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Epigenetic characteristics in inflammatory candidate genes in aggressive periodontitis

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease triggered by the host immune response. Epigenetic modifications also affect the immune response. We assessed CpG methylation in 22 inflammatory candidate genes (ATF2, CCL25, CXCL14, CXCL3, CXCL5, CXCL6, FADD, GATA3, IL10RA, IL12A, IL12B, IL13, IL13RA1, IL15, IL17C, IL17RA, IL4R, IL6R, IL6ST, IL7, INHA, and TYK2) with respect to the occurrence of aggressive periodontitis (AgP).

Patients and methods: In this study 15 AgP patients (53.3% males, 41.4 ± 10.5 years) and 10 controls (40.0% males, 36.9 ± 17.5 years) were included. The methylation patterns of gingival biopsies were quantified using EpiTect® Methyl Signature PCR Array Human Inflammatory Response.

Results: In gingival biopsies taken from patients with AgP, CpG methylation of CCL25 (1.73% vs. 2.59%, $p = 0.015$) and IL17C (6.89% vs. 19.27%, $p = 0.002$) was significantly reduced as compared with periodontally healthy tissues.

Discussion: We showed for the first time a differential methylation pattern for CCL25 and IL17C in periodontitis. CCL25 plays an important role in T-cell development, whereas IL17C regulates innate epithelial immune responses. The decrease in CpG methylation is presumably accompanied by an increase in gene expression. This could lead to a greater availability of CCL25 and interleukin 17C and support periodontal loss of attachment.

1. Introduction

Periodontitis is an infectious disease characterized by inflammation and destruction of tooth-supporting tissue [1]. A variety of extrinsic and intrinsic factors contribute to the manifestation and progression of the disease [2]. An individual's ability to respond to a bacterial challenge plays a crucial role in disease progression. It is widely accepted that some individuals are more susceptible to periodontal disease than others. The same is obviously true for an individual's response to medication. In determining susceptibility to periodontal inflammation intrinsic factors, in particular, need to be taken into consideration. In the last few decades, research focused on associating genetic variations with the etiology of periodontitis. Genetic variations may alter gene expression, which possibly influences an individual's response to microbial load. A variety of case-control studies and genome-wide association studies have been performed in order to analyze the genetic influence on periodontitis [3–7].

An individual's immune response is not only influenced by genetic characteristics, but there is a further level of gene regulation. Gene expression is also triggered by epigenetic modifications not based on an altered DNA sequence [8]. Such epigenetic modifications include distinct methylation of DNA or chemical alterations of DNA-associated proteins, the histones, and the nucleosomes [9]. The best investigated epigenetic modification is DNA methylation occurring at CpG islands, which are mostly located in the promoter regions of genes. Specific epigenetic alterations have been linked to the development of a variety of different diseases, such as cancer and also inflammatory diseases [10]. Initial studies revealed an epigenetic contribution to periodontitis, too

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[11]. Different methylation patterns were shown in TNF-α, E-cadherin, TLR2, and COX-2 [12–15] whereas for IFN-γ and Il-6 no periodontitis-associated changes in methylation status were detected [16,17]. In these studies, mostly one gene was considered (only one study investigated two loci). Up to now, only one study was conducted as a genome wide study to compare a pooled sample of inflamed tissue (chronic periodontitis) with one pooled sample of healthy tissue [18]. Changes in the CpG methylation pattern and associated alterations in gene expression might account for the differences in an individual's susceptibility to periodontal disease [19]. Knowledge of further markers of periodontitis, including epigenetic characteristics, would support the development of an individualized periodontal therapy and preventional regimens [19].

The present study was conducted in order to detect differentially methylated genes involved in immune response between patients with aggressive periodontitis and controls. We screened the promoter methylation status of a variety of genes, including chemokines, cytokines, inflammatory response, and autoimmunity genes (ATF2, CCL25, CXCL14, CXCL3, CXCL5, CXCL6, FADD, GATA3, IL10RA, IL12A, IL12B, IL13, IL13RA1, IL15, IL17C, IL15RA, IL4R, IL6R, IL6ST, IL7, INHA, TYK2) with respect to periodontal inflammation. Most of these genes have already been implicated in the etiology of periodontitis but up to now no investigations concerning epigenetic modifications of these genes in periodontal disease have been carried out. Here, we compared the methylation patterns in gingival biopsies of diseased sites (CAL $\geqslant$ 6 mm) from patients suffering from AgP with sites without attachment loss (CAL $\leqslant$ 3 mm) taken from patients who had no periodontitis or only a mild chronically localized periodontitis in order to assess novel disease associated markers.

2. Material and methods

2.1. Study population and clinical investigations

In all, 25 unrelated persons of the same Caucasian origin from Central Germany were involved in our pilot study. The patient group (n = 15) comprised AgP patients. The control group included 10 participants without or with mild periodontitis. The demographic data are given in Table 1. The study was performed at the Department of Operative Dentistry and Periodontology of the Martin-Luther University Halle-Wittenberg. Gingival biopsies were obtained from all participants and immediately frozen in liquid nitrogen.

All patients and controls were assessed according to the classification system of periodontal diseases [1]. In particular, patients with generalized aggressive periodontitis were only included when there was evidence (from dental history and/or radiographs) that the onset of the disease occurred before the age of 35. The patients showed a clinical attachment loss of 4 mm or more in at least 30% of the teeth. The patients frequently reported a rapid progression of the disease and episodes of acute gingivitis, abscess formation, tooth loosening or dental loss caused by the tooth loosening, and many unsuccessful attempts to heal the disease. Conversely to chronic periodontitis (ChP), the severity of periodontal tissue destruction was inconsistent with the amount of microbial deposits. In the radiographs angular bony defects were often visible. During periodontal surgery gingival biopsies were obtained during flap procedures at sites with CAL $\geqslant$ 6 mm. Control individuals had no or only mild localized periodontitis. The percentage of sites with CAL $\geqslant$ 4 mm of each control was $\leqslant$30%. The teeth from which gingival biopsies were taken did not have CAL values >3 mm. Gingival biopsies from control individuals were obtained, for example, while apically repositioning a flap to extend a clinical crown before restorative therapy or during surgical removal of wisdom teeth at adjacent teeth with CAL $\leqslant$ 3 mm. In general, we excluded persons who were pregnant or were nursing mothers, had a drug-induced gingival hyperplasia, or had taken antibiotics in the last 6 months. Moreover, persons who chronically used anti-inflammatory drugs or had a history of inflammatory diseases of the oral cavity (including herpes simplex infections) or diseases associated with periodontitis were excluded.

The clinical assessment included determining the plaque index (PI), gingival index (GI), clinical probing depth (PDmm), and clinical attachment loss (CALmm) [20,21]. All parameters were assessed at the biopsy site.

All participants gave their written consent to participate 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" [22].

2.2. Epigenetic studies

For epigenetic investigations, gingival biopsies were taken from each participant. Genomic DNA was prepared by using a QIAamp® DNA Micro extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's manual.

The epigenetic methylation pattern of each individual separately was assessed by using EpiTect® Methyl DNA Enzyme Kit (Qiagen, Hilden Germany) for the first step. The analyzed genes comprised chemokines, cytokines, cytokine receptors, and associated proteins as well as other inflammatory response and autoimmunity genes (Table 2). Four cleavage reactions (without enzyme, methylation-sensitive enzyme, methylation-dependent enzyme, and both enzymes) were carried out for each sample. For the restriction analyses, the Mastercycler® gradient (Eppendorf, Hamburg, Germany) was used (conditions: 37 °C for 6 h, 65 °C for 20 min, 4 °C hold). For assessing the CpG methylation pattern the EpiTect® Methyl II Signature PCR Array Human Inflammatory response (catalogue number: EAHS-521ZA-24, Qiagen, Hilden,

Table 1
Demographic and clinical periodontal characteristics.

	Patients with AgP n = 15	Controls n = 10	p-value
Demographic data			
Age, years (mean ± SD)	41.4 ± 10.5	36.9 ± 17.5	0.428*
Male gender (%)	53.3	40.0	0.806**
Periodontal data			
Plaque index at site of biopsies (median, 25th/75th percentiles)	1.0 (0/1.0)	1.0 (0/1.0)	0.482***
Gingiva index at site of biopsies (mean ± SD)	1.33 ± 0.7	0.50 ± 0.5	0.006*
Clinical probing depth at site of biopsies (PD in mm mean ± SD)	7.20 ± 1.6	2.35 ± 0.67	<0.001*
Clinical attachment loss at site of biopsies (CAL in mm mean ± SD)	8.60 ± 2.7	2.35 ± 0.67	<0.001*

* Student's t-test.
** Yates correction.
*** Mann–Whitney-U test.