

Research Article

Polymorphism of CD14 Gene Is Associated with Adverse Outcome among Patients Suffering from Cardiovascular Disease

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Received 12 April 2021; Revised 28 May 2021; Accepted 8 June 2021; Published 8 July 2021

Academic Editor: Dominik Skiba

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Background. The biological link between severe periodontitis and cardiovascular disease is well established. Both complex inflammatory diseases are influenced by genetic background. Therefore, the impact of genetic variations of receptors of the innate immune system—(Toll-like receptors (TLRs)) TLR2, TLR4, cluster of differentiation 14 (CD14), and the transcription factor nuclear factor- κ B (NF- κ B)—was investigated. **Materials and Methods.** In this study (ClinicalTrials.gov identifier: NCT01045070), 1002 cardiovascular (CV) patients were included. In a 3-year follow-up period, new vascular events were assessed. SNPs in CD14 (rs2569190), NF- κ B (rs28362491), TLR2 (rs5743708), and TLR4 (rs4986790) were genotyped. The impact of these genetic variants on severe periodontitis as well as on CV outcome was assessed. **Results.** All investigated genetic variants were not associated with preexisting CV events or severe periodontitis in CV patients. In Kaplan-Meier survival analyses, the CT genotype of CD14 single-nucleotide polymorphism (SNP) rs2569190 was shown to be an independent predictor for combined CV endpoint (log rank: $p = 0.035$; cox regression; hazard ratio: 1.572; $p = 0.044$) as well as cardiovascular death (log rank: $p = 0.019$; cox regression; hazard ratio: 1.585; $p = 0.040$) after three years of follow-up. **Conclusions.** SNPs in CD14, NF- κ B, TLR2, and TLR4 are no risk modulators for preexisting CV events or severe periodontitis in CV patients. The CT genotype of CD14 SNP rs2569190 provides prognostic value for further CV events within 3 years of follow-up.

1. Introduction

The immune system consists of a multitude of innate immune receptors to recognize pathogens. Among these receptors, pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs) and cluster of differentiation 14 (CD14), have long been established to play a key role in the host defense system [1, 2]. In particular, lipopolysaccharides (LPS) are recognized by TLRs, which are localised in the membranes of immune cells and are the first receptors to be activated in the interaction between pathogen and host interaction [3]. TLR2 is capable of recognizing lipoproteins and peptidoglycans from

both Gram-positive and Gram-negative bacteria, as well as LPS and lipoteichoic acid from Gram-positive bacteria [4]. On the other hand, TLR4, in the interaction with the cluster of differentiation (CD14), is able to recognize LPS from Gram-negative bacteria [4]. In the course of the thereby initiated signal transduction cascade, many mediators are produced which contribute to the control of infection. Among these mediators, transcription factor nuclear factor- κ B (NF- κ B) is an important control element that is able to promote the immune response by releasing further proinflammatory cytokines [1].

It was shown that in addition to immune cells, cells such as fibroblasts including oral fibroblasts, epithelial cells, and

endothelial cells can also contribute to the activation of innate immunity via PRRs [3, 5]. In this context, PRRs, including TLRs and CD14, as well as downstream signal transduction cascades have been implicated in pathogen recognition during periodontal infection [6]. Furthermore, activation of PRRs triggers the inflammatory response in various physiological systems, including the cardiovascular (CV) system. PRRs including downstream signal transduction cascades have been discussed as key contributors to the progression of various CV diseases including, e.g., atherosclerosis and heart failure [3].

A biologically plausible connection between periodontal and CV diseases has been assumed for many years [7]. PRRs and the downstream signal transduction cascade could be provided a biologically appropriate link between these two diseases. Among other things, a common genetic predisposition is discussed as an interface between both diseases [8]. The focus of this study will be on genetic variants of PRR candidate genes CD14, TLR2, TLR4, and NF- κ B as a representative of downstream regulation. The main attention will be given to functionally important genetic variants for which an association with both diseases could be suspected. In the CD14 gene, a promoter polymorphism (rs2569190; c.-260C/T) was shown to be associated with increased transcriptional activity resulting in higher levels of soluble CD14 [9]. In the TLR4 gene, the SNP rs4986790 (Asp299Gly) was described to be associated with functional alterations that predispose individuals to respond less sensitively to LPS and to have an increased susceptibility to pathogenic bacterial infections [10–12]. SNP rs5743708 in the TLR2 gene (Arg753Gly) has been implicated in reducing the capability of TLR2 to target bacterial cell wall components [13]. In the NK- κ B gene, the rs28362491 SNP represents a 4 bp ATTG insertion/deletion variation at position –94 bp of the promoter. This polymorphic site has been reported to have a major impact on gene expression, with deletion of ATTG resulting in reduced promoter activity [14]. Due to their functional consequences, all described SNPs are highly involved in the immune response and could thus represent a possible link between periodontal and cardiovascular diseases. And indeed, in a variety of case-control studies as well as in meta-analyses, their impact on periodontitis as well as CV diseases was assessed (CD14 [15–17], NF- κ B [14, 18, 19], TLR2 [20, 21], and TLR4 [4, 22, 23]).

However, the significance of these SNPs in linking periodontitis and CV diseases has not been at the focus of investigations to date. Based on the data available, we assessed in our study the impact of SNPs in TLR2 (rs5743708), TLR4 (rs4986790), CD14 (rs2569190), and NF- κ B (rs28362491), on severe periodontitis as well as on the incidence of new CV events within a three year follow-up in a cohort of CV patients.

2. Materials and Methods

2.1. Cohort of Cardiovascular Patients. The present investigation is a substudy of the longitudinal cohort study “Periodontitis and Coronary Heart Disease (CHD)” ClinicalTrials.gov identifier: NCT01045070. A total of 1002 coronary patients

of the Department of Internal Medicine III were included in the study consecutively from October 2009 to February 2011. The inclusion and exclusion criteria for coronary patients, including periodontal and cardiovascular examinations, and determination of biochemical parameters have already been described in detail [24]. Therefore, it will only be briefly addressed here. All patients were at least 18 years old and of Caucasian descent. They have been suffering from $\geq 50\%$ stenosis of a main coronary artery (angiographically proven). All patients had at least 4 of their own teeth.

The exclusion criteria applied were inability to give written informed consent, pregnancy, subgingival scaling, root planning during the last 6 months, antibiotic therapy during the last 3 months, current alcohol or drug abuse, and medication of drugs that potentially cause gingival hyperplasia (e.g., hydantoin, nifedipine, and cyclosporin A).

All patients underwent a standardized cardiovascular and dental examination and were evaluated for biochemical peculiarities. Regarding cardiovascular parameters, the left ventricular ejection fraction was assessed and serum parameters including haemoglobin, interleukin-6, C-reactive protein, low-density lipoprotein, high-density lipoprotein, triglycerides, and creatinine were measured. The dental examination comprised of determining the plaque index and bleeding on probing after 30 seconds at four sites around each tooth. Both maximal clinical probing depth (PD = distance between gingival margin and the bottom of the pocket) and maximum clinical attachment loss (CAL = distance between the cemento-enamel junction and the bottom of the pocket) were taken at six sites around each tooth applying a pressure-sensitive dental probe UNC 15 (0.2N) (Aesculap, Tuttlingen, Germany). For calculating the mean values, the maximum values for each tooth were considered.

According to the periodontitis classification of Tonetti and Claffey, the coronary patients were evaluated regarding their severity of periodontitis [25]. A severe periodontitis case was diagnosed if $\geq 30\%$ of the teeth that were present showed a proximal attachment loss of ≥ 5 mm. A periodontitis case was considered as the presence of proximal attachment loss of ≥ 3 mm in ≥ 2 nonadjacent teeth. All remaining coronary patients exhibit no periodontitis.

After 3 years, a follow-up was performed from November 2013 until January 2015. Data regarding the predefined combined endpoint (myocardial infarction, stroke/transient ischemic attack (TIA), cardiac death, and death caused by stroke) were collected. Using a standardized questionnaire, the patients (or relatives or patient’s physicians) were interviewed by mail or phone. If follow-up information could not be obtained from these persons, civil registration offices were contacted and information about current address or date of death was requested.

2.2. Genetic Investigations. The genomic DNA was isolated from leucocytes of venous blood using QIAamp® blood extraction kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer’s manual.

For genotyping, specific restriction fragment length polymorphism (RFLP) analyses were established for all SNPs (Table 1).

TABLE 1: Genotyping methods applied for SNPs in CD14, NF- κ B, TLR2, and TLR4.

Gene	SNP	Primer 5' \rightarrow 3'	Reference sequence	Restriction enzyme
CD14	rs2569190	Forward: gtg cca aca gat gag gtt cac Reverse: cct cct ctg tga acc ctg atc ac	AF097335	HaeIII
NF- κ B	rs28362491	Forward: tgc tgc ctg cgt tcc ccg acc atc g Reverse: ccc gca ggg gcc gcg gcg tcc ag	AF213884S1	Taq α I
TLR2	rs5743708	Forward: cat tcc cca gcg ctt ctg caa gct cc Reverse: gga acc tag gac ttt atc gca gct	NM 003264	MspI
TLR4	rs4986790	Forward: gat tag cat act tag acta ct acc tcc atg Reverse: gat caa ctt ctg aaa aag cat tcc cac	NM 138554	NcoI

2.3. Statistical Analyses. Statistical analyses were carried out using the SPSS software 25.0 (SPSS Inc., Chicago, Illinois). Values of $p < 0.05$ were considered to be significant. Metric data were tested for normal distribution using the Kolmogorov-Smirnov test. Categorical variables were documented as percentage and plotted in contingency tables and evaluated using the chi-square test and Yates continuity correction. If the expected cell frequency was < 5 , Fisher's exact test was applied.

For survival, evaluation Kaplan-Meier analyses with log-rank tests were applied. Confounding effects on survival (adjusted hazard ratios) were investigated using Cox regression.

3. Results

In the present longitudinal cohort study, 1002 patients were prospectively involved. Clinical characteristics of the patients are displayed in Table 2. 953 individuals could be evaluated in the 3-year follow-up (follow-up time: 152.9 ± 49.5 weeks). This corresponded to a dropout rate of 4.9% after three years of follow-up. The total incidence of the combined endpoint was 16.4% (stroke/TIA: $n = 23$, myocardial infarction: $n = 33$, cardiovascular death: $n = 93$, and death from stroke: $n = 7$).

3.1. Possible Association of the Genetic Background and the Prevalence of Periodontitis. Possible associations between SNPs in CD14 (rs2569190), NF- κ B (rs28362491), TLR2 (rs5743708), and TLR4 (rs4986790) and the occurrence of severe periodontitis were investigated in the cohort of CV patients. Codominant, dominant, recessive genetic models for all SNPs were tested. In addition, possible associations between allele distribution and severe periodontitis were also included in the assessment. Here, we could not demonstrate any significant associations between genetic markers and the severity of periodontitis (Table 3).

3.2. Genetic Characteristics and the Cardiovascular Prognosis in the 3-Year Follow-Up. The impact of SNPs in CD14 (rs2569190), NF- κ B (rs28362491), TLR2 (rs5743708), and TLR4 (rs4986790) on the incidence of combined endpoint defined as CV death, death from stroke, MI, and stroke/TIA was evaluated. In bivariate analyses without taking survival time into account, neither the genotype nor the allele distribution was shown to be associated with the adverse CV outcome (Table 4). However, when survival time as well as further genetic models were considered, a different conclusion was

obtained. After Kaplan-Meier analysis and log-rank test, the CT genotype of rs2569190 in the CD14 gene was as a prognostic marker for the combined endpoint ($p = 0.035$) (Figure 1(a)). Carriers of the CT genotype had a considerably poorer prognosis than CC + TT genotype carriers (incidence of combined endpoint: CT: 18.9% vs. CC + TT 13.5%, $p = 0.036$). Taking established cardiovascular risk factors, including increasing age, male gender, body mass index, diabetes mellitus, hypertension, and hypercholesterolemia, as well as severe periodontitis into consideration, the CT genotype was proven as an independent risk modulator of the combined endpoint (HR = 1.572, $p = 0.044$) (Table 5(a)). A detailed examination of the CV endpoint showed that the CT genotype had a particular impact on CV death (Kaplan-Meier analysis including log-rank test: $p = 0.019$, Figure 1(b)). Considering further CV risk markers in Cox regression analysis, the hazard ratio for the CT genotype measured 1.585 ($p = 0.040$) (Table 5(b)).

In this study, increasing age (HR = 1.063, $p < 0.001$) and diabetes mellitus (HR = 2.366, $p < 0.001$) were shown to be additional significant predictors for adverse cardiovascular events (combined endpoint and cardiovascular death (Table 5).

All other genetic combinations of the SNPs investigated in the study did not show prognostic value for combined endpoint.

4. Discussion

The focus of this longitudinal cohort study was to examine the influence of SNPs in PRR genes and a gene of the downstream signal transduction cascade on severe periodontitis and to assess their prognostic significance for future CV events in patients with angiographically proven significant stenosis.

4.1. SNPs and Severe Periodontitis. In this cohort of CV patients, no significant associations between SNPs in CD14 (rs2569190), NF- κ B (rs28362491), TLR2 (rs5743708), and TLR4 (rs4986790) and the prevalence of severe periodontitis was proven considering codominant, dominant, and recessive genetic models as well as allele distribution. The studies conducted so far on the possible association of genetic variants in PRRs as well as NF- κ B and periodontitis are very heterogeneous.

Considering SNP rs2569190 in the CD14 gene, an association of this SNP with periodontitis could be shown in highly selected case-control studies [26–28]. But in contrast, other

TABLE 2: Demographical and anamnestic parameters, previous history of diseases, biochemical parameters, and dental parameters of the CV patients.

	CV patients <i>n</i> = 1002
<i>Demographical and anamnestic parameters</i>	
Age (years), median (25 th /75 th IQR)	68.9 (59.8/75)
Male gender (%)	74.0
Current smoking (%)	11.9
Body mass index (kg/m ²), median (25 th /75 th IQR)	28.1 (25.3/30.9)
<i>History of</i>	
Diabetes mellitus (%)	35.7
Hypertension (%)	87.7
MI (%)	38.8
Stroke/TIA (%)	12.7
Peripheral artery disease (%)	9.6
Dyslipoproteinemia (%)	58.8
Severe periodontitis* (%)	47.4
CV disease known by anamnesis (%)	74.3
CV disease family history (%)	39.8
<i>Biochemical parameters</i>	
C-Reactive protein (mg/l), median (25 th /75 th IQR)	8.7 (3.4/31.6)
Leukocytes (Gpt/l), median (25 th /75 th IQR)	7.8 (6.4/9.7)
Interleukin 6 (pg/ml), median (25 th /75 th IQR)	7.4 (3.7/15.8)
Creatinine (mmol/l), median (25 th /75 th IQR)	87 (73/107)
Total cholesterol (mmol/l), median (25 th /75 th IQR)	4.3 (3.7/5.3)
HDL cholesterol (mmol/l), median (25 th /75 th IQR)	1.0 (0.8/1.2)
LDL cholesterol (mmol/l), median (25 th /75 th IQR)	2.6 (2.0/3.3)
Triglycerides (mmol/l), median (25 th /75 th IQR)	1.4 (1.0/1.9)
<i>Periodontal parameters</i>	
Plaque index, median (25 th /75 th IQR)	0.8 (0.5/1.4)
Clinical attachment loss (mm), median (25 th /75 th IQR)	4 (3.2/5.1)
Bleeding on probing (%), median (25 th /75 th IQR)	5.6 (1.9/12.5)
Missing teeth (except 8 th) (<i>n</i>), median (25 th /75 th IQR)	11.0 (5.0/19.0)

*Approximal attachment loss of ≥ 5 mm in $\geq 30\%$ of the teeth present. Continuous variables were presented as median (25th/75th interquartiles (IQR)); TIA: transient ischemic attack; MI: myocardial infarction.

case-control studies did not confirm this association [29, 30]. However, in meta-analyses, no association of rs2569190 in the CD14 gene with periodontitis could be confirmed [15, 31]. Furthermore, applying stratified analysis by ethnicity

and the severity of periodontitis, no significant associations were assessed between CD14 SNP rs2569190 and periodontitis [15, 31]. Investigating the impact of these SNPs on the occurrence of aggressive or chronic periodontitis, a meta-analysis was performed [17]. And again, no significant genetic impact could be evaluated.

Although the importance of NF- κ B in the immune response is undisputed, only few case-control studies have been conducted to investigate possible association of the SNP rs28362491 with periodontitis. The study situation is very inconsistent. Whereas, for example, Toker et al. [19] could not show any association of the SNP with periodontitis and Schulz et al. [18] demonstrated that del/del genotype carriers suffered more frequently from aggressive periodontitis.

Recently, case-control studies on the significance of the SNP rs5743708 in the TLR2 gene in periodontitis were summarized in a meta-analysis [20]. In this analysis, no association of this SNP and periodontitis, including disease-specific subgroup analysis (aggressive, chronic periodontitis), was confirmed. However, ethnic stratification revealed an indication that the SNP is associated with periodontitis in Asians [32].

Regarding rs4986790 in the TLR4 gene, the study situation is also heterogeneous. However, it should be emphasized that meta-analyses have not confirmed the association of this SNP in the TLR4 gene with periodontitis, even after stratification for ethnicity and disease severity [31, 33]. In contrast, in a meta-analysis by Chrzęszczyk et al. [34], this SNP was shown to be in association with chronic periodontitis. Furthermore, specific studies have shown that this genetic variant is associated with the occurrence of periodontitis in male [4] and with periodontitis in the presence of *P. gingivalis* infection [35].

All the meta-analyses or case-control studies presented so far have in common that no study evaluated possible associations between these genetic variants and periodontal diseases in a cohort of CV patients. Since periodontitis as a chronic inflammatory disease is characterized by a complex interaction of different mechanisms, factors interrelating with the immune system (e.g., cardiovascular diseases) must be considered as confounding modulators [7]. For this reason, patients of the present study might be particularly predisposed for periodontal inflammation due to their existing CV disease. Consequently, the current genetic results cannot be compared with the studies presented.

4.2. SNPs and the Cardiovascular Prognosis in the 3-Year Follow-Up. Because of their important role in CV disease, we hypothesized that SNPs in PRRs (CD14, TLR2, and TLR4 gene) and the NF- κ B gene might also have a prognostic value for further CV events.

SNP rs2569190 located in the promoter of the CD14 gene has been shown to modulate inflammatory stimulation by regulating CD14 gene expression and the concentration of soluble CD14 (sCD14) in plasma [36]. Because of these functional effects in the context of the immune response, the SNP has been attributed an important role in inflammatory diseases.

In a genome-wide association study, the CD14 locus was shown to be associated with CV disease [37]. In particular, the SNP rs2569190 was related to the occurrence of CV

TABLE 3: Association of genotype and allele distribution of SNPs in CD14, NF- κ B, TLR2, and TLR4 and periodontal disease severity.

	All patients	No or mild periodontitis	Severe periodontitis	<i>p</i> value
CD14: rs2569190				
	<i>n</i> = 928	<i>n</i> = 487	<i>n</i> = 441	
CC (%)	30.4	29.8	31.1	
CT (%)	46.6	44.8	48.5	0.182
TT (%)	23.0	25.4	20.4	
	<i>n</i> = 1856	<i>n</i> = 974	<i>n</i> = 882	
C (%)	53.7	52.2	55.3	0.186
T (%)	46.3	47.8	44.7	
NF- κ B: rs28362491				
	<i>n</i> = 927	<i>n</i> = 487	<i>n</i> = 440	
II (%)	33.0	31.8	34.3	
ID (%)	51.4	51.5	51.1	0.581
DD (%)	15.6	16.6	14.6	
	<i>n</i> = 1854	<i>n</i> = 974	<i>n</i> = 880	
I (%)	58.7	57.6	59.9	0.341
D (%)	41.3	42.4	40.1	
TLR2: rs5743708				
	<i>n</i> = 939	<i>n</i> = 493	<i>n</i> = 446	
AA (%)	94.5	93.7	95.3	
AG (%)	5.4	6.1	4.7	0.389*
GG (%)	0.1	0.2	0	
	<i>n</i> = 1878	<i>n</i> = 986	<i>n</i> = 892	
A (%)	97.2	96.8	97.6	0.305
G (%)	2.8	3.2	2.4	
TLR4: rs4986790				
	<i>n</i> = 942	<i>n</i> = 494	<i>n</i> = 448	
AA (%)	91.4	92.7	90.0	
AG (%)	8.4	7.1	9.8	0.237*
GG (%)	0.2	0.2	0.2	
	<i>n</i> = 1884	<i>n</i> = 988	<i>n</i> = 896	
A (%)	95.6	96.3	94.9	0.175
G (%)	4.4	3.7	5.1	

*Fisher's exact test.

diseases in case-control studies as well as meta-analysis [16, 38, 39]. In meta-analyses, the TT genotype and T allele of rs2569190 have been associated with ischemic heart diseases [38] and the susceptibility and development of cardiovascular disease [16], particularly in the East Asian population but not European population. In accordance with these studies, we also did not demonstrate a genetic influence of CD14 SNP rs2569190 on the incidence of cardiovascular preexisting conditions (stroke, myocardial infarction, and peripheral arterial disease) in the present European cohort of CV patients.

However, the focus of the present study should be on the impact of SNPs on CV prognosis. In the presented study, the CT genotype of CD14 SNP rs2569190 was demonstrated to be associated with an adverse CV outcome in relation to

the combined CV endpoint (log-rank: $p = 0.035$; cox regression; hazard ratio: 1.572; $p = 0.044$) as well as cardiovascular death (log-rank: $p = 0.019$; cox regression; hazard ratio: 1.585; $p = 0.040$) after three years of follow-up.

So far, only few data are known about a possible prognostic significance of the SNP rs2569190 with regard to the cardiovascular outcome. In a study by Porsch-Ozcürümez et al., T allele carriers were shown to have a 3.6-fold higher risk for nonlethal CV events (log-rank test = 0.029) in a 4-year follow-up period [40]. In contrast, in the present study, the TT genotype or T allele was not proven to be independent predictors for CV prognosis. Although the inclusion criteria of this study were comparable to those of the present study ($\geq 50\%$ stenosis of a main coronary artery; CV patients of a similar geographical region: Central Germany, Saxony-Anhalt),

TABLE 4: Association of genotype and allele distribution of SNPs in CD14, NF- κ B, TLR2 and TLR4 and occurrence of combined endpoint (stroke/TIA, myocardial infarction, Cardiovascular death, Death from stroke).

	All patients	No or mild periodontitis	Severe periodontitis	<i>p</i> value
CD14: rs2569190				
	<i>n</i> = 881	<i>n</i> = 740	<i>n</i> = 141	
CC (%)	30.6	31.9	24.1	
CT (%)	46.2	44.6	54.6	0.074
TT (%)	23.2	23.5	21.3	
	<i>n</i> = 1762	<i>n</i> = 1480	<i>n</i> = 282	
C (%)	53.7	54.2	51.4	0.429
T (%)	46.3	45.8	48.6	
NF- κ B: rs28362491				
	<i>n</i> = 880	<i>n</i> = 738	<i>n</i> = 142	
II (%)	32.7	33.5	28.9	
ID (%)	51.4	50.9	53.5	0.537
DD (%)	15.9	15.6	17.6	
	<i>n</i> = 1760	<i>n</i> = 1476	<i>n</i> = 284	
I (%)	58.4	58.9	55.6	0.332
D (%)	41.6	41.1	44.4	
TLR2: rs5743708				
	<i>n</i> = 892	<i>n</i> = 749	<i>n</i> = 143	
AA (%)	94.5	94.4	95.1	
AG (%)	5.4	5.5	4.9	1.000*
GG (%)	0.1	0.1	0	
	<i>n</i> = 1784	<i>n</i> = 1498	<i>n</i> = 286	
A (%)	97.2	97.1	97.6	0.840
G (%)	2.8	2.9	2.4	
TLR4: rs4986790				
	<i>n</i> = 895	<i>n</i> = 751	<i>n</i> = 144	
AA (%)	91.2	91.5	89.6	
AG (%)	8.6	8.3	10.4	0.590*
GG (%)	0.2	0.2	0.0	
	<i>n</i> = 1790	<i>n</i> = 1502	<i>n</i> = 288	
A (%)	95.5	95.6	94.8	0.650
G (%)	4.5	4.4	5.2	

*Fisher's exact test.

the CV endpoints differed. While Porsch-Ozcürümez et al. defined nonlethal CV events as endpoints in the present study, cardiovascular death and death from stroke were also included as endpoints. Furthermore, the enrolled patients in the present study were $n = 1002$, which is significantly higher than the number of patients in the study of Porsch-Ozcürümez et al. ($n = 69$).

SNP rs28362491 in the ubiquitous transcription factor NF- κ B was reported to be involved in the expression of immune-modulating genes due to its impact on NF- κ B gene transcription [41]. Therefore, this SNP has been implicated in inflammatory diseases including cardiovascular diseases [14]. In the meta-analysis by Wang et al., the deletion allele

was shown to be associated with CV diseases even after stratification by ethnicity and gender [14]. However, a corresponding association could not be confirmed in the present study in the cohort of CV patients. The SNPs including all genetic models were independent of stroke, myocardial infarction, and peripheral arterial disease. However, when comparing the studies, it is important to note that the present study only included patients with a history of cardiovascular disease (inclusion criterion: $\geq 50\%$ stenosis of a main coronary artery). Furthermore, it was shown that this SNP has no predictive power for an adverse CV outcome in the present study. As this issue has not been addressed in previous studies, no comparisons with the existing evidence can be provided here.

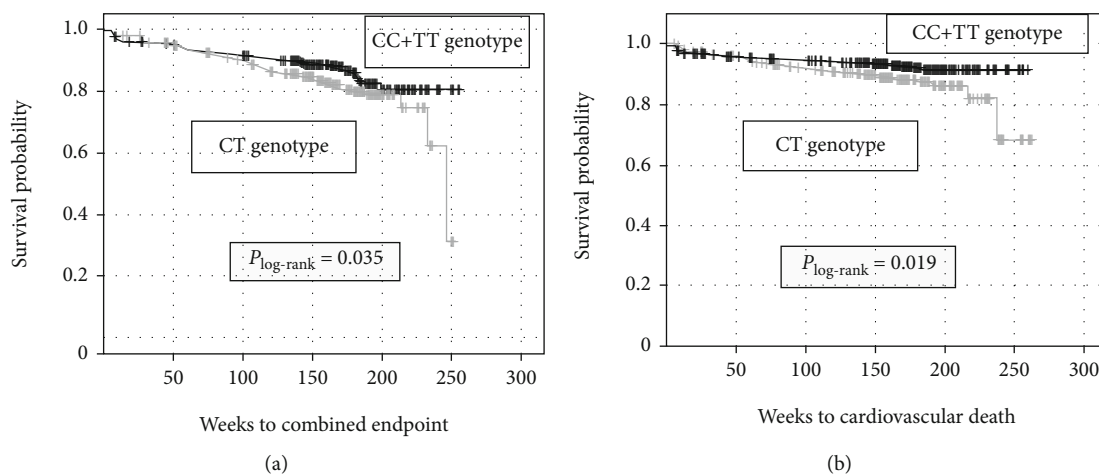


FIGURE 1: Kaplan-Meier plot for the incidence of the (a) combined endpoint (stroke/TIA, myocardial infarction, cardiovascular death, and death from stroke) and (b) cardiovascular death within a 3-year follow-up period according genotype distribution of CD14 SNP rs2569190. Statistical comparison was made by the log-rank test.

TABLE 5: Cox regression analysis evaluating the hazard ratio (HR) of the CT genotype of CD14 SNP rs2569190 for the incidence of the (a) combined endpoint (stroke/TIA, myocardial infarction, cardiovascular death, and death from stroke) and (b) cardiovascular death within a 3-year follow-up period (adjusted for age, gender, smoking, body mass index, hypertension, hyperlipoproteinemia, diabetes, and severe periodontitis).

Confounding variables	Hazard ratio	95% lower	CI upper	P values
(a) Endpoint: stroke/TIA, myocardial infarction, cardiovascular death, and death from stroke				
<i>CT genotype rs2569190</i>	1.572	1.012	2.439	0.044
Age	1.063	1.035	1.092	<0.001
Diabetes	2.366	1.501	3.729	<0.001
Male gender	1.302	0.784	2.162	0.309
Current smoking	1.572	0.469	5.262	0.463
Hypertension	1.413	0.706	2.828	0.329
Hyperlipoproteinemia	1.037	0.657	1.636	0.876
Severe periodontitis	1.121	0.721	1.742	0.613
(b) Endpoint: cardiovascular death				
<i>CT genotype rs2569190</i>	1.585	1.020	2.500	0.040
Age	1.063	1.035	1.092	<0.001
Diabetes	2.366	1.501	3.729	<0.001
Male gender	1.280	0.772	2.125	0.339
Current smoking	1.563	0.467	5.233	0.469
Hypertension	1.411	0.705	2.825	0.331
Hyperlipoproteinemia	1.052	0.6668	1.658	0.826
Severe periodontitis	1.116	0.717	1.736	0.626

TLR2 is involved immune response due to its recognition of many pathogen-associated molecular patterns. The SNP rs5743708 has been described to have immediate functional significance regarding altered signaling [42] and might be implicated in inflammatory diseases [21]. A few studies were conducted in order to evaluate the possible association between this SNP and CV diseases; however, results are controversial. Whereas Golovkin et al. did not reveal a genetic influence on infective endocarditis

[21], Guven et al. reported on an association with CV susceptibility in Turkish patients [43]. However, in the present study, an influence on cardiovascular burden (stroke, myocardial infarction, and peripheral arterial disease) could not be confirmed in the cohort of CV patients of Central Germany. Since the inclusion criteria of the present study were comparable to those of Guven et al., ethnic characteristics could possibly play a role in the discrepancy of the results.

With regard to a possible prognostic CV power of this SNP, only limited evidence is available so far. Hamann et al. were able to prove an influence of this SNP on restenosis and recommended the inclusion of the SNP in the individual CV profile for risk stratifying as well as for preventive and therapeutic measures [44]. In the present study, however, the influence of this genetic factor on the CV outcome could not be confirmed. The reason for these differences could be found in the follow-up period, which was only 6 months in the study by Hamann et al. and 3 years in the present study. On the other hand, different CV endpoints were referred to (restenosis; present study: CV death, death from stroke, MI, and stroke/TIA).

The TLR4 SNP rs4986790 has been implicated in attenuation of receptor signaling and diminishing the inflammatory response to Gram-negative pathogens. Therefore, its impact on inflammatory diseases including CV diseases was assessed in different studies [23, 45, 46]. But here, as well, the study evidence is very heterogeneous. In an early study by Kiechl et al., investigating 810 individuals, an association of this SNP with CV disease was shown [45]. By contrast, Morange et al. demonstrated that this SNP is not a significant predictor of the CV risk in healthy individuals [46]. These data are in line with the results of two meta-analyses based on 12 and 20 case-control studies, in which nonassociation of this SNP with CV diseases was proven [23, 47]. And also, in the present study, no correlation between this genetic variant and the occurrence of preexisting CV diseases could be shown. Furthermore, SNP rs4986790 in the TLR4 gene has not been associated with cardiovascular prognosis in this study cohort. This result is in line with early studies which demonstrated that in patients with documented CV disease, this SNP was no predictor of future CV events [48] and was not associated with an increased risk of target vessel revascularization or angiographic restenosis after percutaneous coronary intervention [49].

4.3. Limitations of the Study. The presented study was performed as a longitudinal cohort study. It was conducted to establish assumptions of the possible prognostic value of genetic variants of CD14 (rs2569190), NF- κ B (rs28362491), TLR2 (rs5743708), and TLR4 (rs4986790) on adverse CV events during a 3-year follow-up period. Considering the study design, the verification of these assumptions is not realizable. The results of the current study are representative for coronary patients in Central Germany and cannot be generally extrapolated to the overall population or other patient cohorts. Furthermore, it is advisable to verify the study results in an additional study.

In the 3-year follow-up, the combined CV endpoint (stroke/TIA, myocardial infarction, CV death, and death from stroke) was investigated. These data were obtained from existing medical records or from civil record offices or by interviewing patients or their relatives (standardized questionnaire, telephone interview). Thus, it is possible that false information from patients or their relatives cannot be excluded due to possible personal incorrect interpretations of the state of health.

5. Conclusions

- (1) At baseline, SNPs in TLR2 (rs5743708), TLR4 (rs4986790), CD14 (rs2569190), and NF- κ B (rs28362491) were not associated with the occurrence of severe periodontitis in CV patients
- (2) In the 3-year follow-up, the CT genotype of CD14 SNP rs2569190 was proven to be an independent prognostic marker for further cardiovascular events, especially cardiovascular death, considering classical cardiovascular risk factors. All other genetic variants included in the study (NF- κ B (rs28362491), TLR2 (rs5743708), and TLR4 (rs4986790)) had no predictive cardiovascular value

Confirmation of the prognostic value of the CD14 SNP rs2569190 for CV disease should be obtained in further studies. Potentially, this SNP could complement the individual CV risk profile. Integration of stable genetic markers in the CV risk profile assists in the identification of higher risk patients and supports the improvement of individualized therapy.

Data Availability

All data are available on request from the corresponding author (Susanne Schulz, Martin Luther University Halle-Wittenberg, Medical Faculty, Department of Operative Dentistry and Periodontology, Magdeburger Str. 16, 06112 Halle (Saale), Germany).

Ethical Approval

All investigations were carried out in accordance with the ethical guidelines of the "Declaration of Helsinki" and its amendment in "Tokyo and Venice." The study was approved by the ethics committee of the Martin Luther University of Halle-Wittenberg on 16.11.2016 (approval number: 2016-86).

Consent

Informed written consent was obtained from each patient.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

S. Schulz and S. Reichert equally contributed to this work, designed the study, conducted statistical data analysis, confirmed the accuracy of the data, and drafted the manuscript. Dental examinations were performed under the instructions of S. Reichert and H-G. Schaller. Clinical investigations were carried out under the supervision of B. Hofmann and A. Schlitt. M. Zielske and S. Schneider conducted the genetic analyses under the instruction of S. Schulz. All authors have read and agreed to the published version of the manuscript. All authors have given their consent for publication.

Acknowledgments

We would like to thank all the patients with coronary heart disease for their cooperation. The study was supported by a grant from the Deutsche Herzstiftung, Frankfurt am Main, Germany (F/34/08), and by an unrestricted grant from HAIN-Diagnostica®, Nehren, Germany. We acknowledge the financial support of the Open Access Publication Fund of the Martin Luther University Halle-Wittenberg.

References

- [1] K. Lucas and M. Maers, "Role of the Toll like receptor (TLR) radical cycle in chronic inflammation: possible treatments targeting the TLR4 pathway," *Molecular Neurobiology*, vol. 48, no. 1, pp. 190–204, 2013.
- [2] C. R. Pinheiro, A. L. Coelho, C. E. de Oliveira et al., "Recognition of *Candida albicans* by gingival fibroblasts: the role of TLR2, TLR4/CD14, and MyD88," *Cytokine*, vol. 106, pp. 67–75, 2018.
- [3] R. I. Jaén, A. Val-Blasco, P. Prieto et al., "Innate immune receptors, key actors in cardiovascular diseases," *JACC: Basic to Translational Science*, vol. 5, no. 7, pp. 735–749, 2020.
- [4] J. M. V. Zacarias, J. B. de Alencar, P. Y. Tsuneto et al., "The influence of TLR4, CD14, OPG, and RANKL polymorphisms in periodontitis: a case-control study," *Mediators of Inflammation*, vol. 2019, Article ID 4029217, 10 pages, 2019.
- [5] A. Matucci, E. Maggi, and A. Vultaggio, "Cellular and humoral immune responses during tuberculosis infection: useful knowledge in the era of biological agents," *The Journal of Rheumatology. Supplement*, vol. 91, pp. 17–23, 2014.
- [6] B. Song, Y. L. Zhang, L. J. Chen et al., "The role of Toll-like receptors in periodontitis," *Oral Diseases*, vol. 23, no. 2, pp. 168–180, 2017.
- [7] M. Sanz, A. Marco Del Castillo, S. Jepsen et al., "Periodontitis and cardiovascular diseases: consensus report," *Journal of Clinical Periodontology*, vol. 47, no. 3, pp. 268–288, 2020.
- [8] G. Aarabi, T. Zeller, H. Seedorf et al., "Genetic susceptibility contributing to periodontal and cardiovascular disease," *Journal of Dental Research*, vol. 96, no. 6, pp. 610–617, 2017.
- [9] T. D. LeVan, J. W. Bloom, T. J. Bailey et al., "A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity," *Journal of Immunology*, vol. 167, no. 10, pp. 5838–5844, 2001.
- [10] N. C. Arbour, E. Lorenz, B. C. Schutte et al., "TLR4 mutations are associated with endotoxin hyporesponsiveness in humans," *Nature Genetics*, vol. 25, no. 2, pp. 187–191, 2000.
- [11] C. Schmitt, A. Humeny, C. M. Becker, K. Brune, and A. Pahl, "Polymorphisms of TLR4: rapid genotyping and reduced response to lipopolysaccharide of TLR4 mutant alleles," *Clinical Chemistry*, vol. 48, no. 10, pp. 1661–1667, 2002.
- [12] D. M. Agnese, J. E. Calvano, S. J. Hahm et al., "Human Toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of Gram-negative infections," *The Journal of Infectious Diseases*, vol. 186, no. 10, pp. 1522–1525, 2002.
- [13] N. W. Schroder, C. Hermann, L. Hamann, U. B. Gobel, T. Hartung, and R. R. Schumann, "High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR," *Journal of Molecular Medicine*, vol. 81, no. 6, pp. 368–372, 2003.
- [14] Y. Wang, B. Wu, M. Zhang, H. Miao, and J. Sun, "Significant association between rs28362491 polymorphism in NF- κ B1 gene and coronary artery disease: a meta-analysis," *BMC Cardiovascular Disorders*, vol. 20, no. 1, p. 278, 2020.
- [15] C. Chen, X. Fan, S. Yu et al., "Association between periodontitis and gene polymorphisms of hBD-1 and CD14: a meta-analysis," *Archives of Oral Biology*, vol. 104, pp. 141–149, 2019.
- [16] J. J. Xu, K. Q. Liu, Z. M. Ying et al., "Effect of CD14 polymorphisms on the risk of cardiovascular disease: evidence from a meta-analysis," *Lipids in Health and Disease*, vol. 18, no. 1, p. 74, 2019.
- [17] M. X. Han, C. Ding, and H. M. Kyung, "Genetic polymorphisms in pattern recognition receptors and risk of periodontitis: evidence based on 12,793 subjects," *Human Immunology*, vol. 76, no. 7, pp. 496–504, 2015.
- [18] S. Schulz, L. Hierse, W. Altermann et al., "The del/del genotype of the nuclear factor-kappaB -94ATTG polymorphism and its relation to aggressive periodontitis," *Journal of Periodontal Research*, vol. 45, no. 3, pp. 396–403, 2010.
- [19] H. Toker, E. P. Görgün, and E. M. Korkmaz, "Analysis of IL-6, IL-10 and NF- κ B gene polymorphisms in aggressive and chronic periodontitis," *Central European Journal of Public Health*, vol. 25, no. 2, pp. 157–162, 2017.
- [20] C. Shan, A. Aisaiti, Z. P. Wu, T. T. Wang, and J. Zhao, "Association of TLR-2 gene polymorphisms with the risk of periodontitis: a meta-analysis," *Disease Markers*, vol. 6, Article ID 9353958, 2020.
- [21] A. S. Golovkin, A. V. Ponasenko, A. E. Yuzhalin et al., "An association between single nucleotide polymorphisms within *_TLR_* and *_TREM-1_* genes and infective endocarditis," *Cytokine*, vol. 71, no. 1, pp. 16–21, 2015.
- [22] S. H. Jin, X. Y. Guan, W. H. Liang, G. H. Bai, and J. G. Liu, "TLR4 polymorphism and periodontitis susceptibility: a meta-analysis," *Medicine (Baltimore)*, vol. 95, no. 36, article e4845, 2016.
- [23] R. Chen, N. Gu, Y. Gao, and W. Cen, "TLR4 Asp299Gly (rs4986790) polymorphism and coronary artery disease: a meta-analysis," *PeerJ*, vol. 26, no. 3, article e1412, 2015.
- [24] S. Schulz, H. Lüdi, M. Lierath et al., "C-reactive protein levels and genetic variants of CRP as prognostic markers for combined cardiovascular endpoint (cardiovascular death, death from stroke, myocardial infarction, and stroke/TIA)," *Cytokine*, vol. 88, pp. 71–76, 2016.
- [25] M. S. Tonetti and N. Claffey, "European Workshop in Periodontology group C, advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology," *Journal of Clinical Periodontology*, vol. 32, pp. 210–213, 2005.
- [26] D. Ishaan, H. Parthasarathy, D. Ponnaiyan, and A. Tadepalli, "The CD14 rs2569190 TT genotype is associated with chronic periodontitis," *Genetic Testing and Molecular Biomarkers*, vol. 21, no. 9, pp. 560–564, 2017.
- [27] M. Folwaczny, J. Glas, H. P. Török, K. Fricke, and C. Folwaczny, "The CD14 -159C-to-T promoter polymorphism in periodontal disease," *Journal of Clinical Periodontology*, vol. 31, no. 11, pp. 991–995, 2004.
- [28] M. L. Laine, S. A. Morré, L. S. Murillo, A. J. van Winkelhoff, and A. S. Peña, "CD14 and TLR4 gene polymorphisms in adult

- periodontitis," *Journal of Dental Research*, vol. 84, no. 11, pp. 1042–1046, 2005.
- [29] S. Schulz, N. Zissler, W. Altermann et al., "Impact of genetic variants of CD14 and TLR4 on subgingival periodontopathogens," *International Journal of Immunogenetics*, vol. 35, no. 6, pp. 457–464, 2008.
- [30] L. I. Holla, D. Buckova, A. Fassmann, T. Halabala, A. Vasku, and J. Vacha, "Promoter polymorphisms in the CD14 receptor gene and their potential association with the severity of chronic periodontitis," *Journal of Medical Genetics*, vol. 39, no. 11, pp. 844–848, 2002.
- [31] J. Zheng, T. Hou, L. Gao et al., "Association between CD14 gene polymorphism and periodontitis: a meta-analysis," *Critical Reviews in Eukaryotic Gene Expression*, vol. 23, no. 2, pp. 115–123, 2013.
- [32] G. Zhu, C. Li, Z. Cao, E. F. Corbet, and L. Jin, "Toll-like receptors 2 and 4 gene polymorphisms in a Chinese population with periodontitis," vol. 39, Quintessence International, Berlin, Germany:1985, 2008.
- [33] G. G. Song, J. H. Kim, and Y. H. Lee, "Toll-like receptor (TLR) and matrix metalloproteinase (MMP) polymorphisms and periodontitis susceptibility: a meta-analysis," *Molecular Biology Reports*, vol. 40, no. 8, pp. 5129–5141, 2013.
- [34] D. Chrzęszczyk, T. Konopka, and M. Ziętek, "Polymorphisms of Toll-like receptor 4 as a risk factor for periodontitis: meta-analysis," *Advances in Clinical and Experimental Medicine*, vol. 24, no. 6, pp. 1059–1070, 2015.
- [35] R. M. Sellers, J. B. Payne, F. Yu, L. V. TD, C. Walker, and T. R. Mikuls, "TLR4 Asp299Gly polymorphism may be protective against chronic periodontitis," *Journal of Periodontal Research*, vol. 51, no. 2, pp. 203–211, 2016.
- [36] T. Griga, W. Klein, J. T. Epplen, U. Hebler, A. Stachon, and B. May, "CD14 expression on monocytes and soluble CD14 plasma levels in correlation to the promotor polymorphism of the endotoxin receptor CD14 gene in patients with inactive Crohn's disease," *Hepato-Gastroenterology*, vol. 52, pp. 808–811, 2004.
- [37] A. P. Reiner, E. M. Lange, N. S. Jenny et al., "Soluble CD14: genomewide association analysis and relationship to cardiovascular risk and mortality in older adults," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 33, no. 1, pp. 158–164, 2013.
- [38] H. F. Zhang, B. L. Zhong, W. L. Zhu et al., "CD14 C-260T gene polymorphism and ischemic heart disease susceptibility: a HuGE review and meta-analysis," *Genetics in Medicine*, vol. 11, no. 6, pp. 403–408, 2009.
- [39] T. A. Nakada, W. Takahashi, E. Nakada, T. Shimada, J. A. Russell, and K. R. Walley, "Genetic polymorphisms in sepsis and cardiovascular disease: do similar risk genes suggest similar drug targets?," *Chest*, vol. 155, no. 6, pp. 1260–1271, 2019.
- [40] M. Porsch-Ozcürümez, J. Hucke, S. Westphal, J. A. Hubáček, G. Schmitz, and C. Luley, "Post-hoc analysis on the CD14 C(-260)T promoter polymorphism and coronary heart disease," *Physiological Research*, vol. 56, no. 6, pp. 727–733, 2007.
- [41] A. S. Karban, T. Okazaki, C. I. Panhuysen et al., "Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis," *Human Molecular Genetics*, vol. 13, no. 1, pp. 35–45, 2004.
- [42] U. Buwitt-Beckmann, H. Heine, K. H. Wiesmüller et al., "TLR1- and TLR6-independent recognition of bacterial lipopeptides," *The Journal of Biological Chemistry*, vol. 281, no. 14, pp. 9049–9057, 2006.
- [43] M. Guven, Z. Ismailoglu, B. Batar et al., "The effect of genetic polymorphisms of TLR2 and TLR4 in Turkish patients with coronary artery disease," *Gene*, vol. 558, no. 1, pp. 99–102, 2015.
- [44] L. Hamann, A. Gomma, N. W. Schröder et al., "A frequent Toll-like receptor (TLR)-2 polymorphism is a risk factor for coronary restenosis," *Journal of Molecular Medicine*, vol. 83, no. 6, pp. 478–485, 2005.
- [45] S. Kiechl, E. Lorenz, M. Reindl et al., "Toll-like receptor 4 polymorphisms and atherogenesis," *New England Journal of Medicine*, vol. 347, no. 3, pp. 185–192, 2002.
- [46] P. E. Morange, L. Tiret, N. Saut et al., "TLR4/Asp299Gly, CD14/C-260T, plasma levels of the soluble receptor CD14 and the risk of coronary heart disease: the PRIME study," *European Journal of Human Genetics*, vol. 12, no. 12, pp. 1041–1049, 2004.
- [47] B. W. Wu, J. Zhu, H. M. Shi, B. Jin, and Z. C. Wen, "Association between Toll-like receptor 4 Asp299Gly polymorphism and coronary heart disease susceptibility," *Brazilian Journal of Medical and Biological Research*, vol. 50, no. 9, article e6306, 2017.
- [48] S. M. Boekholdt, W. R. Agema, R. J. Peters et al., "Variants of Toll-like receptor 4 modify the efficacy of statin therapy and the risk of cardiovascular events," *Circulation*, vol. 107, no. 19, pp. 2416–2421, 2003.
- [49] M. A. Beijk, S. M. Boekholdt, S. Z. Rittersma et al., "Toll-like receptor 4 gene polymorphisms show no association with the risk of clinical or angiographic restenosis after percutaneous coronary intervention," *Pharmacogenetics and Genomics*, vol. 20, no. 9, pp. 544–552, 2010.