Are Genetic Variants of C-Reactive Protein Prognostic Markers for Further Cardiovascular Events in Patients With Coronary Heart Disease?

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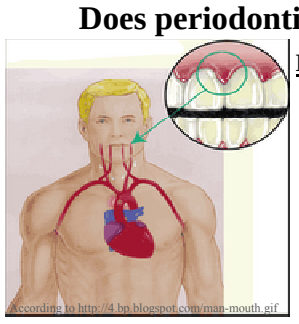
Introduction

Does periodontitis influence CAD?



Biological plausability

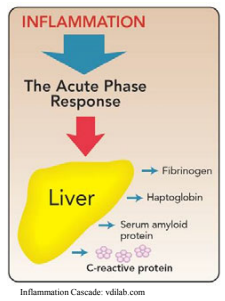
- Periodontopathogens can enter the bloodstream and affect coronary vessels
- Bacterial toxins can enter the bloodstream and influence coronary processes
- Both diseases share same inflammatory mediators

C-reactive protein is involved in both diseases

- CRP plays as an acute phase protein an important role in inflammation
- CRP levels are increased in perio-dontitis and cardiovascular diseases

Periodontopathogens can stimulate CRP secretion

CRP is involved in **cardiovascular remodelling**



Hypotheses of the study

- Genetic variants in CRP (#1q21-q23) rs1800947 and rs 1417938 can influence its expression

SNPs:
- are associated with CRP expression in CAD patients?
- are prognostic markers for cardiovascular events (myocardial infarction, stroke, cardiovascular death)?

CRP:
- Circulating CRP level is a prognostic marker for cardiovascular events (myocardial infarction, stroke, cardiovascular death)?

Material and Methods

Cardiovascular patients

Longitudinal cohort study (Clinical Trials.gov Identifier: NCT01045070; 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
- **Baseline clinical examination:**
  Anamnesis → age, gender, smoking status, medication, existing diseases
  Dental examination → plaque index, bleeding on probing, pocket depth, clinical attachment loss

CAD patients with severe periodontitis (n = 447)
Clinical attachment loss:
≥ 5mm in ≥30% of the teeth

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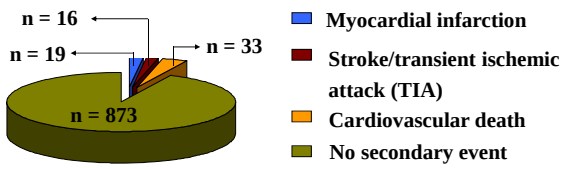
CAD patients: no or mild periodontitis (n = 493)
Clinical attachment loss:
< 5mm

- **1 year follow up:**
  Evaluation of possible secondary cardiovascular events (telephone surveys or written surveys)



Genomic investigations

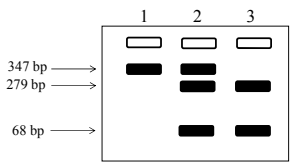
DNA-isolation from EDTA-blood

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

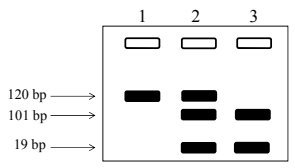
Genotyping of rs 1800947 and rs 1417938

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)
PCR reactions were carried out using Mastermix (Promega, Mannheim, Germany)
PCR-program (2min 94°C; 10 cycles: 15sec 94°C, 1min 64°C; 20 cycles: 15sec 94°C, 50sec 61°C, 30sec 72°C)
rs1800947 → forward: cag ttt tac agt ggg tgg gtc, reverse: ccc gcc agt tca gga cat tag, restriction enzyme: BsiHKA I
rs1417938 → forward: acc ccc at acct cag atc gaa, reverse: gac gtg acc atg gag aag ct, restriction enzyme: Tfi I

Schematic illustration of PCR fragments after restriction cleavage



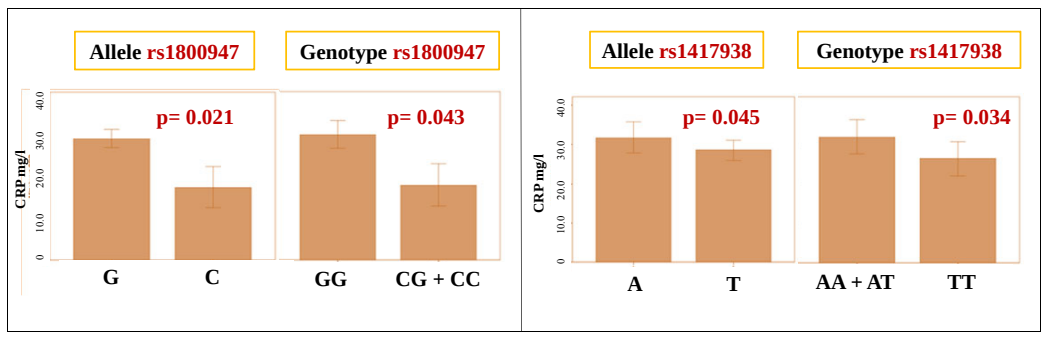
rs1800947
1 homozygous GG
2 heterozygous CG
3 homozygous CC



rs1417938
1 homozygous AA
2 heterozygous AT
3 homozygous TT

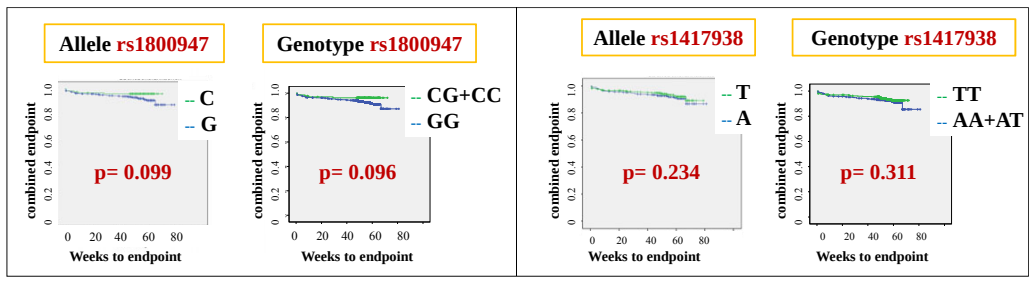
Results and discussion

CRP serum level in dependence of SNPs



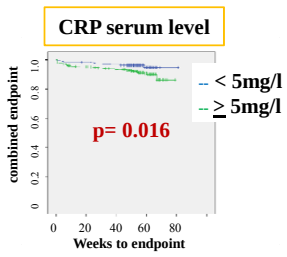
- **Allele and genotype of both SNPs rs1800947 (G-allele, GG-genotype) and rs1417938 (A-allele and AA + AT-genotypes) are significantly associated with increased CRP serum level.**

CRP SNPs as prognostic markers for CAD
→ Kaplan-Meier survival curves and log-rank-test



- **The genotype and allele frequencies of both SNPs rs1800947 and rs1417938 were not prognostic markers for adverse cardiovascular events (myocardial infarction, stroke/TIA, cardiovascular death) regarding the one-year outcome.**

Increased CRP serum level as a prognostic marker for CAD
→ Kaplan-Meier survival curve, log-rank-test, cox regression



Variable	Regression coefficient	p-value	Hazard Ratio	95 CI lower - upper
CRP level ≥5mg/l	0.790	0.017	2.203	1.15 – 4.22
Age	0.004	0.731	1.004	0.98 – 1.03
Male gender	0.172	0.522	1.188	0.70 – 2.02
BMI	-0.066	0.028	0.936	0.88 – 0.99
Smoking	0.086	0.835	1.090	0.49 – 2.45
Diabetes	0.524	0.039	1.689	1.03 – 2.78

- **In a complex risk model (cox regression) considering age, gender, body mass index (BMI), smoking, and diabetes as potential confounders, the CRP serum level could be proven as an independent prognostic indicator for adverse cardiovascular events regarding the one-year outcome.**

Conclusions

CRP polymorphisms rs1800947 and rs1417938 are associated with CRP serum level in our cohort of CAD patients. Despite the CRP level as an independent prognostic marker for adverse cardiovascular events (myocardial infarction, stroke/TIA, cardiovascular death), neither polymorphism could be proven to have prognostic value regarding the one year outcome.

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