

Prevalence of OPG and IL-1 gene polymorphisms in chronic periodontitis

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Abstract

Aim: To investigate the association of polymorphisms in the osteoprotegerin (OPG) and interleukin 1 (IL-1) genes with chronic periodontitis (CP).

Material and Methods: One hundred and ninety-four individuals (97 CP patients, 97 controls) were genotyped for the OPG polymorphisms Lys3Asn and Met256Val and for the IL-1 polymorphisms IL-1A (–889C/T) and IL-1B (+3953C/T).

Results: The homozygous variants coding for Lys3 were present at a higher frequency, whereas Asn3 and Met256 were present at a lower frequency in CP patients/controls (Lys3: 31%/25%, Asn3: 23%/32% and Met256: 66%/73%). Heterozygosity for Lys3Asn was observed at a higher frequency in CP patients/controls (46%/43%). Homozygosity for the Val256 genotype was observed in two CP patients (one in controls). Met256Val heterozygosity was more prevalent in CP patients/controls (32%/20%). All differences were statistically not significant between CP patients and controls. In contrast, both IL-1 polymorphisms were statistically significant. The heterozygous variant for IL-1A was present in 32% of the CP patients and in 20% of the controls (homozygosity (patients/controls) CC: 10%/21% and TT: 55%/33%). Heterozygosity for IL-1B was observed in 37% of the CP patients versus 34% in the controls (homozygosity (patients/controls) CC: 26%/57% and TT: 37%/9%).

Conclusion: While the association between the IL-1 polymorphisms and CP was confirmed, no association between the OPG polymorphisms and CP could be found.

Key words: chronic periodontitis; interleukin; interleukin-1A; interleukin-1B; osteoprotegerin; polymorphism

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Periodontitis is a multifactorial disease (Page et al. 1997, Ehmke et al. 2003). While the bacterial infection is a primary cause, the incidence and severity of the disease is determined by the host response to this infection. The host response is influenced by several possible risk factors including variable

factors, such as oral hygiene and smoking, and invariable factors including age, gender and genetic factors. Initial studies indicated that not only aggressive periodontitis but also chronic periodontitis (CP) may have a genetic background (Michalowicz et al. 2000). In the past, several studies have failed to show an association between periodontal disease and polymorphisms in candidate genes including interleukin (IL) IL-1, IL-2, IL-4, tumor necrosis factor (TNF) TNF- α , TGF- β 1, matrix metalloproteinase (MMP) MMP-1, MMP-9 and TIMP-2 (Borrell & Papanou 2005, Loos et al. 2005, Astolfi et al. 2006). In contrast, other authors reported an association between polymorphisms in the IL-1A and IL-1B

genes and the incidence of periodontitis (Pociot et al. 1992, Kornman et al. 1997, McDevitt et al. 2000, Cullinan et al. 2001). The involvement of IL-1 gene polymorphisms in the pathogenesis of CP is still controversial (Rogers et al. 2002, Sakellari et al. 2003, Borrell & Papanou 2005, Heitz-Mayfield 2005, Loos et al. 2005, Tonetti & Claffey 2005).

Bone resorption by osteoclasts is one of the key symptoms of periodontal disease. Osteoclastogenesis is controlled by three members of the TNF and TNF receptor superfamily: receptor activator of nuclear factor κ B ligand (RANKL), receptor activator of nuclear factor κ B (RANK) and osteoprotegerin (OPG). RANKL, which is expressed on the

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membrane of bone marrow-derived stromal cells, activates the differentiation of precursor cells into osteoclasts, enhances the activity of mature osteoclasts and inhibits osteoclast apoptosis by binding to its functional receptor RANK. OPG is a soluble receptor blocking the interaction between RANK and RANKL. Several studies have investigated the influence of a change of the OPG/RANKL ratio on the periodontal tissues. Their observations suggest that local changes of the OPG/RANKL ratio may be of pathogenic relevance for the occurrence of periodontal disease (Crotti et al. 2003, Mogi et al. 2004, Bostanci et al. 2007).

Polymorphisms in the OPG gene have a potential impact on the structural and functional properties of the protein and, therefore, may change the OPG/RANKL ratio as well. So far, the influence of allelic variants on the expression and biological activity of OPG in the context of periodontitis has not yet been investigated. For this reason, in the present study the hypothesis was appraised that the genotype and allele frequency of the two polymorphisms in the OPG gene differ in patients with CP compared with periodontally healthy controls.

The following allelic variants were investigated: OPG Lys3Asn (rs2073618, exon 1, chromosome 8, G/C) and OPG Met256Val (rs7844577, exon 4, chromosome 8, T/C). The OPG Lys3Asn dimorphism has been associated with loss in bone mineral density and increased susceptibility to osteoporosis (Zhao et al. 2005). Met256Val is a recently identified single nucleotide polymorphism (SNP) that has not yet been analysed in detail. It may potentially affect the OPG function, because this dimorphism involves a non-conservative amino acid substitution within the mature OPG protein. We included the analysis of the allele frequencies of two IL-1A and IL-1B polymorphisms [IL-1A at position -889 (rs1800587, promoter, chromosome 2, C/T) and IL-1B at position +3953 (rs1143634, exon 5, chromosome 2, C/T)], which have been associated previously with CP (Kornman et al. 1997).

Material and Methods

Study cohorts

A total of 194 unrelated, non-smoking [not smoked in the last 5 years

Table 1. Characteristics of the study population

	Patients	Controls
Age (years) median (25/75 percentiles)	55 (46/60)	50 (41/57)
Ethnic origin <i>n</i> (%)		
Caucasian	97 (100)	97 (100)
Gender <i>n</i> (%)		
Male	54 (56)	58 (60)
Female	43 (44)	39 (40)
Smoking habit <i>n</i> (%)		
Non-smoking	97 (100)	97 (100)
Number of patients with at least three teeth in at least two different quadrants with CAL>3.5 mm, PPD≥3 mm <i>n</i> (%)	97 (100)	0 (0)

PPD, probing pocket depth; CAL, clinical attachment level.

(Kornman et al. 1997)] Caucasian individuals 35–77 years of age (112 males, 82 females) were recruited from the patient pool of the Department of Operative Dentistry and Periodontology at the University of Regensburg (Table 1). All patients had to have at least 18 remaining teeth and to be in good general health. Exclusion criteria were: oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, a history of systemic or local disease with influence on the immune system, diabetes mellitus, hepatitis or HIV infection, immunosuppressive chemotherapy or current pregnancy or lactation. After completion of personal medical and dental questionnaires, written informed consent was obtained. The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Regensburg according to the declarations of Helsinki (1975) and Tokyo (1983).

The diagnosis of CP was established on the basis of radiographic and clinical parameters and included physical examination, medical and dental history, as well as measurements of probing pocket depth (PPD), clinical attachment level (CAL), tooth mobility and bleeding on probing. Furthermore, the periodontal screening index (PSI) was recorded (Meyle & Jepsen 2000). All subjects were assigned to clinical categories with regard to their PPD severity and PSI code. Periodontally diseased patients (test, *n* = 97) had to have at least three teeth with CAL>3.5 mm, PPD≥3 mm in at least two different quadrants and PSI codes of three or four in at least two different sextants. The control group comprised *n* = 97 individuals with no symptoms of periodontitis.

Genotype distribution for all investigated SNPs in both study cohorts was tested concerning their being in

Hardy–Weinberg equilibrium (Guo & Thompson 1992).

Sample collection and DNA isolation

Venous blood anticoagulated with EDTA was obtained from each individual and genomic DNA was extracted from 2 ml whole blood using the QIAamp Blood DNA Midi Kit (Qiagen, Hilden, Germany). A BIOMEK FX pipeting robot was used for DNA sample handling (Beckman Coulter, Fullerton, CA, USA). The DNA was transferred into microtitre plates, the concentration was determined using the Picogreen Assay (Molecular Probes, Eugene, OR, USA) and all DNA samples were normalized to a concentration of 10 ng/μl. DNA normalization was performed on a Biomek FX pipeting robot using the BFX-Normalization Software (Beckman Coulter).

SNPs

The following allelic variants were investigated: OPG Lys3Asn (rs2073618, exon 1, chromosome 8, G/C), OPG Met256Val (rs7844577, exon 4, chromosome 8, T/C) as well as IL-1A at position -889 (rs1800587, promoter, chromosome 2, C/T) and IL-1B at position +3953 (rs1143634, exon 5, chromosome 2, C/T).

Oligonucleotide primers and probes

Oligonucleotide primers specific for the human OPG and IL-1 genes, respectively, were designed on the basis of published sequence information from the GenBank database. Detailed primer and probe sequences can be requested from the authors. TaqMan probes were labelled with a fluorophore at their 5' end (FAM or VIC) and a non-fluorescent quencher attached to a minor-groove binder at their 3' end (Kutyavin

et al. 2000). After PCR amplification, the FAM and VIC fluorescence signals were assessed and the genotypes were determined in a two-dimensional panel.

TaqMan-based genotyping

TaqMan PCR assays were performed in an MWG standard thermocycler (MWG, Ebersberg, Germany); the end-point fluorescence intensity was measured using an ABI Prism 7900 HT Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The PCR master mixture contained 2.5 μ l of 2 \times TaqMan Universal PCR Master Mix, 0.25 μ l of a 20 \times primer and probe mix (Applied Biosystems), 1 μ l of DNA (10 ng/ μ l) and 1.25 μ l H₂O. Cycling was performed in 384-well optical plates. Following a 10-min. incubation at 95°C, PCR amplification was performed for 40 cycles (cycle profile: 15 s at 92°C, 1 min. at 60°C). For each SNP, samples with known OPG and IL-1 genotypes, respectively, were used as controls. The Sequence Detector Software SDS 2.0 (Applied Biosystems) was used for data analysis.

Statistical analyses

Genotypes for all polymorphisms were determined by simple counting. The χ^2 -test was used to evaluate statistical differences between periodontitis patients and the control group. *p*-values ≤ 0.05 were considered to be statistically significant.

Results

The median age of the CP patients was 55 years (25 and 75 percentiles: 46 and 60). The median age of the control population was 50 years (25 and 75 percentiles: 41 and 57). The distributions of gender for the CP-positive patients were 44% female and 56% male, and for the healthy controls 40% female versus 60% male, respectively (Table 1). Genotype distribution for all investigated SNPs for the control group is in Hardy-Weinberg equilibrium (χ^2 -test values and *p*-values: Lys3Asn 0.19 and 0.22, Met256Val 0.85 and 1, IL-1A - 889 0.53 and 0.52, IL-1B +3953 0.27 and 0.28).

Genotyping of the patient and control groups revealed more CP patients displaying homozygosity for the wild-type Lys3 allele in the OPG gene in comparison with the healthy individuals (Table 2). Asn3 homozygosity was present in less CP patients compared with the healthy subjects. The genotype frequencies for Lys3Asn heterozygous individuals were marginally higher in the test group than in the control group.

Homozygosity for the Met256 allele tended to occur in less CP patients than in periodontically healthy patients. The Val256Val genotype was found in two individuals of the CP cohort and in one of the healthy controls. Met256Val heterozygosity was more frequent in the patient group than in the control population.

Taken together, the present study did not reveal statistically significant differ-

ences in the genotype frequencies for the OPG Lys3Asn and Met256Val dimorphisms between patients with CP and healthy individuals (Lys3Asn: *p* = 0.352 and Met256Val: *p* = 0.146) (Table 2).

Furthermore, the analysis of the subgroups did not reveal any significant influence of gender or age regarding the distribution of the genotype or allele frequency of these investigated SNPs (data not shown).

Assessment of the genotype frequencies for the IL-1A - 889 polymorphism demonstrated that only a minority of the CP patients (10%) but many of the healthy individuals displayed C/C homozygosity (Table 2). The IL-1A - 889 T/T genotype was present in the majority of the patients and only in a few controls. IL-1A - 889 C/T heterozygosity was found in less CP patients than in healthy individuals.

Genotyping of the IL-1B +3953 C/T SNP revealed the presence of C/C homozygosity in less CP patients than in healthy subjects (Table 2). Homozygosity for the IL-1B +3953 T-allele was observed in a majority of the CP patients and a minority of the healthy subjects.

The frequencies of the IL-1B +3953 C/T heterozygosity were higher in the patient than in the control group. To recapitulate, these results revealed significant differences (*p* < 0.001) in the genotype frequencies of both IL-1 polymorphisms between patients with CP and healthy controls (Table 2).

Table 2. Distribution of the investigated genotypes for osteoprotegerin (OPG) and interleukin-1 (IL-1) in patients with chronic periodontitis and healthy controls

Genotype	All	Patients	Controls	<i>p</i> value for patients versus controls
OPG Lys3Asn	<i>n</i> = 188 (100%)	<i>n</i> = 93 (100%)	<i>n</i> = 95 (100%)	NS, <i>p</i> = 0.352
GG	53 (28%)	29 (31%)	24 (25%)	
CC	51 (27%)	21 (23%)	30 (32%)	
GC	84 (45%)	43 (46%)	41 (43%)	
OPG Met256Val	<i>n</i> = 183 (100%)	<i>n</i> = 90 (100%)	<i>n</i> = 93 (100%)	NS, <i>p</i> = 0.146
TT	132 (72%)	59 (66%)	73 (79%)	
CC	3 (2%)	2 (2%)	1 (1%)	
TC	48 (26%)	29 (32%)	19 (20%)	
IL-1A	<i>n</i> = 184 (100%)	<i>n</i> = 95 (100%)	<i>n</i> = 89 (100%)	S, <i>p</i> < 0.001
CC	29 (16%)	10 (10%)	19 (21%)	
TT	81 (44%)	52 (55%)	29 (33%)	
CT	74 (40%)	33 (35%)	41 (46%)	
IL-1B	<i>n</i> = 185 (100%)	<i>n</i> = 93 (100%)	<i>n</i> = 92 (100%)	S, <i>p</i> < 0.001
CC	77 (41%)	24 (26%)	53 (57%)	
TT	42 (23%)	34 (37%)	8 (9%)	
CT	66 (36%)	35 (37%)	31 (34%)	

NS, not significantly different; S, significantly different.

Discussion

In the present study, the association of CP with polymorphisms in the gene coding for OPG was investigated. OPG is an attractive candidate gene for involvement in CP for several reasons. First, the protein has been directly implicated in the homeostasis of bone metabolism. Second, OPG is expressed by human periodontal ligament cells, and finally, an increased RANKL/OPG ratio in the GCF has been associated with periodontal disease (Crotti et al. 2003, Mogi et al. 2004).

Our results dismiss an association of the two OPG variants with CP. These findings do not preclude the possibility of other allelic variants in the OPG gene being associated with the pathogenesis of CP. In this context, it is conceivable that different polymorphisms in the promoter regions and the 3' UTR (untranslated region) of the OPG gene may show an association with CP.

In the present study, the lack of differences in the prevalence of the investigated polymorphisms between CP patients and healthy controls may also be attributed to the relatively small sample size. Nevertheless, our sample size resembles or outnumbers the sample sizes of many studies in the current literature (Scarel-Caminaga et al. 2002, 2003, Sakellari et al. 2003, de Souza et al. 2005). A by far greater population might be necessary to rule out small differences. However, this appears unlikely because the frequencies of the investigated polymorphisms in our control cohort corresponded well with those reported in the NCBI SNP database (NCBI SNP database 2006a,b,c,d), suggesting that our study populations were representative. Additionally, the genotype distribution for the investigated SNPs for the control group is in Hardy–Weinberg equilibrium (Guo & Thompson 1992).

Another important reason for the observed lack of a significant association may be the fact that a threshold for periodontitis, i.e. case definition was set at a relatively low severity (at least three teeth with CAL \geq 4 mm in at least two different quadrants and PSI codes of three or four in at least two different sextants).

Two allelic variants in the IL-1A and IL-1B genes (IL-1A – 889, IL-1B +3953) have been implicated in CP (Kornman et al. 1997, McDevitt et al. 2000, Cullinan et al. 2001). We therefore determined whether the

observed absence of an association of the OPG SNPs with CP is paralleled by a lack of association of polymorphisms in the IL-1A and IL-1B genes with CP. In contrast to the investigated OPG SNPs, statistically significant differences could be found for the IL-1A – 889 and the IL-1B +3953 gene polymorphisms between CP patients and the control group. Additionally, the genotype distribution for the investigated SNPs for the control group is in Hardy–Weinberg equilibrium (Guo & Thompson 1992). Thus, our data indicate an association of IL-1A – 889 T and the IL-1B +3953 T alleles with CP. This confirms previous findings by Kornman et al. (1997), who found an increased risk for severe CP in non-smoking Caucasian patients being positive for these two alleles. An additional study demonstrated accordant findings (McDevitt et al. 2000), which additionally may valorize our study population. However, several other recent studies failed to find an association between IL-1 polymorphisms and the incidence of periodontitis (Rogers et al. 2002, Sakellari et al. 2003, Borrell & Papapanou 2005, Heitz-Mayfield 2005, Loos et al. 2005, Tonetti & Claffey 2005). The still ongoing controversy concerning the role of IL-1 SNPs in the pathogenesis of CP strongly suggests the involvement of additional factors that may codetermine the susceptibility to this disease.

Conclusion

Within the limits of this study, genotyping could not reveal an association between the two investigated polymorphisms in the OPG gene with CP. In contrast, the investigated polymorphisms of IL-1A/B confirmed previous findings of an increased susceptibility for CP.

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Clinical Relevance

Scientific rationale for the study: In the past, many studies have investigated the association between allelic variants of certain genes and periodontitis. Because the OPG/RANKL system is an important factor regulat-

ing osteoclastogenesis and bone metabolism, the association of polymorphisms in the OPG gene with CP has been investigated.

Principal findings: Analysis failed to show statistically significant associa-

tions between the investigated OPG polymorphisms and CP.

Practical implications: The investigated OPG polymorphisms cannot be used for detection of patients having a higher risk for CP.