β polymorphisms investigated (c.7G>A, IVS1+90A>G, C13R, T60N) on TNF-α gene expression could be demonstrated. These effects were shown to be more pronounced in pa-

tients suffering from coronary stenosis. These differences in TNF-α regulation due to changes in coronary status, provide evidence of a sensitive regulation of TNF-α gene expression under different metabolic influences. Moreover, this result suggests, that genomic variants of TNF-β, located in the vicinity of the TNF-α gene, could influence its expression. This corresponds to investigations of Stüber *et al*. (1996) [29], who showed that a polymorphic variant of the TNF-β locus is responsible for TNF-α expression and poor outcome in sepsis. However, it remains unclear whether these polymorphisms influence the TNF-α gene expression directly or by influencing TNF-β expression. In previous studies, it was shown that the IVS1+90A>G polymorphism of the TNF-β gene could also influence TNF-β expression [9].

However, at the translational level, no association between the genetic background and the plasma level of TNF-α could be determined in the whole patient group. In accordance with our results, no effect of the TNF-β polymorphism IVS1+90A>G, on TNF-α expression could be shown in Korean patients with lupus nephritis [9]. In contrast, in a previous study investigating the influence of different haplotypes on TNF-α expression, Poicot *et al*. (1993) [30] demonstrated that the IVS1+90A>G polymorphism has a significant effect on TNF-α expression in patients with diabetes mellitus. However, these controversial data may be caused by different experimental and clinical settings, since only 15.5% of our patient group suffered from diabetes mellitus.

Our data concerning the varying importance of genetic background on the TNF-α gene and protein expression respectively, may reflect the sensitive regulation of TNF-α expression at both the transcriptional and translational level.

Effect of the coagulation/fibrinolysis balance: acute phase protein fibrinogen

For the first time, we were able to describe significant relationships between TNF-β polymorphisms and one factor of the coagulation/fibrinolysis balance, fibrinogen. It was shown previously, that its expression is triggered either by genetic variants within the fibrinogen gene itself [31], or within other genes, *e.g*. the plasminogen-activator inhibitor gene [32] and eNOS [33]. However, we showed that genetic variants of TNF-β are significantly associated with patients with marked coronary stenosis. In the patient group without coronary stenosis, no similar significant associations were observed. This might imply that the genetic background of TNF-β might only influence the expression of fibrinogen in patients with coronary symptoms. Since fibrinogen acts as an acute phase protein, its potential role as an inflammatory modulator of cardiovascular disease has already been described [34].

Multivariate evaluation of the importance of the genetic background to the incidence of coronary stenosis

Previously unexplored, complex interactions of the genetic background and the development of significant coronary stenosis were investigated, taking into account coronary risk factors shown to be associated with TNF-β polymorphisms. However, no association between genetic background and the incidence of angiographically-determined stenosis could be shown in a multivariate model, when adjusting for possible confounding effects.

On a genetic level, a variety of clinical studies were performed in order to evaluate the effect of TNF-β polymorphisms on the development of atherosclerosis [10-13, 35, 36]. However, the data obtained are inconsistent. This may be due to the fact that the number and/or ethnicity of the coronary patients selected, and the endpoints investigated differed between the studies. Another reason may be the lack of more complex evaluation, taking into account further coronary risk factors.

Limitations of the study

A limitation of this present study may have been that the control group does not represent a group of healthy individuals with respect to all confounding factors for coronary atherosclerosis. However, the inclusion criteria for the present study were assigned in order to evaluate the importance of the genetic background of TNF-β to the incidence of significant coronary stenosis. In the control group, coronary stenosis could be excluded by angiography. Since quantitative angiography is the method of choice for proving significant coronary stenosis, only persons undergoing this procedure for a clinical indication were included in the present study, because for ethical reasons, angiography could not be used to screen healthy individuals.

CONCLUSION

In the present clinical study, the genetic background of TNF-β could not be shown to be a significant predictor for the development of angiographically-proven coronary stenosis in bivariate analyses. Investigations on the influence of the genetic background of TNF-β on classical coronary risk factors revealed significant associations with the incidence of hypertension, as well as body mass index, the mRNA expression of TNF-α and fibrinogen. However, in a stepwise binary logistic regression analysis, we could not prove a significant association between the genetic background of TNF-β and the incidence of coronary stenosis. These results imply that the genomic variants of TNF-β indeed do influence coronary risk markers, but do not predict the development of coronary stenosis.

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