Clinical Periodontal and Microbiologic Parameters in Patients With Crohn’s Disease With Consideration of the CARD15 Genotype

Jamal M. Stein,* Frank Lammert,† Vincent Zimmer,‡ Marie Granzow,§ Stefan Reichert,‖ Susanne Schulz,‖ Christina Ocklenburg,‡ and Georg Conrads#

Background: Crohn’s disease (CD) was reported to have oral manifestations. However, data on periodontal parameters and oral microbiology in CD are rare. Recent studies showed associations of variants in the caspase recruitment domain (CARD)15 gene with CD that are involved in the immune response toward bacterial products. Our aim is to investigate the periodontal status and prevalence of periodontal pathogens in patients with CD under consideration of the CARD15 polymorphism.

Methods: Oral soft tissue alterations and periodontal parameters of 147 patients with CD were assessed. Subgingival plaque samples were analyzed for the periodontal pathogens Aggregatibacter actinomycetemcomitans (Aa; previously Actinobacillus actinomycetemcomitans), Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf; previously T. forsythensis), Prevotella intermedia (Pi), and Campylobacter rectus (Cr) using dot-blot hybridization. CARD15 genotyping was performed with the a polymerase chain reaction (PCR) based assay.

Results: A total of 36.7% of patients had oral manifestations predominated by gingival swellings (27.2%) and hyperplastic lesions of the buccal mucosa (20.4%). The mean probing depth and mean clinical attachment level were 3.6 and 3.8 mm, respectively. A total of 57.8% of the patients had a Community Periodontal Index of Treatment Needs (CPITN) score 3, and 31.3% of had a CPITN score 4. The prevalence of Aa, Pg, Pi, Tf, and Cr was 76.9%, 62.6%, 79.6%, 64.6%, and 94.6%, respectively. Pi was significantly less frequent in carriers of CARD15 mutations compared to the wild type (69.7% versus 87.7%; P = 0.008). All other pathogens and clinical periodontal parameters did not differ significantly as to the CARD15 polymorphism.

Conclusions: Our findings suggest that patients with CD have an increased prevalence and moderate severity of periodontitis. The colonization of periodontal pathogens, in particular Cr, might be of particular value for the periodontal manifestation of CD. Although a modulating impact on periodontal microbiota can be supposed, our data do not support the role of CARD15 in oral symptoms and periodontal lesions in patients with CD. J Periodontol 2010;81:535-545.

KEY WORDS
Bacteria; Campylobacter rectus; CARD15 protein; Crohn’s disease; inflammatory bowel diseases; periodontitis.

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Crohn’s disease (CD) is a chronic inflammatory bowel disease (IBD) that is characterized by a dysregulation of the mucosal immune response. CD is most common in northern Europe and North America and occurs fairly equally among men and women with an age of onset between 15 and 30 years. In contrast to ulcerative colitis (UC), which is restricted to the colon and rectum, CD can affect any part of the gastrointestinal tract, most commonly in the distal ileum (regional enteritis) but has extra intestinal manifestations as well. Oral symptoms of IBD were shown by several author groups. In particular, CD was associated with aphthous-ulcerative lesions, a cobblestone pattern and/or swelling of the oral mucosa. These lesions can precede or coincide with intestinal symptoms.

CD was also reported to have periodontal manifestations. The results of three previous cross-sectional studies on CD and UC point to a higher prevalence but moderate severity of periodontitis in patients with CD and UC. Unfortunately, oral microflora were not investigated in these studies. However, in an earlier study by Van Dyke et al., an unusual predominance of the Gram-negative Wolinella recta (the former name of Campylobacter rectus [Cr]) was detected in 20 patients with CD and UC with and without signs of periodontitis. Extracts of this bacterium revealed an inhibition of neutrophil chemotaxis in a dose-response fashion. The authors concluded that this bacterium might play a role in the pathogenesis of IBD. Unfortunately, to our knowledge, no investigation verified these pilot findings.

Furthermore, several studies found an association of single-nucleotide polymorphisms (SNPs) in the caspase recruitment domain family, member 15 (CARD)15 genes with CD. The CARD15-gene cluster encodes an intracellular protein that is involved in the innate immune response by recognition of invading bacteria and induction of the inflammatory response. CARD15 induces apoptosis and activates nuclear factor-kappa B. This factor plays an important role in proinflammatory responses by regulating the transcription of cytokine genes. A strong association with the CARD15 variants was shown in patients with CD, with carrier frequencies of one or more variant CARD15 alleles (SNPs) between 22% and 60%. It seems conceivable that variants of CARD15 might be involved in cytokine-mediated inflammatory responses in periodontal tissues and, therefore, affect interactions between CD and periodontitis.

In summary, there are several similar features in the pathophysiology of CD and periodontitis that point to possible interactions: both are chronic inflammatory diseases that have intermittent courses with acute episodes, similar cytokine profiles (interleukin-1, -6, and -8) are involved in tissue damage, environmental factors such as stress and smoking influence the disease, and genetic background factors influence the susceptibility to both diseases. Thus, these similarities raise the question of whether CD might have extra intestinal (periodontal) manifestations that may be reflected by clinical periodontal parameters and by changes in oral microbiologic flora. Also, it is unclear whether mutations in the CARD15 genes, which modify the innate immune response toward bacteria, have an impact on the oral (periodontal) manifestation in patients with CD. The knowledge of these interactions may help to improve the understanding of periodontal symptoms of CD, which could be of diagnostic value. Therefore, the purpose of our study is to examine a group of patients with CD for the prevalence of oral soft tissue lesions, the prevalence and severity of periodontal disease, and the presence and number of periodontal pathogens in the patients’ pockets under consideration of and stratification for CARD15-gene mutations.

Materials and Methods
Study Population
The study was performed with a cross-sectional design. A total of 147 subjects with CD participated in the study. The diagnosis of CD was previously established by clinical, radiologic, endoscopic, and histologic criteria. All subjects were outpatients who attended the Department of Internal Medicine, University Hospital Aachen, between February 2004 and May 2005. Patients were excluded from the study if they: 1) were <18 years old, 2) had a systemic disease other than CD or CD-associated spondylarthropathies, 3) were pregnant, or 4) had undergone antibiotic therapy <2 months prior to the study. Patients who were edentulous and those who underwent a periodontal treatment within the preceding 6 months were also excluded. All study participants were of white descent and unrelated to each other.

For evaluation of anamnestic data, all participants were interviewed according to a standardized protocol. Subjects were asked about their medical history, medications, smoking habit, and history of periodontitis and oral hygiene. The study protocol was approved by the Ethics Committee of the University of Aachen, Aachen, Germany, and written informed consent was obtained from all subjects before their examinations.

Clinical Oral and Periodontal Examinations
The oral examination assessed mucosa lesions, oral hygiene, and the prevalence and severity of periodontal disease. Therefore, in all patients, intraoral mucous membranes were evaluated with regard to the presence of swelling of the gingiva and/or lips.
ulcerations of the mucosa, leukoplakia, and other lesions.

The periodontal examination was performed on all present teeth (except third molars) and comprised the assessment of the plaque index (PI), gingival index (GI), probing depths (PDs), and clinical attachment level (CAL). PD and CAL values were recorded at six sites per tooth. The CAL (the distance between the cemento-enamel junction and bottom of the pocket) was obtained by adding the PD values to gingival recession values (the distance between the gingival margin and cemento-enamel junction). Furthermore, the Community Periodontal Index of Treatment Needs (CPITN) was recorded. Therefore, the oral cavity was divided into sextants; for each sextant, the highest index found was recorded by applying the following scores: 0 = periodontal health, 1 = gingival bleeding, 2 = calculus and/or overhanging restorations, 3 = PD of ≥4 mm but <6 mm; and 4 = PD ≥6 mm. Finally, the prevalence of the highest CPITN score of each subject was calculated. The presence of bleeding on probing (BOP) was also recorded for all sites measured.

All measurements were performed by the same examiner (JMS) throughout the study using a millimeter-graded, pressure-sensitive probe set to a probing force of 0.25 N. Prior to the study, intraexaminer reliability was assessed by computing Cohen’s κ coefficient. The simple κ value was 0.905 (95% confidence interval [CI]: 0.851 to 0.958), and the weighted κ was 0.937 (95% CI: 0.902 to 0.972), which were considered acceptable.

Identification of Periodontal Pathogens

For the identification of the periodontal pathogens Aggregatibacter actinomycetemcomitans (Aa; previously Actinobacillus actinomycetemcomitans), Porphyromonas gingivalis (Pg), Tannarella forsythia (Tf; previously T. forsythensis), Prevotella intermedia (Pi), and Cr, subgingival microbial samples were obtained from all patients. The deepest pocket of each arch quadrant was selected for sampling. After supragingival debridement, one sterile paper point was inserted to the bottom of each selected pocket for 15 seconds. All four paper points with subgingival plaque samples were pooled together into a transfer tube and stored at a temperature of −25°C for further laboratory analysis.

The detection of the periodontal pathogens was performed with dot-blot hybridization using oligonucleotide probes derived from 16S rRNA and labeled with digoxigenin-11-deoxyuridine 5-triphosphate. The DNA probes were developed according to the methods of Conrads et al. and optimized in a computerized comparison against 20,000 bacterial 16S rRNA/DNA sequences and tested empirically against various bacterial strains and against the human genome. They proved to be >99.99% specific for Aa, Pg, Tf, Pi, and Cr. Nucleic acids were isolated by the aid of a tissue kit. Hybridization was performed following the instructions of the manufacturer and standard procedures.

According to the manufacturer, the dot-blot hybridization assay has a detection limit of 10² to 10³ cells and is highly specific, reacting negatively with up to 10⁹ oral competitor cells. Laboratory analyses were performed in a masked manner.

CARD15 Genotyping

CARD15 genotyping was performed according to the protocols described by Hampe et al. Peripheral venous blood was obtained by all participants, and genomic DNA was extracted from whole blood by standard procedures using a DNA purification kit. DNA was diluted in water to a final concentration of 15 ng/μl, and 5 μl (75 ng) was used per reaction. The three CD-associated CARD15 variants, SNP 8 (rs2066844; Arg702Trp), SNP 12 (rs2066845; Gly908Arg), and SNP 13 (rs2066847; Leu1007finsC), were genotyped using solution-phase hybridization reactions with 5’-nuclease and subsequent fluorescence detection. For each SNP, two alleles were discriminated (allele 1 = mutant; allele 2 = wild type). Genotyping was performed twice masked to clinical data.

Statistical Analyses

Primary outcome values of continuous variables were given as the mean and SD. The unpaired t test was used for comparison of these values between the mutant and wild-type subgroups of the CARD15 polymorphism. The investigation of associations between allele type (mutant or wild type) and various categoric variables was performed with the Fisher exact test. The statistical comparison of the three CARD15 subgroups (SNPs 8, 12, and 13) with the CARD15 wild type regarding the prevalence of the five periodontal bacteria was done with the χ² test with the Yates correction. In cases of comparisons with n <5, the Fisher exact test was performed.

Analysis of covariance (ANCOVA) was used to determine the effect of the variables age, gender, smoking, PI, GI, the detection level of the five bacteria (as a four-level categoric variable: <10³, ≥10³, ≥10⁴, and ≥10⁵), and the presence/absence of a mutation in the CARD15 gene on periodontal parameters PD and CAL. Because of the large number of independent
factors, univariable analyses (simple logistic or linear regression or one-factorial analysis of variance, depending on the types of independent and dependent variables investigated) were previously conducted to select relevant risk factors (factors with $P < 0.2$) for the final multivariable model. In a similar way, multiple regression analyses were used to determine the effect of the variables age, gender, smoking, PI, GI, PD, and the presence/absence of a mutation in the CARD15 gene on the prevalence of the five periodontal bacteria. Once again, univariable tests were previously performed to select the relevant risk factors for the final multiple model.

All statistical tests were conducted at a global significance level of $a = 5\%$ in an explorative manner only. Data processing and statistical analyses were performed using software packages.

### RESULTS

#### Descriptive Results (Table 1)

Among all 147 patients with CD, 34 patients (23.1%) were carriers of the mutant allele of CARD15 SNP 8, 15 patients (10.2%) had the mutant allele for SNP 12, and 29 patients (19.7%) had the mutant allele for SNP 13. Sixty-six patients (44.9%) had at least one mutant allele for SNPs 8, 12, or 13 (mutant group). In 81 patients (55.1%), only wild-type alleles (wild-type group) were found. Allele frequencies were consistent with the Hardy-Weinberg equilibrium (all $P$ values >0.05).

Age and gender and the percentage of smokers did not significantly differ among all CARD15 subgroups ($P > 0.05$). Among smokers, the mean number of pack-years was 7.0 ± 10.4. Carriers of SNP 12 had significantly less mean pack-years than carriers of the wild-type ($P = 0.034$), whereas the mean pack-years in all other CARD15 subgroups were similar to those in the wild-type group. Patients received standard medical treatment including corticosteroids, immunosuppressants (azathioprine and methotrexate), and aminosalicylates as mono- or combination therapy. There were no significant differences among the percentages of patients in the subgroups who took these medications.

### Intraoral Examination (soft tissue alterations; Table 2)

Overall, 54 of the 147 patients (36.7%) had oral soft tissue alterations. From these, 42 patients presented one lesion only, whereas 12 patients showed more than one lesion simultaneously.

Most common alterations were gingival swellings (27.2%) and hyperplastic lesions on the buccal mucosa (20.4%). Less frequent alterations were mucocutaneous lesions such as leukoplakia (2.0%) and lichen planus (2.7%). Also, five cases (3.4%) with candidiasis were found. Aphthous ulcers of the mucosa occurred in six patients (4.1%). Because oral manifestations were reported to coincide or precede gastrointestinal symptoms of CD, the frequency of gingival swelling, aphthous ulceration, and/or the presence of mucocutaneous alterations were evaluated. Fifteen patients with CD (10.2%) had oral lesions during the flare-up of their CD.

For all examined oral soft tissue alterations, there were no relevant differences between the different

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**Table 1. Descriptive Parameters in Different CARD15 Subgroups of Patients With CD**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (N = 147)</th>
<th>SNP 8 (n = 34)</th>
<th>SNP 12 (n = 15)</th>
<th>SNP 13 (n = 29)</th>
<th>Mutant* (n = 66)</th>
<th>Wild Type† (n = 81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± SD [range])</td>
<td>36.6 ± 9.9 (18 to 62)</td>
<td>34.9 ± 11.0 (18 to 62)</td>
<td>32.7 ± 10.5 (18 to 54)</td>
<td>36.2 ± 8.8 (22 to 56)</td>
<td>35.5 ± 9.8 (18 to 62)</td>
<td>37.5 ± 9.8 (20 to 59)</td>
</tr>
<tr>
<td>Females (n [%])</td>
<td>77 (52.4)</td>
<td>16 (47.1)</td>
<td>9 (60.0)</td>
<td>18 (62.1)</td>
<td>33 (50.0)</td>
<td>44 (54.3)</td>
</tr>
<tr>
<td>Smokers (n [%])</td>
<td>55 (37.4)</td>
<td>15 (44.1)</td>
<td>4 (26.7)</td>
<td>13 (44.8)</td>
<td>29 (43.9)</td>
<td>26 (32.1)</td>
</tr>
<tr>
<td>Pack-years (mean ± SD)</td>
<td>7.0 ± 10.4</td>
<td>5.8 ± 8.7</td>
<td>2.0 ± 4.0</td>
<td>6.6 ± 9.6</td>
<td>6.6 ± 9.3</td>
<td>8.5 ± 11.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medications</th>
<th>Corticosteroids (n [%])</th>
<th>62 (42.2)</th>
<th>11 (32.4)</th>
<th>7 (46.7)</th>
<th>12 (41.4)</th>
<th>25 (37.9)</th>
<th>37 (45.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunosuppressants (n [%])</td>
<td>70 (47.6)</td>
<td>13 (38.2)</td>
<td>7 (46.7)</td>
<td>13 (44.8)</td>
<td>28 (42.4)</td>
<td>42 (51.9)</td>
</tr>
<tr>
<td></td>
<td>Aminosalicylates (n [%])</td>
<td>48 (32.7)</td>
<td>13 (38.2)</td>
<td>6 (40.0)</td>
<td>9 (31.0)</td>
<td>22 (33.3)</td>
<td>26 (32.1)</td>
</tr>
</tbody>
</table>

For all comparisons between the mutant and wild type: $P > 0.05$; for the comparison of mean pack years between SNP 12 and the wild type: $P = 0.034$; for all other comparisons between CARD15 subgroups (SNPs 8, 12, and 13) and the wild-type group: $P > 0.05$.

* Presence of least one mutant allele for SNPs 8, 12, or 13.
† Only wild-type alleles present.

*** SAS, Version 9.1, SAS Institute, Cary, NC.
††† Microsoft Office Excel, Version 11.1.1, Redmond, WA.
CARD15 subgroups and the CARD15 wild-type group.

**Periodontal Parameters (Table 3)**
The number of missing teeth (6.1 ± 3.7) was similar in all subgroups compared to the total group. There were no significant differences between the mutant and wild-type groups regarding the parameters PI, GI, mean PD, mean CAL, BOP, and all CPITN values.

Among all subjects with CD, the mean values for PD and CAL were 3.6 and 3.8 mm, respectively. More than half of the patients (57.8%) had PD values...
between 4 and 5 mm (corresponding to CPITN score 3), whereas 31.3% of the patients had at least one site with PD ≥6 mm (corresponding to CPITN score 4). In accordance with the PDs, 57.9% of all patients with CD had CAL values between 4 and 5 mm, whereas in 32.0% of the patients, at least one site with CAL ≥6 mm was found (data not shown Table 3). BOP occurred in 23.9% of all measured sites of all patients. All presented values did not differ significantly among CARD15 subgroups.

**Microbiologic Results**

Among all 147 patients, Aa was observed in 76.9% of the patients, Pg in 62.6% of the patients, Pi in 79.6% of the patients, and Tf in 64.6% of the patients. Cr had the highest frequency, being found in 94.6% of the patients. When the prevalence of the periodontal pathogens in all CARD15 subgroups was compared to the wild-type group, the frequency of Pi was found to be significantly decreased in carriers of the CARD15 SNP 8 (70.6% versus 87.6%; P = 0.036), SNP 12 (53.3% versus 87.6%; P = 0.004) and SNP 13 (69.0% versus 87.6%; P = 0.032). The comparisons for Aa, Pg, Tf, and Cr did not show any statistically significant differences (Fig. 1).

A comparison of the mutant group (at least one mutant CARD15 allele) with the wild-type group confirmed the significantly negative association of Pi (69.7% versus 87.7%; P = 0.008). The prevalence of all other bacteria did not differ significantly between the groups, even though there was a trend for a decreased frequency in the mutant group (Fig. 2).

**Multivariable Analyses**

Multivariable analyses were performed to determine PD and CAL as well as the examined periodontopathic bacteria in all patients with CD.

**Multivariable analyses for PD and CAL.** Out of all variables tested (see Statistical Analyses), univariable analyses revealed the variables age, GI, PI, smoking, and all bacteria (Aa, Pg, Tf, Pi, and Cr) to be potentially relevant risk factors (P < 0.2) for PD and CAL. These variables were included in ANCOVA models. The results of these analyses demonstrate that PD has a significant impact on Aa and Cr. Moreover, smoking was
a significant predictor for the presence of $T_f$, whereas age was a significant confounder for $P_g$. The presence of a mutation in the CARD15 gene was significantly negatively associated with the presence of $P_l$ (Table 5).

**DISCUSSION**

CD and periodontitis are multifactorial diseases that were shown to be dependent on environmental, microbiologic, and genetic factors. Gingivitis and periodontitis were reported in many of the early studies on oral manifestations of CD mucosal alterations. Because we supposed that genetic and microbiologic parameters may have an impact on the oral findings in CD, the potential influence of CD and its associated genetic variants in the CARD15 gene cluster on the prevalence of oral lesions, clinical periodontal parameters, and distribution of periodontal pathogens was investigated.

In 36.7% of the patients in this study, soft tissue alterations were found. The kind and frequency of the lesions (Table 2) confirmed the findings in a group of patients with IBD reported by Harty et al. and Grössner-Schreiber et al. The frequencies of gingival swelling (27.2%) as well as mucosal edema and hyperplasia of the mucosa (20.4%) predominated, whereas lichen planus, leukoplakia, and candidiasis were rare lesions. The latter finding was also in agreement with the findings of Flemmig et al. and Brito et al. Although in a few cases, hyperplasia in the oral mucosa tended to resemble a kind of lobular lymphoedema, which has a typical cobblestone pattern (epithelial hyperplasia scored by linear fissures and ulcers) as described in earlier articles, and pyostomatitis vegetans with pustules were not observed. Assumably, immunosuppressants, antiinflammatories, and other medications (Table 1) depressed the oral manifestation compared to cases in older reports. Oral lesions were reported to precede or to appear concomitantly with flare-ups of gastrointestinal symptoms. In our patients, only 10.2% of oral lesions appeared at the same time as flare-ups of the CD, whereas the majority of oral lesions occurred in the remission phase. The latter observation was supported by data from other reports. Therefore, oral lesions do not appear to be a reliable diagnostic symptom for acute phases of CD.

An evaluation of the periodontal parameters revealed a mean PD of 3.6 mm and a nearly identical mean CAL of 3.8 mm (Table 3). Both values were higher than in previous studies. There may be two reasons for these differences: First, in contrast to Flemmig et al. and Grössner-Schreiber et al., who recorded PD and CAL values in two sites of only two quadrants, periodontal measurements in the present study were performed on six sites of all teeth. As partial-mouth studies were proven to underestimate the severity of periodontitis, this might have led to different mean values. Second, in contrast to the studies of Flemmig et al. and Brito et al., we found a higher trend for gingival swellings/hyperplasia in our patients (Table 2) that might have produced increased PD values rather than clinical attachment loss. Thus, gingival recessions were minimal in our patient group. This also explains the observation that the amounts of patients with at least one site with a CAL of 4 to 5 mm (57.9%) and ≥6 mm (32.0%) were nearly identical to the percentages of patients with a CPITN score 3 (57.8%) and CPITN score 4 (31.3%) (Table 3).

On the other hand, the prevalence of periodontitis in our patient group was not relevantly increased when the CPITN scores of our study were compared to population data from a clinical study of oral health in Germany in which 52.7% of subjects ranging in age between 35 and 45 years had a CPITN score 3, and 20.5% of subjects had a CPITN score 4. This observation was in agreement with the previously cited studies on IBD and CD. Grössner-Schreiber et al. reported that 81% of their patients with IBD had at least one site with a CAL ≥4 mm, which was not significantly increased compared to their control probands (61%). In the present investigation, the corresponding value for the CD group was 89.9% representing a similar result. In the study of Brito et al., periodontitis was diagnosed in 81% of all patients with CD. Thereby, periodontitis was defined as the presence of at least four sites with CAL ≥3 mm. When these criteria were applied to our study participants, the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age</th>
<th>Smoking</th>
<th>PI</th>
<th>GI</th>
<th>$A_o$</th>
<th>$P_g$</th>
<th>$T_f$</th>
<th>$P_l$</th>
<th>$Cr$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>&lt;0.0001</td>
<td>0.3170</td>
<td>0.5816</td>
<td>&lt;0.0001</td>
<td>0.1930</td>
<td>0.0155</td>
<td>&lt;0.0001</td>
<td>0.1814</td>
<td>0.6418</td>
</tr>
<tr>
<td>CAL</td>
<td>&lt;0.0001</td>
<td>–</td>
<td>0.7908</td>
<td>&lt;0.0001</td>
<td>0.2692</td>
<td>0.1132</td>
<td>0.0366</td>
<td>0.0606</td>
<td>0.8221</td>
</tr>
</tbody>
</table>

$= $ variable was not included for the multiple model due to the result of the univariable analysis ($P>0.2$). Significant $P$ values ($P<0.05$) are marked in bold type.
patients showed a similar periodontitis prevalence of 85.2%. In summary, the majority of our patients with CD were associated with moderate periodontitis, and only a smaller percentage of patients with CD had a susceptibility for severe periodontitis. Further, the prevalence of periodontitis did not significantly differ from the prevalence data of the corresponding population and those of previous studies.\(^\text{15-17}\)

To our knowledge, one aspect that was not previously evaluated is the potential influence of genetic polymorphisms on the oral and periodontal manifestation of CD. Three variants of the CARD15-gene cluster were associated with CD.\(^\text{19-22}\) They are involved in the recognition of peptidoglycans derived from bacterial lipopolysaccharides\(^\text{42}\) and, therefore, might affect interactions between CD and periodontitis. This is why we compared the frequency of patients with CD harboring the CARD15-gene variants (SNPs 8, 12, and 13) with those who did not carry this polymorphism (wild type), regarding the presence of soft tissue lesions and periodontal parameters. Demographic parameters such as age, smoking, and intake of medications did not show any relevant differences among the wild-type and three SNP groups. We were unable to find any significant differences of oral lesions and periodontal parameters among the different SNPs and wild type. Concordant results were found in studies that assessed the association of CARD15 variants in chronic\(^\text{43}\) and aggressive\(^\text{44}\) periodontitis. In both investigations, no association of oral manifestations and clinical periodontal parameters in patients with CD.

Beside the clinical and genotypic parameters, we determined the prevalence of periodontal pathogens in periodontal pockets of patients with CD. All examined species occurred with high detection rates > 63%. With a frequency of 95%, \(Cr\) had the highest prevalence in our study. In multivariable analysis, \(Tf\) and \(Pg\) were significant predictors for an increased CAL (Table 4). Because both pathogens belong to the red complex, which is characterized by a high periodontal pathogenicity,\(^\text{45,46}\) this might explain the association. Despite their higher prevalence, \(Cr\) and \(Pl\) (of the orange complex) were not significant confounders for periodontal attachment loss; however, they might have a symbiotic influence on the colonization of \(Tf\) and \(Pg.\text{47}\) On the other hand, besides smoking (\(Tf\) and age (\(Pg\)), the depth of the periodontal pockets (\(Aa\) and \(Cr\)) positively confounded the colonization of periodontal pathogens. The latter finding is plausible because deep pockets are known to favor the colonization of periodontal pathogens\(^\text{48}\). Consequently, there seems to be a mutual influence of colonization of periodontal pathogens and development of periodontal pockets.

Interestingly, there was a significantly negative association of \(Pl\) with a CARD15 mutation in the univariable (Fig. 2) and multivariable (Table 5) analyses, whereas all other bacteria showed a trend toward a decreased occurrence in the mutant group. A decreased frequency of periodontal pathogens in CARD15-positive subjects was reported by Laine et al.\(^\text{45}\) who observed a higher number of CARD15 mutations in patients without the presence of \(Aa\) and \(Pg\). The reason for this association is not entirely clear. In carriers of the CARD15 mutation, it is possible that a hyperactive response toward \(Pl\) or other pathogens may result in the elimination or reduction of these bacteria and maintain inflammation in periodontal and/or gastrointestinal tissues. Nevertheless, this remains speculative and should be verified in further studies.

The high frequency of periodontopathic bacteria in this study can, at least partially, be explained by three facts: 1) a very sensitive culture-independent, molecular

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**Table 5. Multiple Logistic Regression Analyses for Periodontopathic Bacteria in Patients With CD**

| Variable\(^\text{a}\) | Age | | | | | |
|---|---|---|---|---|---|
| | \(P\) | OR (95%CI) | \(P\) | OR (95%CI) | \(P\) | OR (95%CI) |
| \(Aa\) | 0.6826 | 1.0 (1.0 to 1.0) | 0.1249 | 2.3 (0.8 to 6.7) | 0.7164 | 0.8 (0.2 to 2.7) |
| \(Pg\) | 0.2088 | 1.0 (1.0 to 1.1) | 0.4224 | 0.7 (0.3 to 1.7) | 0.0725 | 2.6 (0.9 to 7.4) |
| \(Tf\) | **0.0221** | 1.1 (1.0 to 1.1) | – | – | 0.9443 | 1.0 (0.5 to 2.2) |
| \(Pl\) | 0.0903 | 1.0 (1.0 to 1.1) | – | – | – | – |
| \(Cr\) | – | – | – | – | – | – |

\(OR = \) odds ratio; \(\_\_\_\_\) = variable was not included for the multiple model due to the result of the univariable analysis (\(P >0.2\)).

Significant \(P\) values (\(P <0.05\)) are marked in bold type.

\(^{\text{a}}\) Prevalence of periodontopathic pathogens with a number \(\geq10^3\).

\(^{\text{b}}\) Presence of at least one mutant allele for SNPs 8, 12, or 13.
Table 5. (continued)

**Multiple Logistic Regression Analyses for Periodontopathic Bacteria in Patients With CD**

<table>
<thead>
<tr>
<th>Smoking</th>
<th>PD</th>
<th>CARD15†</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>OR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0.0136</td>
</tr>
<tr>
<td>0.0388</td>
<td>2.4 (1.0 to 5.4)</td>
<td>0.0760</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0.0808</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0.1360</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0.0449</td>
</tr>
</tbody>
</table>

OR = odds ratio; — = variable was not included for the multiple model due to the result of the univariable analysis (P>0.2).

Significant P values (P<0.05) are marked in bold type.

* Prevalence of periodontal pathogens with a number ≥10^3.
† Presence of least one mutant allele for SNPs 8, 12, or 13.

A diagnostic method with a detection limit of 100 rRNA genes was used; 2) because of the reclassification within the genus *Wolinella* (*W. recta* became *Cr*) and *Actinobacillus* (became *Aggregatibacter*), the criteria for including certain genotypes might have been changed or are different among the studies compared in the present study; and 3) nearly half of all patients with CD took immunosuppressant medications, which influence T- and B-cell functions and, therefore, might have impaired the antibacterial immune response. This could favor the colonization of periodontal pathogens. Nevertheless, the striking predominance of *Cr* supports the first and, to our knowledge, the only study of oral microflora in patients with IBD by Van Dyke et al. who reported an unusual colonization of *Cr* (*W. recta*) in periodontal lesions of patients with IBD. They noticed a serum-mediated defect in neutrophil chemotaxis in 10 IBD patients with periodontitis in response to *Cr*. Although the neutrophil function was not analyzed in the present study, we can confirm the striking predominance of this bacterium in a sample size (*N* = 147) that was considerably higher than in the report of Van Dyke et al. This bacterium has favorable conditions to colonize the oral cavity in these patients and might play a particular role for IBD or CD. Assumingly, it also favors the colonization of other Gram-negative bacteria, which would explain the remarkable frequency of the concomitant species *Aa*, *Pg*, *Tf*, and *Pl* found in our study. One of the characteristics of *Cr* is the existence of a surface layer (S-layer) external to the outer membrane. This structure was shown to confer resistance to complement-mediated killing and to cause the down regulation of proinflammatory cytokines. Hinode et al. reported a sequence homology between a 64-kDa heat-shock protein (HSP) and the S-layer protein of *Cr*. Also, Tanabe et al. showed that *Helicobacter pylori* and *Cr* share an antigen, which has homologies to the HSP of the HSP60 family. Because polymorphisms of HSP genes are known, and an impaired humoral immune response to several HSP proteins was demonstrated in patients with CD, the molecular mimicry with cross-reactivity of antibodies against HSP and *Cr* may represent another mechanism that could explain the predominance of this bacterium in the CD group. However, this remains hypothetical and should be verified in further studies.

**CONCLUSIONS**

From the data shown in the present study, the following conclusions can be drawn: 1) our results support previous studies that showed a high prevalence but moderate severity of periodontitis among patients with CD; 2) soft tissue lesions such as gingival swelling and mucosal hyperplasia may occur in patients with CD; however, they do not necessarily coincide with gastrointestinal symptoms; 3) our data do not support a role of CARD15 in the oral manifestation and clinical periodontal status in CD; however, the frequency of *Pl* was significantly reduced in carriers of CARD15 mutations; and 4) along with other periodontal pathogens, *Cr* appeared to be preferably harbored in periodontal pockets of patients with CD and might play a particular role for the periodontal manifestation of CD.

The present study has potential limitations that must be considered. First, the results, in particular the genotypic findings, are only applicable to white individuals. Periodontal, genotypic and microbiologic data in patients with CD of other ethnic populations might differ from those presented in our study. Second, the presented results are based on an explorative cross-sectional study. Confirmative studies should verify and extend the presented data by case-control designs. The role of the microbiologic periodontal results for the manifestation and course of CD should be...
studied to improve the knowledge of the interaction between CD and periodontal symptoms, which may offer new options in diagnostics of CD.

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