Genetic variants of interferon-γ and periodontitis in patients with coronary heart disease

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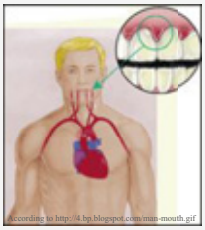
Introduction

Does periodontitis influence CAD?

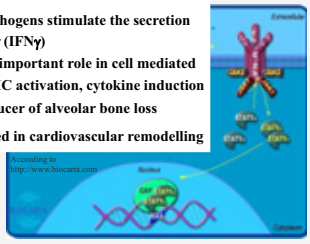


Biological plausability

- Periodontopathogens can enter the bloodstream and affect coronary vessels
- Bacterial toxins can enter the bloodstream and influence coronary processes
- Both diseases share same inflammatory mediators

Interferon-γ is involved in both diseases

- Periodontopathogens stimulate the secretion of interferon-γ (IFNγ)
- IFNγ plays an important role in cell mediated immunity, MHC activation, cytokine induction
- IFNγ is an inducer of alveolar bone loss
- IFNγ is involved in cardiovascular remodelling



Hypotheses of the study

- Genetic variant in IFNγ at position c.-874T>A can influence its expression (AA: low, AT: intermediate, TT: high producer)

SNP c.-874T>A:

- is associated with severity of periodontitis
- is associated with periodontal risk markers (Approximal plaque index, Bleeding on probing, Pocket depth, Clinical attachment loss)
- is associated with the occurrence of periodontopathogens (*A.actinomycetemcomitans, P.intermedia, P.gingivalis, T.forsythia, T.denticola, P.micros, F.nucleatum , C.rectus , E.nodatum , E.corrodens* , and a combination of *C.sputigena, C.gingivalis, and C.ochracea (Csp)*)

Material and Methods

Cardiovascular patients

Longitudinal cohort study (n = 940)

Period of investigation: 10/2009-02/2011, Follow-Up: 11/2010-04/2012

- **Inclusion criteria:**
  in-patient stay subjects with ≥50% stenosis of the main coronary artery
  German caucasian, ≥ 18 years of age, Presence of ≥ 4 teeth
- **Exclusion criteria:**
  periodontal treatment during the last 6 months,
  antibiotic therapy during the last 3 month, pregnancy

CHD patients with severe periodontitis	⟷	CHD patients: no and mild periodontitis
n = 447		n = 493
Clinical attachment loss: ≥ 5mm in ≥30% of the teeth		Clinical attachment loss: < 5mm

Genomic investigations

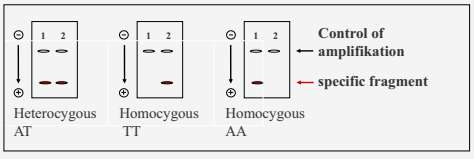
DNA-isolation from EDTA-blood

Preparation of genomic DNA was carried out using the blood extraction kit (Qiagen, Hilden, Germany).

Genotype specific PCR of IFNγ

- For Genotyping CYTOKINE Genotyping array CTS-PCR-SSP Tray kit of the Collaborative Transplant Study, Department of Transplantation Immunology of the University Clinic of Heidelberg was applied.
- The PCRs were performed using sequence specific primers for detection of possible alleles prepipetted and lyophilized in thin-walled plastic 96-well PCR trays.
- For every PCR 10µl of a Mastermix containing 1U Taq-Polymerase (Invitek), 100ng genomic DNA, 5% glycerol, and PCR reaction buffer was added.
- PCR-program (2min 94°C; 10 cycles: 15sec 94°C, 1min 64°C; 20 cycles: 15sec 94°C, 50sec 61°C, 30sec 72°C)
- After cycling was completed, the PCR products were loaded onto a 2% agarosegel for electrophoresis.

After electrophoresis, the ethidium bromide stained gel is photographed and interpreted.



Evaluation of periodontopathic bacteria in subgingival pockets

Subgingival sampling

Paper points for collection of subgingival samples were used to bind periodontopathogens of the deepest pocket of each quadrant.

DNA-isolation, Multiplex-PCR and bacteria specific hybridization

11 periodontopathogens were evaluated *(A.actinomycetemcomitans, P.intermedia, P.gingivalis, T.forsythia, T.denticola, P.micros, F.nucleatum , C.rectus , E.nodatum , E.corrodens* , and a combination of *C.sputigena, C.gingivalis, and C.ochracea (Csp))*

All procedures were carried out by a commercial laboratory applying reagent of HAIN-Diagnostik

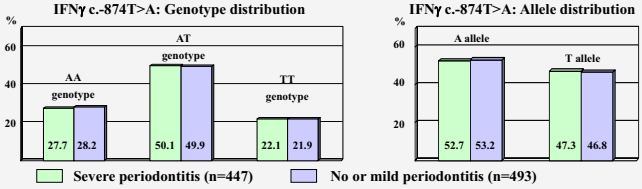
Results and discussion

Clinical characterization of the patient group

Patients with	Severe periodontitis	No or mild periodontitis	p-value
Age (mean years)	67.4±10.8	66.3±11.2	
Female gender (%)	21.4	30.2	
Current smokers (%)	16.0	8.9	0.377
Plaque index	1.4±0.8	0.75±0.6	0.003
Bleeding on probing (%)	12.4±15.0	7.2±12.4	0.001
Clinical probing depth (mm)	4.1±0.8	3.0±0.6	<0.001
Clinical attachment loss (mm)	5.5±1.3	3.3±0.7	<0.001
Diabetes mellitus (%)	37.0	31.6	<0.001
			0.098

Genetic evaluation

Genetic association to severity of periodontitis



- The genotype and allele frequencies of the c.-874T>A polymorphism were no risk indicators for the severity of periodontitis in patients with coronary heart disease.

Genetic association to periodontal risk markers

- The genotype and allele frequencies of the c.-874T>A polymorphism were no risk indicators for clinical periodontal risk markers including plaque index, bleeding on probing, clinical probing depth and clinical attachment loss.

Genetic association to the occurrence of periodontopathogens

IFNγ c.-874T>A: *P.intermedia*

	p-value	OR	95% CI
smoking	0.004	0.535	0.351-0.817
AA genotype	0.008	0.744	0.599-0.924
Age	0.004	1.016	1.005-1.027
Plaque index	0.003	0.994	0.990-0.998
A allele	0.009	0.751	0.605-0.932

IFNγ c.-874T>A: *E.corrodens*

	p-value	OR	95% CI
age	0.009	1.31	1.07-1.603
AA genotype	0.001	0.97	0.957-0.983
Age	0.012	1.296	1.059-1.587
gender	0.033	0.784	0.626-0.981
A allele	0.001	0.972	0.962-0.981

- In a complex risk model (forward stepwise binary logistic regression analysis) considering age, gender, smoking diabetes, plaque index as potential confounders the SNPs c.-874T>A could be proven as a independent indicator for the occurrence of *P.i.* and *E.c.*

Despite the c.-874T>A polymorphism of the gene encoding for IFNγ could be shown to be associated with subgingival occurrence of *P.intermedia* and *E.corrodens* there was no evidene that it is a risk indicator for the severity of periodontitis in coronary patients.