Objective: Polymorphisms of the toll-like receptor 4 (TLR4) have been reported to confer differences in the inflammatory response to gram-negative infections. Since periodontal diseases are caused by gram-negative microorganisms, we performed a pilot study to investigate the association of two polymorphisms (Asp299Gly and Thr399Ile) of the TLR4 gene with the risk of chronic periodontitis.

Study design: In a monocentric cross-sectional study, 75 caucasians with chronic periodontitis and 54 periodontally healthy controls were investigated. Asp299Gly and Thr399Ile genotypes were detected by polymerase chain reaction (PCR) and subsequent cleavage by NcoI and HinfI restriction endonucleases.

Results: In total, 9.3% (12/129) of the individuals, 13.3% (10/75) of the periodontitis group, and 3.7% (2/54) of the periodontally healthy controls were heterozygous for both polymorphisms. However, an increased prevalence of the Asp299Gly and Thr399Ile genotypes in the periodontitis group was shown, but it failed to reach the level of statistical significance either in the univariate method (p = 0.073) and also in the multivariate logistic regression model [odds ratio (OR): 4.000; 95%-confidence interval (95%CI) 0.839–19.061; p = 0.063].

Conclusions: Our results suggest that the presence of Asp299Gly and Thr399Ile polymorphisms of the TLR4 gene might have implications for the risk for chronic periodontitis, but larger studies are needed to confirm these data.

Introduction

Microorganisms quickly colonise tooth surfaces, if not prevented by oral hygiene procedures. Within a few days, their accumulation leads to the development of clinical signs of gingival inflammation. Gingivitis is seen as a necessary condition for the development of periodontitis. However, not all patients with gingivitis develop periodontitis. Periodontitis results from a complex interplay between chronic bacterial infection and the inflammatory host response, leading to irreversible destruction of the tooth-supporting tissues. The so-called attachment loss is therefore an indicator for the sum of all
destructive inflammatory processes in this area during lifetime. Periodontal pathogens, mostly gram-negative anaerobes, produce a variety of enzymes and toxins that can create local tissue inflammation\(^3\) and induce inflammatory and immune responses of the host. As a result, various inflammatory molecules such as proteases, cytokines and prostaglandins initiate the breakdown of the periodontal tissues. Several risk factors, such as age, smoking and socio-economic factors, are reported to modify this process\(^2\).

In addition, local characteristics such as carious lesions, insufficient margins of restorations or an unfavourable anatomic situation may also increase the risk of periodontitis. Genetic factors were hypothesised to play an important role in predisposing subjects to periodontal breakdown. Functional genetic polymorphisms of the innate immune response appear to influence the individual susceptibility for chronic periodontitis\(^5\)–\(^8\).

Such important functional genetic variants of the innate immunity are the polymorphisms of the toll-like receptor 4 (TLR4), a central pattern recognition receptor for lipopolysaccharide. These functional polymorphisms are presumed to affect the incidence and progression of gram-negative infection- and inflammatory-related diseases\(^9\),\(^10\). Therefore, one might think that the presence of these polymorphisms would result in a higher degree of periodontitis, indicated by a greater loss of the tooth-supporting structures. To investigate this hypothesis we studied the prevalence of the two common single nucleotide polymorphisms (SNPs) of TLR4 gene in patients with chronic periodontitis and in control subjects.

### Study design

We recruited 129 individuals between 30 and 60 years of age from a population-based sample of the Heidelberg area. All participating individuals gave informed consent to study participation, and separately for radiography. The study was approved by the local ethics board. Exclusion criteria were pregnancy, inability to give informed consent or to cooperate in the dental examination, and any known condition in which a prophylactic antibiotic treatment before dental examination is required. Participants had to be caucasians and to speak German to a degree that allowed them to follow the interview.

### Interview and dental examination

All subjects were interviewed by trained interviewers using a standardised questionnaire and examined in a standardised way in a dental unit under optimum conditions, as reported previously\(^11\),\(^12\).

### Genotyping

Genomic DNA was isolated from leukocytes using a commercial kit according to the instructions of the supplier (Qiagen, Valencia, CA). The TLR4 Asp299Gly (reference number rs4986790) and Thr399Ile (reference number rs4986791) polymorphisms were analysed by polymerase chain reaction (PCR), and subsequent cleavage was by NcoI and HinfI restriction endonucleases. Each polymorphism was investigated in a separate reaction as published previously\(^13\).

### Statistical analysis

All data were entered twice into a data bank to minimise data input errors. Dichotomous variables are presented as percentages and continuous variables are presented as mean and standard deviation or median and quartiles as appropriate. Differences were tested for statistical significance first by univariate methods (Student’s t test or \(\chi^2\) test, or two tailed Fisher’s exact test). Multivariate testing was performed by multiple logistic regression analysis including well-known risk factors for periodontitis such as age, smoking and sex. Odds ratios and 95%-confidence intervals are given for all factors. The software package SPSS 11.0 (SPSS Inc., Chicago IL, USA) was used for the analyses\(^14\).

### Results

The study was performed as a monocentric, cross-sectional pilot study, in which 129 (85 male, 44 female) individuals were examined. According to the definition of Macht\(^15\) periodontitis was diagnosed in 75 individuals (58.1%). Periodontally healthy individuals (54; 41.9%) served as control. The groups are characterised in Table 1.
In total, 12 individuals (9.3%) were heterozygous for both polymorphisms; 10 (13.3%) were detected in the periodontitis group and 2 (3.7%) in the control group. No carriers heterozygous for one or the other allele and no homozygous carriers were identified. In the univariate analysis the association of periodontitis and the polymorphisms of TLR4 just failed to reach the level of statistical significance (p = 0.073) (Table 1). However, in individuals with the Asp299Gly and Thr399Ile genotypes, a statisti-
cally significant clinical attachment loss ($p = 0.042$) and a statistically significant radiographic bone loss ($p = 0.032$) were found when compared with the Asp299Asp and Thr399Thr genotype (Table 2).

In the multivariate logistic regression analysis including the presence of the TLR4 polymorphisms, smoking, age and sex revealed a strong trend between TLR4-polymorphisms and periodontitis (OR: 4.000; 95% CI: 0.839–19.061; $p = 0.063$).

**Discussion**

Periodontitis is a chronic inflammatory disease, which is closely related to the presence of gram-negative pathogens between periodontal tissues and the tooth and the inflammatory and immune response of the host organisms to these pathogens and their products\(^2\). The loss of tooth-supporting tissues, being the clinical and radiographic sign of chronic periodontitis, is the direct result of the local inflammatory processes. As the destruction is not reversible, the amount of attachment loss can be seen as an indicator for the individual interplay between gram-negative infections and the host defence mechanisms in general. Immune responses against microbial agents are partly genetically determined. Genetic conditions that alter the immune response to gram-negative microorganisms, such as the known polymorphisms of the TLR4\(^9,10\) might, therefore, result in a higher risk for periodontitis\(^6,7\).

In this pilot study we failed to show any statistically significant association between the functional polymorphisms of TLR4 and chronic periodontitis either in the univariate or in the multivariate analysis. However, both results showed a strong trend, and they may reach the statistical significance level in a larger cohort. In such a small cohort the failure to reach the significant association may be influenced by some unconsidered environmental factors. However, in individuals carrying the polymorphisms of TLR4, statistically significant increases in clinical attachment loss and radiographic bone loss were found. According to these results, a possible role of these polymorphisms in chronic periodontitis is not excluded, but further investigations are needed.

The prevalence of the Asp299Gly and Thr399Ile polymorphisms was comparable to other studies, which reported a prevalence between 6% and 11%\(^16\), and they were in Hardy-Weinberg equilibrium.

To date, there are controversial results in the literature about the association between TLR4-polymorphisms and periodontitis, affected perhaps by the different clinical selection criteria, various ethnic populations and demographic data\(^7,17–19\). The major problem of all these studies (including ours) is implicated in the rather low population size investigated, which is not large enough to get adequate statistical power for a definitive assertion. Therefore, larger studies are needed to evaluate the final influence of TLR4-polymorphisms in the aetiology of chronic periodontitis, as others have previously suggested\(^20\).

Identifying genetic, clinical, laboratory markers for patients at high risk of periodontitis is the key for prevention. We investigated one possible genetic marker for periodontitis risk. Taken together, under the limitations of the small sample size our results support that the polymorphisms in the TLR4 gene are not sufficiently powerful genetic markers for susceptibility for chronic periodontitis in clinical practice.

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