Epigenetic characteristics in inflammatory candidate genes in aggressive periodontitis: The role of interleukin 17C and CCL25

S Schulz [1], HG Schaller [1], U. Immel [2], L. Just [3], C Gläser [4], S Reichert [1]

[1] University School of Dental Medicine, Department of Operative Dentistry and Periodontology, Martin-Luther-University Halle-Wittenberg, Germany

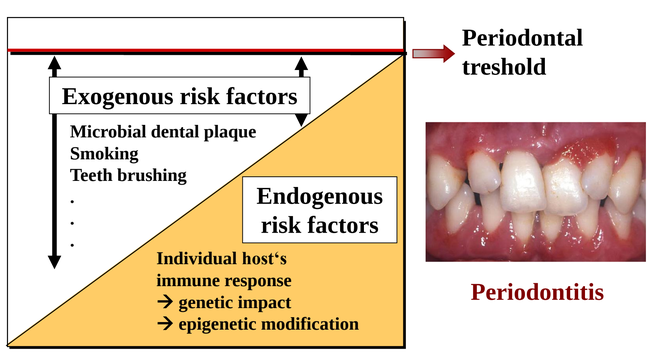
[3] Department of Oral and Maxillofacial Surgery and Facial Plastic Surgery, Martin-Luther-University Halle-Wittenberg, Germany

[2] Clinic of Legal Medicine, Martin-Luther-University Halle-Wittenberg, Germany

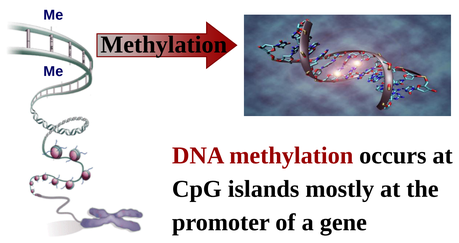
[4] Institute of Human Genetics and Medical Biology, Martin-Luther-University, Halle-Wittenberg, Germany

Introduction

Periodontitis a complex inflammatory disease



Epigenetic mechanisms



DNA methylation occurs at CpG islands mostly at the promoter of a gene

DNA-methylation is involved in transcriptional regulation

→ leads to gene silencing

Epigenetic - Cytokines - Periodontitis

- **Cytokines are inducers of alveolar bone loss and collagen degradation**
- **Cytokines promote inflammatory processes of periodontitis**
- **Epigenetic pattern can influence cytokine expression**

Hypotheses and aims of the present study:

- **Because of the possible epigenetic control of cytokine expression and its potential role in periodontitis manifestation an development we investigated the CpG methylation pattern of 22 inflammatory candidate genes** (ATF2, CCL25, CXCL14, CXCL3, CXCL5, CXCL6, FADD, GATA3, IL10RA, IL12A, IL12B, IL13, IL13RAI, IL15, IL17C, IL17RA, IL4R; IL6R, IL6ST, IL7, INHA, TYK2) **in dependence of periodontal status.**

Material and Methods

Patients and controls

Preliminary case-control study

n = 21

All patients and healthy controls were of Caucasian descent

Patients with aggressive periodontitis n = 11		**Periodontitisfree controls** n = 10
clinical manifestation < 30 years	⟺	age ≥ 30 years
Clinical Attachmentloss of > 4mm in 30% ot teeth		Clinical Attachmentloss: ≤ 3.5mm

Exclusion criteria for all participants:

- periodontal treatment during the last 6 months,
- antibiotic therapy during the last 3 month,
- pregnancy
- Occurrence of systemic diseases

Genomic investigations

Sample preparation: gingival biopsies

During dental surgery gingival biopsies were obtained from inflammed tissue from patients with aggressive periodontitis and from periodontal healthy controls

DNA-isolation from gingival biopsies

Preparation of genomic DNA was carried out using the QIAamp® DNA Micro Kit (Qiagen, Hilden, Germany).

Epigenetic methylation pattern

•DNA samples were cleaved using **EpiTect ® Methyl DNA Restriction Kit** (Qiagen, Hilden Germany)

Cleavage reactions for every sample were carried out:

1. no enzyme
2. methylation sensitive enzyme
3. enzyme that is not sensitive for methylation
4. both enzymes

conditions for cleavage: 37°C, 6h; 65°C, 20min; 10°C hold

•For analysing CpG methyltaion pattern **EpiTect® Methyl II Signature PCR Array Human Inflammatory response** (Qiagen, Hilden, Germany) was applied.

Real-Time-PCR was carried out using SYBR-Green (*RT² SYBR Green ROX qPCR Mastermix*, Qiagen, Hilden, Germany)

PCR-System: Applied Biosystems 7500 Real-Time PCR System

PCR-Programm:

1 cycle: 95°C, 10min, hot start for activation of DNA polymerase
3 cycles: 99°C, 30sec, 72°C, 1min
40 cycles: 97°C, 15sec, 72°C, 1min, detection of SYBR-green fluorescence in every cycle4 cleavage reaction

Results and discussion

Clinical characterization of the patient groups

	Patients with aggressive Periodontitis n=11	**Periodontitisfree Controls n=10**	**p values** * Student's T-Test ** Yates corrected p-values
Age mean (±SD), years)	40.6±11.5	37.7±17.1	0.646*
Female gender (%)	54.5	40.0	0.819**
Probing sites			
Approx. plaque index	0.91±0.7	0.6±0.5	0.268*
Bleeding on probing	1.45±0.69	0.6±0.5	0.003*
Clinical probing depth (mm)	7.0±1.7	2.9±1.9	<0.001*
Clinical attachment loss (mm)	8.45±3.0	3.0±2.2	<0.001*

Epigenetic evaluation

Genesymbol	**Patients with aggressive Periodontitis n=11**	**Periodontitisfree Controls n=10**	**p values** * Student's T-Test ** Mann-Whitney-U-Test
CpG methylation (%)			
AFT2	0.1±0.1	0.2±0.3	0.361*
CCL25	**1.7±0.4**	**8.1±16.2**	**0.002****
CXCL14	8.7±4.6	5.7±5.6	0.185*
CXCL3	2.0±1.2	1.6±1.4	0.410*
CXCL5	9.9±16.0	8.9±16.5	0.105**
CXCL6	6.6±3.8	10.1±18.2	0.291**
FADD	0	0	0.602*
GATA3	12.3±15.9	8.3.3±15.5	0.105**
IL10RA	0	0	0.462*
IL12A	0.6±0.3	0.8±0.4	0.090*
IL12B	0.2±0.3	0.4±0.3	0.221*
IL13	2.0±2.4	1.5±1.4	0.573*
IL13RA1	9.1±10.2	23.9±25.8	0.095*
IL15	0.3±0.3	0.2±0.3	0.311*
IL17C	**6.1±1.9**	**26.4±22.0**	**0.007***
IL17RA	0	0.2±0.3	0.622**
IL4R	6.1±17.1	1.5±1.1	0.481**
IL6R	0.2±0.2	0.3±0.3	0.347*
IL7	6.5±20.5	0	0.597**
INHA	0.2±0.2	0.3±0.3	0.683*
TYK2	1.9±1.4	1.7±1.9	0.865*

In gingival inflamed tissue of patients with aggressive periodontitis there was a sginificant reduction in CpG methylation pattern of CCL25 and interleukin 17C compared with tissue of periodontal healthy persons.

Conclusions

In this preliminary study we showed for the first time a differential methylation pattern for CCl25 and IL17C in periodontitis. CCL25 is involved in T-cell development and IL17C plays a role in epithelial immune response induced by bacterial challenge and inflammatory stimuli. The decrease in methylation is presumably accompanied by an increase in gene expression. This could result in a greater availability of CCL25 and IL17C and the induction and progression of periodontal inflammation.

Martin-Luther-Universität Halle-Wittenberg

UKH Universitätsklinikum Halle (Saale)