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Single nucleotide polymorphisms in interleukin-1gene cluster and subgingival colonization with *Aggregatibacter actinomycetemcomitans* in patients with aggressive periodontitis

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ABSTRACT

Periodontitis is initiated by the subgingival occurrence of periodontopathogens. It is triggered by a specific host-dependent immune response that is influenced by genetic predisposition. Polymorphisms in the interleukin-1 (IL-1) gene cluster have been suggested to influence the pathogenesis of periodontitis. A total of 159 periodontitis patients (chronic disease: $n = 73$, aggressive disease: $n = 86$) and 89 periodontitis-free controls were included in the study. Polymorphisms IL-1α (rs1800587), IL-1β (rs16944, rs1143634), IL-1 receptor (rs2234650), and IL-1 receptor antagonist (rs315952) were determined by polymerase chain reaction with sequence-specific primers (PCR-SSP). Subgingival bacterial colonization was assessed using a polymerase chain reaction/DNA probe test (micro-Ident). Haplotype block structure was determined using Haploview 4.2. Statistical analyses were performed applying SPSS 17.0 considering dominant, recessive, and codominant genetic models. In this case-control study, no association between genomic variants of the IL-1 gene cluster and the incidence of severe periodontitis could be shown. Carriers of the rare genotypes of rs1800587 ($p_{corr} = 0.009$), rs1143634 ($p_{corr} = 0.009$) and composite genotype (rs1800587+rs1143634) ($p_{corr} = 0.031$) had a twofold higher risk for subgingival occurrence of *Aggregatibacter actinomycetemcomitans*. In forward stepwise binary logistic regression analyses considering age, gender, smoking, and approximal plaque index as potential confounders these significant associations were demonstrated. Despite the genetic background of IL-1 gene cluster could be shown to be associated with subgingival colonization of *A actinomycetemcomitans*, there is no evidence that it is an independent risk indicator for periodontitis.

1. Introduction

Periodontitis, as a chronic infectious inflammatory disease, is characterized by destructive immune response of the supporting tissue [1,2]. The subgingival occurrence of periodontopathogens as disease initiating factor is required but does not necessarily result in periodontal destruction [3,4]. The distinct ability to response to bacterial challenge is the basis for the individual susceptibility to periodontitis. Mechanisms modulating individual host's immune response to periodontopathogens play a crucial role in the progression of the disease. The interplay of pro- and anti-inflammatory cytokines has been described as a critical step in the etiopathology of periodontitis [5–8].

Among the factors mediating host's immune response genes of the interleukin-1 cluster (interleukin-1α [IL-1α], and -1β [IL-1β], and interleukin-1 receptor antagonist [IL-1RA]) have received most attention as potential predictors of disease progression [9,10]. IL-1α and IL-1β are mediating as pro-inflammatory cytokines their biologic activity via binding to its receptor (IL-1R). They compete for receptor binding with IL-1RA, which is diminishing their role in immune activation.

The role of genetic variants in these genes, especially in IL-1α and IL-1β, on the risk for severe periodontitis has been extensively studied [9,11,12]. Kornman et al. described a periodontitis-associated genotype comprising the rare genotypes of the SNPs rs1800587 (IL-1α) and rs1143634 (IL-1β) [10]. Functional consequences of these SNPs could be shown *in vitro* and *in vivo*, respectively. In cell culture experiments a dosage dependent effect on secretory capacity regarding the genotype of SNP rs1143634 could be proved [13]. The occurrence of the rare genotypes of the SNP rs1800587 was associ-

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ated with an almost fourfold increase in IL-1α protein levels in gingival crevicular fluid [14]. These findings give rise to the assumption of a more pronounced pro-inflammatory response in individuals carrying the rare genotypes. A variety of clinical studies were conducted to investigate the role of these genetic variants as risk indicators for severe periodontitis [12]. However, the results obtained are highly contradictory. The review by Grigoriadou et al. concluded that there is currently less proof of a genotype associated susceptibility to chronic periodontitis in white populations [9]. Furthermore, there is limited evidence of an association of SNPs in IL-1α and IL-1β, and aggressive periodontitis [15]. However, in large clinical trials, it could be shown that these SNPs in IL-1α and IL-1β could influence the risk of severe periodontitis in patients with diabetes [16] or in patients who smoke [17,18].

Another important molecule in the IL-1 pathway is the receptor (IL-1R) for IL-1α and IL-1β, supposed to play a vital role in the development of periodontal disease. The expression of IL-1R was shown to be increased in inflamed gingiva *in vitro* [19]. Furthermore, an IL-1–dependent receptor expression was evident, because a treatment with IL-1 increased the IL-1R level on gingival fibroblasts. A genetic variant in IL-1R, namely SNP (rs2234650), was demonstrated to be associated with inflammatory diseases [20,21]. Therefore, the implication of this SNP in periodontitis, described as an inflammatory disease, is conceivable. However, the biologic function of this polymorphic variant remains to be elucidated.

The IL-1RA gene, located in close proximity to the IL-1 genes on chromosome 2q13–q21, was also implicated in the etiopathology of periodontitis [22]. The IL-1–mediated expression of additional factors that amplify the inflammatory response could be diminished by administration of IL-1RA. A polymorphism in IL-1RA (rs315952) was significantly associated with systemic lupus erythematosus, an autoimmune disease [23], and an association between genetic variants and transcriptional level of IL-1RA was demonstrated.

Components of periodontopathogens have been proved to induce the secretion of Il-1β [24-26], leading to increased inflammation and tissue destruction. On one hand, in a clinical study investigating the impact of SNPs in IL-1α and IL-1β on the occurrence of oral bacteria, no significant association could be found in human immunodeficiency virus (HIV)–infected patients [27]. By contrast, another clinical study was able to describe characteristic differences in microbial pattern based on IL-1 gene status [28].

On the basis of the clinical data, functionally important polymorphisms in the genes of the IL-1 cluster (IL-1α, IL-1β, and IL-1RA) as well as in IL-1R gene may have an impact on the composition of subgingival plaque and/or the inflammatory response to periodontopathogens contributing to the development of periodontitis. The current clinical study was performed to evaluate genetic variants of these genes in association with aggressive and chronic periodontitis as well as in association with key periodontal bacteria in bivariate and multivariate models considering established confounders for periodontitis, such as age, gender, smoking, and the plaque index.

2. Subjects and methods

2.1. Study population and clinical investigations

A total of 248 consecutive, unrelated persons of the same Caucasian origin from Central Germany were involved in our study. The patient groups (n = 159) comprised 86 patients with generalized aggressive periodontitis (AP) and 73 patients with generalized chronic periodontitis (CP). The control group included 89 periodontitis-free participants. Demographic characteristics are given in Table 1. The study was performed at the Department of Operative Dentistry and Periodontology of the Martin-Luther-University (Halle-Wittenberg, Germany) from April 1996 to August 2010.

Table 1
Clinical characteristics of both patient groups and periodontitis-free controls

Variable	Aggressive periodontitis (n = 86)	Chronic periodontitis (n = 73)	Periodontitis-free controls (n = 89)
Demographic and clinical parameters			
Average age at diagnosis (years)	40.4 ± 9.8[a]	49.1 ± 9.4	46.2 ± 10.8
Female (%)	64.0	63.0	53.3
Current smoker (%)	34.9	23.6	21.3
Past smokers (%)	12.8	12.5	15.7
Early tooth loss because of periodontitis among relatives (%)	57.0[a]	40.9[a]	9.1
No. of lost teeth (n)	3.6 ± 3.8[a]	4.0 ± 3.3[a]	2.5 ± 2.8
Approximal plaque index (%)	53.3 ± 28.7[a]	62.0 ± 25.6[a]	47.2 ± 21.4
Bleeding on probing (%)	78.7 ± 23.2[a]	70.6 ± 24.7[a]	45.2 ± 23.9
Clinical probing depth (PD in mm)	5.7 ± 1.4[a]	5.3 ± 1.3[a]	2.6 ± 0.7
Clinical attachment loss (CAL, mm)	6.5 ± 1.5[a]	6.0 ± 1.5[a]	3.0 ± 0.8
Teeth with CAL 4–6 mm (%)	39.5[a]	45.8[a]	3.4
Teeth with CAL >6 mm (%)	57.0[a]	44.4[a]	1.1
$PD_{bacteria}$ (mm)	7.5 ± 1.5[a]	7.0 ± 1.6[a]	3.1 ± 0.4
$CAL_{bacteria}$ (mm)	8.5 ± 1.7[a]	7.8 ± 1.9[a]	3.3 ± 0.5
Individual occurrence of periodontal bacteria in subgingival pockets			
A actinomycetemcomitans (%)	40.7[a]	34.2	18.0
P gingivalis (%)	76.7[a]	87.7[a]	22.5
P intermedia (%)	61.6[a]	61.6[a]	31.5
T forsythiae (%)	86.0[a]	97.3[a]	68.5
T denticola (%)	86.0[a]	98.6[a]	62.9
Red complex *P.g+T.f+T.d* (%)	69.8[a]	83.6[a]	22.5

[a]Significant ($p \leq 0.05$ vs periodontitis-free controls).

All patients and control subjects were assessed in accordance with the classification system of periodontal diseases [1]. Inclusion and exclusion criteria for all participants were previously described in detail [29]. In particular, patients with generalized chronic periodontitis were selected if they showed an attachment loss in at least 30% of the teeth with a minimum pocket depth of 4 mm. The amount of the attachment loss was consistent with the presence of calculus. More horizontal than vertical approximal bone loss was visible in the radiographs. Patients with generalized aggressive periodontitis were included only in case of evidence (from dental history and/or radiographs) that the onset of the disease occurred in the period before the age of 35. They had a clinical attachment loss of 4 mm and more in at least 30% of the teeth. To exclude localized aggressive periodontitis, at least three of the affected teeth had to include no first molars or incisors. Contrary to chronic periodontitis, the severity of attachment loss was inconsistent with the amount of calculus, and more vertical than horizontal approximal bone loss was visible in the radiographs. Periodontitis-free individuals were included if they were at least 30 years old and had no attachment loss, (probing depth ≤3.5 mm, no gingival recession resulting from periodontitis [30]. Clinical attachment loss of >3.5 mm caused by traumatic tooth brushing and overhanging dental restorations were not considered as periodontitis. Although a definite clinical correlation of traumatic tooth brushing and attachment loss could be determined, a strict relation between dental filling and ensuing periodontitis could not be assumed. It could not be completely excluded that the periodontal lesion existed before the dental filling was made. However, the strictly localized coexistence of periodontitis and an overhanging filling in an otherwise healthy periodontium is most likely induced by dental restoration.

In general, we excluded women who were pregnant, persons who had drug-induced gingival hyperplasia, and individuals who received antibiotics in the last 6 months. Moreover, persons were excluded who had chronic use of anti-inflammatory drugs or a history of disease associated with periodontitis.

The clinical assessment included the determination of the approximal plaque index (approximal plaque index [API], %), bleeding on probing (BOP, %), pocket depth (PDmm), and clinical attachment loss (CALmm).

All participants gave their written consent to participation in this study. The study was approved by the ethics committee of the Medical School of the Martin-Luther-University Halle. The investigations were carried out in accordance with the ethical guidelines of the Declaration of Helsinki and its amendment in Tokyo and Venice.

2.2. Molecular assessment of periodontopathic bacteria

For each participant, the mean pocket depth and clinical attachment loss of these test sites ($PD_{bacteria,}$ $CAL_{bacteria}$) were recorded. Subgingival plaque samples were harvested before subgingival scaling was done. They were taken from the deepest pocket of each quadrant by an insertion of a sterile paper point for 20 seconds. All samples of each individual were pooled in one tube. Preparation of bacterial DNA was carried out using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For specific amplification of *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and bacteria of the "red complex" (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*), the micro-Ident test (Hain Lifescience, Nehren, Germany) was used according to the manufacturer's instructions as described previously [31].

2.3. Genetic studies

For genetic investigations, fresh venous blood was obtained from the subjects in ethylenediaminetetraacetic acid (EDTA)–treated tubes. Preparation of genomic DNA was carried out using a QIAamp blood extraction kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's manual.

The analysis of SNPs in IL-1α (rs1800587, C→T), IL-1β (rs16944, C→T, rs1143634, C→T), IL-1R (rs2234650, C→T), and IL-1RA (rs315952, T→C) was carried out using the CYTOKINE Genotyping array CTS-PCR-SSP Tray kit (Collaborative Transplant Study, Department of Transplantation Immunology of the University Clinic of Heidelberg, Heidelberg, Germany) as previously described [31].

2.4. Statistical evaluation

Statistical analyses were carried out using SPSS 17.0 software (SPSS, Inc., Chicago, IL). Values of $p < 0.05$ were considered significant. The genotype distributions of the polymorphisms were tested in accordance with the Hardy–Weinberg equilibrium. Categorical variables were plotted in contingency tables and evaluated using χ^2 analysis and Yates continuity correction. If $n < 5$, Fisher's exact test was performed. For power evaluation, the program nQuery Advisor 4.0 was applied. Metric parameters are presented as mean ± standard error (SE). These data were analyzed using the Kolmogorov–Smirnov-Test (test of normal distribution). For statistical evaluation, the Student's *t* test or one-way analysis of variance (for normally distributed values) and the Mann–Whitney *U* test or Kruskal–Wallis test (for values not distributed normally) were used. Binary logistic regression analysis was used for investigating the impact of polymorphic variants on the subgingival colonization periodontopathogens considering established confounders. Calculation of LD between SNPs and the definition of haplotype blocks were carried out using Haploview 4.2 [32].

3. Results

3.1. Clinical evaluation of the study groups

All study participants were assessed according to their age, gender, smoking status, and family history of periodontitis. When comparing the patient groups with the periodontitis-free healthy controls, no statistically significant differences in gender and smoking status could be detected. In accordance with the inclusion criteria, the mean age of the AP group was significantly lower.

In comparison with the periodontitis-free controls, the clinical parameters of periodontitis, such as API% (AP patients vs periodontitis-free controls, not significant (NS)), BOP%, PDmm, and CALmm were significantly elevated in the two patient groups. In both patient groups, there was an increase in the development of periodontopathogen bacteria. However, there was no significant difference with respect to *A actinomycetemcomitans* between patients with CP and the control group. Clinical and demographic data are given in Table 1.

3.2. Haplotype analysis

The haplotype block structure was determined for all five SNPs in the different patient groups (Fig. 1). The SNP of IL-1R was shown not to be included in the haplotype block of SNPs investigated in IL-1α, IL-1β, and IL-1RA. In the patient groups as well as in periodontitis-free controls, the SNPs of the composite genotype rs1800587 (IL-1α) and rs1143634 (IL-1β) were shown to be in strong linkage disequilibrium with each other (logarithm of odds (LOD) > 10).

3.3. Allele and genotype distribution of SNPs in IL-1a (rs1800587), IL-1b (rs16944, rs1143634), IL-1R (rs2234650), and IL-1RA (rs315952)

All SNPs were investigated according to a co-dominant genetic model. No statistical significant association between genotype distribution and severe periodontitis could be proved for any SNP investigated. On the basis of a dominant model regarding the rare genotypes, no significant disease-dependent associations could be proved as well (Table 2). Furthermore, the allele frequencies of all SNPs showed no significantly different distribution pattern in the patient groups compared with those in periodontitis-free controls. According to the haplotype analysis, the impact of the composite genotype of IL-1α (rs1800587) and IL-1β (rs1143634) on the development of severe periodontitis was investigated. Again, however, no significant influence of the genetic pattern and the occurrence of severe periodontitis could be proved (Table 2).

3.4. Subgingival occurrence of periodontopathic bacteria and genetic variants

Only in the group of patients with aggressive periodontitis was a significant association of genetic variants and the subgingival occurrence of a periodontopathogen, namely *A actinomycetemcomitans*, proved. Carriers of the rare genotypes of rs1800587 (p_{corr}= 0.009) and rs1143634 (p_{corr}= 0.009) as well as the rare genotypes of the composite genotype (rs1800587+rs1143634, p_{corr}=0.031) were at twofold greater risk for the subgingival colonization with *A actinomycetemcomitans* (Table 3). The occurrence of the other bacteria (*P gingivalis, P. intermedia, T. forsythiae,* and *T denticola*) was not significantly associated with the specific SNPs investigated.

3.5. Binary logistic regression analysis

Binary logistic regression analyses were carried out to investigate the impact of the genetic background of the rare genotypes of rs1800587, rs1143634, and composite genotype (rs1800587+ rs1143634) on the occurrence of *A actinomycetemcomitans*. Established confounders for periodontitis such as age, gender, smoking, and approximal plaque level were included. In these multivariate

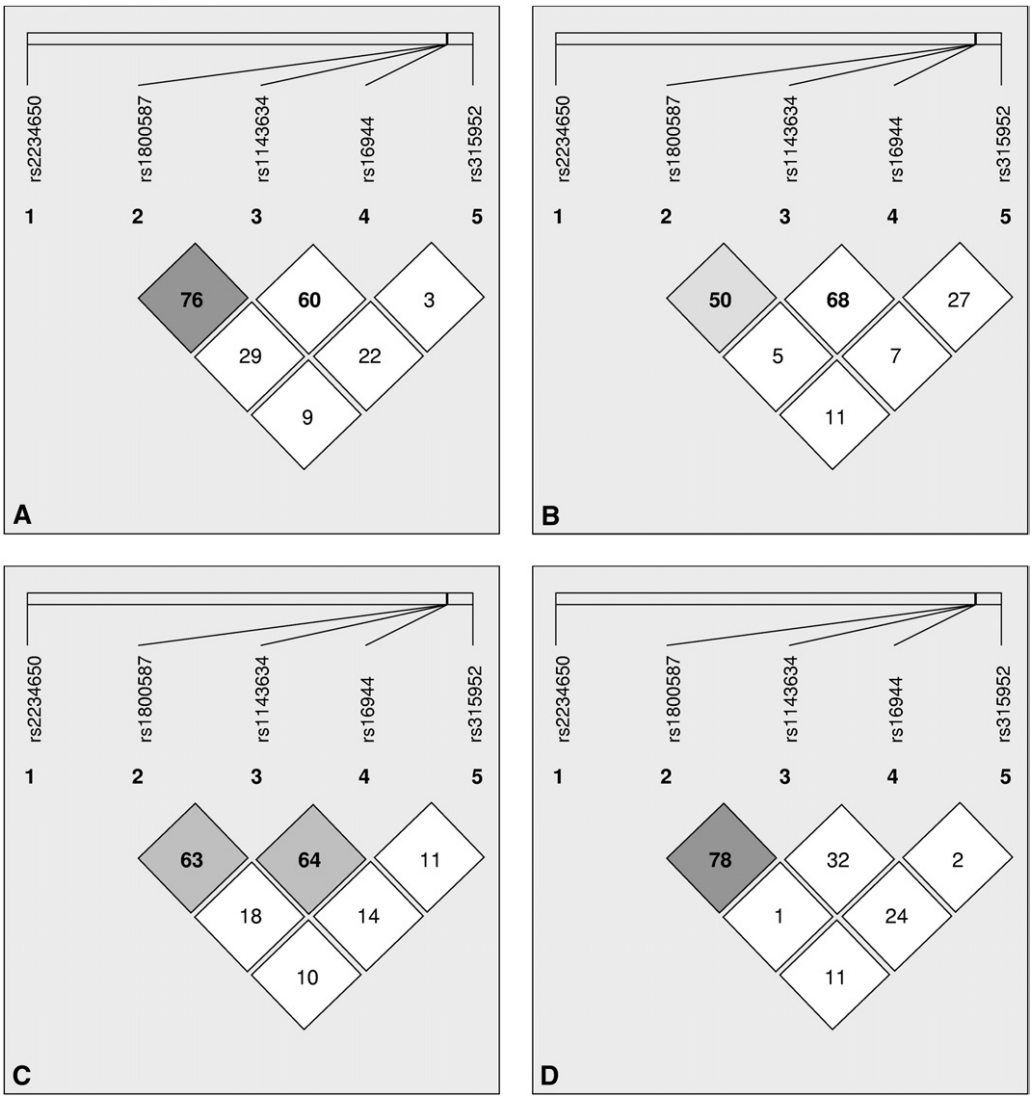

Fig. 1. Haplotype block structure determined using Haploview 4.2. Haplotype structure in patients with aggressive periodontitis (a), chronic periodontitis (b), aggressive and chronic periodontitis (c), and periodontitisfree controls (d). Squares: linkage disequilibrium and LOD score between SNPs Numbers in boxes: D' (measure of LD) × 100.

analyses, the rare genotypes have been proved as significant predictors for the subgingival colonization with *A actinomycetemcomitans* in aggressive periodontitis (Table 4). Further significant indicators of the occurrence of *A actinomycetemcomitans* were age and male gender in these statistical models.

4. Discussion

The etiopathology of periodontitis is initiated and maintained by subgingival bacterial infection. However, further exogenous and endogenous risk indicators have been established to modulate disease initiation and progression. In addition, the impact of individual genetic variation on the development of periodontitis, including its severity and extend has been demonstrated in studies of twins [33,34]. In recent years, research was promoted to characterize susceptibility genes for periodontitis [12]. Especially of interest are genetic variants of genes involved in the individual immune response, including IL-1 genes [11,12].

4.1. Impact of SNPs on severe periodontitis

In the present clinical case-control study, no association of SNPs in IL-1α (rs1800587), IL-1β (rs1143634, rs16944), IL-1R (rs2234650), and IL-1RA (rs315952) with eiether aggressive or chronic periodontitis could be determined. In our study groups of patients with severe periodontitis as well as in the group of periodontits-free controls, strong linkage disequilibrium of rs1800587 and rs1143634 could shown (LOD $>$ 10). These data are in accordance with the profile obtained from the HapMap database as well as with results of clinical association studies [35]. A weak linkage disequilibrium (LOD = 2,65) could be detected between rs1143634 and rs16944 only in the group of patients with severe periodontitis (CP+AP, Fig. 1c, LOD = 2.65). In all other study groups (AP patients, CP patients, and periodontitis-free controls; Figs. 1a, 1b, 1d), LOD scores clearly less than 3 were assessed, assuming there was no linkage between the two loci. Therefore, the two SNPs were evaluated separately.

A number of clinical studies were performed to investigate the impact of the IL-1α and IL-1β and SNP rs1800587 and rs1143634 as well as rs16944, with conflicting results. On one hand, the SNP rs1143634 was associated with disease severity [36,37] or with occurrence of periodontitis in patient groups stratified for clinical markers of periodontitis, such as smoking [38]. Kornman et al. postulated first a composite genotype of rs1800587 and rs1143634, which they linked to the severity of

Table 2
Genotype and allele distribution of SNPs in IL-1 gene cluster in dependence on the occurrence of aggressive periodontitis (AP) and chronic periodontitis (CP)

	All patients	AP	CP	Periodontitis-free controls
IL-1α: rs1800587 (c. −889C>T)	(*n* = 157)	(*n* = 85)	(*n* = 72)	(*n* = 89)
CC (%)	52.2	54.1	50.0	49.4
CT (%)	40.8	38.8	43.1	42.7
TT (%)	7.0	7.1	6.9	7.9
CT+TT (%)	47.8	45.9	50.0	50.6
C-allele (%)	72.6	73.5	71.5	70.8
T-allele (%)	27.4	26.5	28.5	29.2
IL-1β: rs16944 (c. −511C>T)	(*n* = 159)	(*n* = 86)	(*n* = 73)	(*n* = 89)
CC (%)	43.4	43.0	43.8	40.4
CT (%)	44.7	41.9	48.0	45.0
TT (%)	11.9	15.1	8.2	14.6
CT+TT (%)	56.6	57.0	56.2	59.6
C-allele (%)	65.7	64.0	67.8	62.9
T-allele (%)	34.3	36.0	32.2	37.1
IL-1β: rs1143634 (c. +3962C>T)	(*n* = 154)	(*n* = 82)	(*n* = 72)	(*n* = 88)
CC (%)	61.7	62.2	61.1	56.8
CT (%)	29.2	30.5	27.8	37.5
TT (%)	9.1	7.3	11.1	5.7
CT+TT (%)	38.3	37.8	38.9	43.2
C-allele (%)	76.3	77.4	75.0	75.6
T-allele (%)	23.7	22.6	25.0	24.4
IL-1R: rs2234650 (pstI, c.1970C > T)	(*n* = 159)	(*n* = 86)	(*n* = 73)	(*n* = 89)
CC (%)	45.9	48.8	42.5	46.1
CT (%)	45.3	45.3	45.2	38.2
TT (%)	8.8	5.8	12.3	15.7
CT+TT (%)	54.1	51.2	57.5	53.9
C-allele (%)	68.6	71.5	65.1	65.2
T-allele (%)	31.4	28.5	34.9	34.8
IL-1RA: rs315952 (mspaI, c.11100C > T)	(*n* = 159)	(*n* = 86)	(*n* = 73)	(*n* = 88)
TT (%)	45.9	45.3	46.6	40.9
CT (%)	40.3	41.9	38.4	43.2
CC (%)	13.8	12.8	15.1	15.9
CT+CC (%)	54.1	54.7	53.4	59.1
C-allele (%)	34.0	33.7	34.2	37.5
T-allele (%)	66.0	66.3	65.8	62.5
Composite genotype (I) IL-1α: rs1800587 (c. −889C>T) + IL-1β: rs1143634 (c. +3962C>T)	(*n* = 152)	(*n* = 81)	(*n* = 71)	(*n* = 88)
Carrier of all other SNP combination (%)	32.2	33.3	31	40.9
Carriers of at least one rare allele at each SNP (%)	67.8	66.7	69	59.1

*Significance was estimated considering a rare-dominant genetic model (frequent/frequent vs frequent/rare+rare/rare).

periodontitis in nonsmoking patients [35]. By contrast, other groups could not prove similar associations [15]. The conflicting results may be traced back to varying influence of the SNPs in different ethnicity [39–41] or to different clinical settings [11,12,42]. In our study, no statistically significant influence of any SNP investigated on the occurrence of severe periodontitis could be proved even after stratifying for clinical markers of periodontitis, including smoking (data not shown). An almost twofold increase in TT-genotype carriers (IL-1β, rs1143634) was detected in the group of patients with chronic periodontitis compared with periodontitis-free controls (NS, 11.1% vs 5.7%, Table 2). This was in line with an increase in CC-genotype carriers in the patient group (61.1% vs 56.8%, Table 2). A similar trend in genotype distribution was shown for patients with aggressive periodontitis. This result is in accordance with a previous published study by Parkhill et al. [38], who could also show an increase in CC- and TT-genotype carriers among patients with early onset periodontitis. Because a genotype-dependent influence on IL-1β secretion because of lipopolysaccharide (LPS) stimulation could be assessed [13] it could be supposed that this

Table 3
Association of the genotype distributions of rs1800587 (IL-1α), rs1143634 (IL-1β), and the composite genotype I (rs1800587 + rs1143634) and the subgingival occurrence of *A actionmycetemcomitans* in the aggressive periodontitis group

	A actinomycetemcomitans (%)	*p* Value[a]	Odds ratio (95% CI)	Power (1-b)
IL-1α: rs1800587 (c. −889C>T)				
CC (*n* = 46)	26.1			
CT+TT (*n* = 39)	56.4	0.009	3.66 (1. 74–9.14)	75%
IL-1β: rs1143634				
CC (*n* = 51)	29.4			
CT+TT (*n* = 31)	61.3	0.009	3.80 (1.48–9.74)	75%
Composite genotype (I) IL-1α: rs1800587 (c. −889C>T) + IL-1β: rs1143634 (c. +3962C>T)				
Carriers of at least one rare allele at each SNP (*n* = 54)	59.3			
Carrier of all other SNP combination (*n* = 27)	31.5	0.031	3.16 (1.21–3.12)	56%

CI, confidence interval.
[a]Yates corrected *p* value.

Table 4
Forward stepwise binary logistic regression investigating the adjusted[a] odds ratio of the rare genotypes of Il-1α rs1800587, IL-1β rs1143634, and composite genotype I (rs1800587 + rs1143634) for the occurrence of *A. actinomycetemcomitans* in patients with aggressive periodontitis

Significant variables	Regression coefficient	SE	*p* Value	Odds ratio	95% CI	
					Lower	Upper
IL-1α: rs1800587 (c. −889C>T)						
Genotype: CT+TT	1.59	0.57	**0.006**	4.88	1.59	14.92
Age	−0.08	0.03	**0.012**	0.93	0.87	0.98
Male gender	1.77	0.58	**0.002**	5.91	1.89	18.49
IL-1β: rs1143634 (c. +3962C>T)						
Genotype: CT+TT	1.48	0.57	**0.009**	4.41	1.45	13.33
Age	−0.086	0.03	**0.008**	0.92	0.86	0.98
Male gender	1.54	0.58	**0.007**	4.67	1.51	14.40
Composite genotype (I) IL-1α: rs1800587 (c. −889C>T) +IL-1β: rs1143634 (c. +3962C>T)						
Genotype:	1.39	0.59	**0.019**	4.00	1.26	12.66
Age	−0.079	0.03	**0.012**	0.92	0.87	0.98
Male gender	1.72	0.59	**0.004**	5.57	1.75	17.72

CI, confidence interval; SE, standard error.
[a]Adjusted for potential confounders age, gender, smoking, and approximal plaque index (API).

SNP could influence disease pathogenesis directly because of differences in IL-1β production.

Although a distinct decrease in TT-genotype carrier of IL-1 receptor SNP rs2234650 was detected in AP patients compared with periodontitis-free controls (NS, 5.8% vs 15.7%, Table 2) no significant association of this SNP with the occurrence of severe periodontitis could be proved in our study. Scapoli et al., who investigated 70 markers in the IL-1 gene region in patients with aggressive periodontitis [43], could also not demonstrate an association of SNPs in IL-1R with the occurrence of periodontitis.

The functionally important SNP rs315952 in IL-1RA, which was shown to be associated with inflammatory disease [20,21,23], could not be proved as a risk indicator for either aggressive or chronic periodontitis in this German cohort. However, our results do not exclude the possibility that other functionally important SNPs in these genes could be implicated in the occurrence of severe periodontitis [40,44].

4.2. Impact of SNPs on subgingival occurrence of periodontitis

In this study, a significant association of SNPs in IL-1α (rs1800587) and IL-1β (rs1143634) as well as the composite genotype on the colonization with *A actinomycetemcomitans* could be described in aggressive periodontitis for the first time. In multivariate analysis, the impact of the rare genotypes of these SNPs could be emphasized as confounders of occurrence of *A actinomycetemcomitans* after adjustment for age, approximal plaque index, gender, and smoking status. The role of *A actinomycetemcomitans* in the manifestation and progression of aggressive periodontitis in particular is well established [45]. Furthermore, clinical studies were conducted to assessed the genetic impact of IL-1 genes on the subgingival microbiota [27,28,46]. In HIV-infected Brazilian subjects, no significant association between the composition of subgingival microbiota and genetic variation in IL-1α and IL-1β could be demonstrated [27]. In contrast, in another clinical study, it was supposed that the individual genetic constellation could influence the composition of subgingival microbiota [27,46]. Carriers of the rare genotypes were shown to be more frequently affected with certain species, primarily those of the red and orange complex. This interdependence was mainly obvious with increasing pocket depth [46].

In accordance with this clinical study, one can speculate that a genetically dependent change in IL-1α and IL-1β expression could increase gingival inflammation and gingival crevice fluid flow, and thereby promote the colonization with subgingival periodontopathogens.

4.3. Limitations of the study

The current study was performed as a case-control study. It was conducted to establish assumptions of possible associations between genetic variants and periodontitis, and periodontopathogens, respectively. However, considering the study design, the verification of these assumptions is not realizable.

For the group of periodontitis-free (defined by previously published criteria [26]) control subjects, the possibility of periodontitis development at a later time cannot be excluded. That is why an underestimation of the genotype-dependent effect because of this possible selection bias may be feasible.

However, as pointed out in Table 3, the power of the statistical evaluation does not reach the required power of 80% to obtain statistical reliable results (power range, 56%–75%). This implies a definite level of uncertainty regarding the study results.

Finally, the data presented can be considered applicable for Caucasian individuals of central Germany only, and must therefore be interpreted with caution. Extrapolation to the general population is not rationally supported.

4.4. Conclusion

Despite the limitations mentioned, our results emphasize the importance of the rare gentoypes of IL-1α (rs1800587), IL-1β (rs1143634), and composite genotypes on the subgingival bacterial colonization with *A actinomycetemcomitans* in this German cohort of patients with aggressive periodontitis.

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