Periodontitis and SNPs in TNFα gene in patients with Crohn's disease

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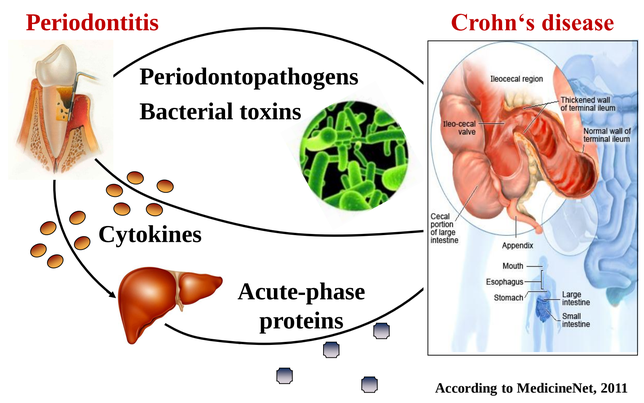
Introduction

Putative interrelation between periodontitis and Crohn's disease



According to MedicineNet, 2011

Biological plausability

- Periodontopathogens can enter the bloodstream and affect tissue of bowels
- Bacterial toxins can enter the bloodstream and influence inflammatory processes
- Both chronic inflammatory diseases share same mediators

TNF-α is involved in both chronic inflammatory diseases

- TNF-α is an inducer of alveolar bone loss and collagen degradation leading to periodontitis
- TNF-α promotes inflammatory processes which can causes clinical problems associated with CD
- Functional important SNPs in TNF-α gene are discussed to be invloved in the etiology of both diseases

Hypotheses and aims of the present study:

- Because of the biological plausibility of the relationship of both inflammatory diseases, we hypothesize comparable genetic risk pattern regarding TNF-α gene in patients suffering from CD and in patients with generalized periodontitis.
- Investigation of the impact of TNF-α SNPs rs1800629 and rs361525
  - on the occurrence of Crohns's disease
  - on the occurrence of generalized periodontitis including chronic and aggressive periodontitis

Material and Methods

Patients and controls

Case-control study

n = 400

All patients and healthy controls were of Caucasian descent

Patients with Crohn's disease
n = 142
-diagnosis of CD was established by clinical, radiologic, endoscopic, and histologic criteria (according to Lennard-Jones (1989))
- age ≥ 18years

Healthy controls
n = 91
-age ≥ 30 years
- Periodontitis free
-CAL: ≤ 3.5mm
- Crohn's disease free

Patients with periodontitis
n = 167
- Aggressive periodontitis (AgP)
-Age of onset > 35 years
-CAL: ≥ 4mm ≥ 30% teeth
-CAL inconsistent with amount of mineralized plaque, **n=90**
- Chronic periodontitis (CP)
-CAL: ≥ 4mm ≥ 30% teeth
-amount of CAL was consistent with presence of mineralized plaque, **n=77**

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

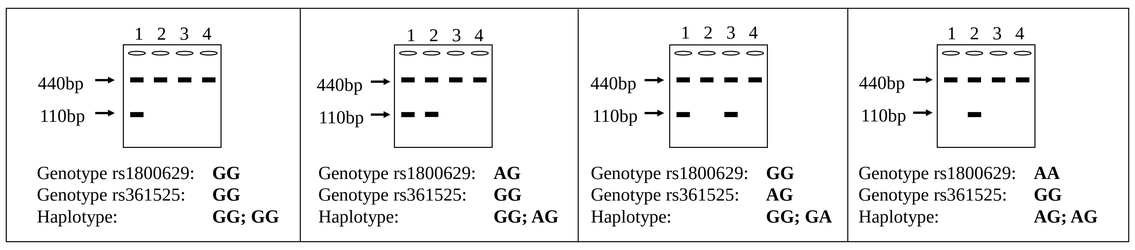
DNA-isolation from EDTA-blood

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

Genotype specific PCR of TNFα

• 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.
Lane 1: sequence specific fragment at 110bp: **G** at rs1800629; **G** at rs361525
Lane 2: sequence specific fragment at 110bp: **A** at rs1800629; **G** at rs361525
Lane 3: sequence specific fragment at 110bp: **G** at rs1800629; **A** at rs361525
Lane 4: sequence specific fragment at 110 bp: **A** at rs1800629; **A** at rs361525

Observed gel patterns



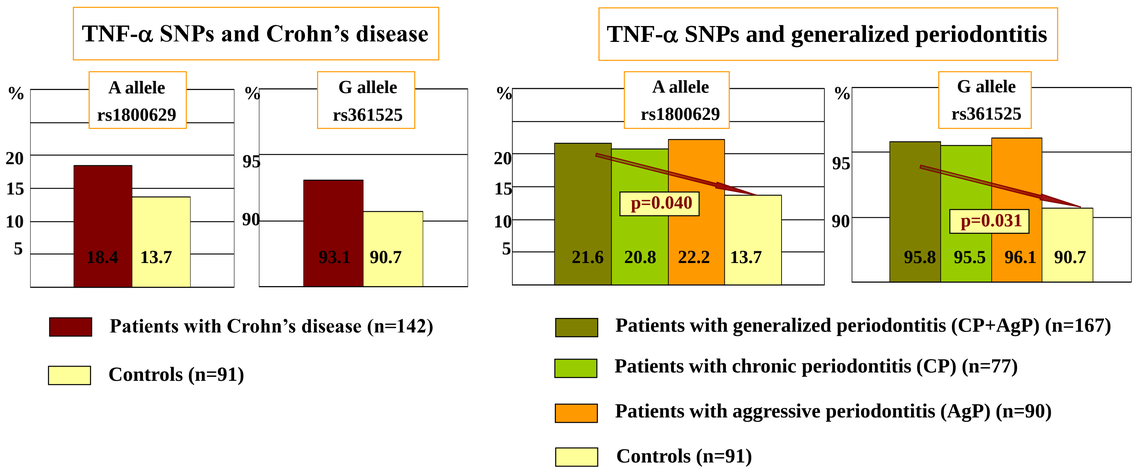
Results and discussion

Clinical characterization of the patient groups

Patients with	Crohn's disease n=142	Periodontitis CP+AgP n=169	Periodontitis Chronic n=77	Periodontitis Aggressive n=90	Controls n=91
Age mean (±SD), years)	36.8±9.8*	44.4±10.4	49.0±9.2	40.5±9.7*	46.7±11.0
Female gender (%)	51.4	62.9	62.3	63.3	52.2
Current smokers (%)	38.0**	30.1	23.7	35.6**	20.7
Approx. plaque index (%)	40.2±19.7	52.5±31.1	55.9±30.8	49.6±31.3	47.0±21.1
Bleeding on probing (%)	72.4±23.3	73.1±27.2***	69.1±27.5***	76.5±26.7***	45.8±24.0
Clinical probing depth (mm)	3.6±0.8***	5.4±1.4***	5.1±1.3***	5.6±1.5***	2.6±0.7
Clinical attachment loss (mm)	3.8±1.0*	6.2±1.6*	5.8±1.6*	6.5±1.5*	3.0±0.8

*p<0.05, Mann-Whitney U-Test
** p<0.05, Yates corrected p-values
*** p<0.05, Student's T-Test

Genetic evaluation: bivariate analyses



In bivariate analyses the A-allele of TNF-α SNP rs1800629 and G-allele of TNF-α SNP rs361525 were risk indicators for generalized periodontitis but not for Crohn's disease.

TNF-α SNPs and generalized periodontitis: multivariate analyses

A-allele TNF-α SNP rs1800629

	p-value	OR	95% CI
Gender (♂)	0.009	1.65	1.13 – 2.41
Appr. plaque ind.	0.014	1.008	1.002 – 1.01
Age	0.049	1.02	1.00 – 1.04
Smoking	0.047	1.56	1.005 – 2.43
A-allele	0.035	1.73	1.04 – 2.86

G-allele TNF-α SNP rs361525

	p-value	OR	95% CI
Gender (♂)	0.011	1.63	1.12 – 2.39
Appr. plaque ind.	0.013	1.009	1.002 – 1.02
Age	0.041	1.02	1.001 – 1.04
Smoking	0.043	1.58	1.01 – 2.46
G-allele	0.017	2.51	1.17 – 5.35

In a complex risk model (forward stepwise binary logistic regression analysis) considering age, gender, smoking, approximal plaque index as potential confounders the A-allele (rs1800629) and G-allele (rs361525) could be proven as independent risk indicators for generalized periodontitis.

Conclusions

In this case-control study the A allele of TNF-α polymorphism rs1800629 and the G allele of TNF-α polymorphism rs361525 were proven to be significant indicators for generalized periodontitis but not for Crohn's disease.

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