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No correlation of five gene polymorphisms with periodontal conditions in a Greek population

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Abstract

Background: Various studies have examined possible correlations between a number of cytokine gene polymorphisms and periodontal disease in populations of different origins. The present study sought the correlation between four single-nucleotide polymorphisms (*IL1A*+3954, *IL1B*+4845, *TNFA* −308, *COL1A1* Sp1), a variable number of tandem repeats polymorphism (*IL1RN* intron 2) and periodontal conditions in subjects of Greek origin.

Methods: One hundred and ninety-two healthy subjects, stratified as non-periodontitis and periodontitis (chronic and aggressive) cases, participated in the present study. Genotyping was performed by polymerase chain reaction-based techniques using the primers and conditions described in the literature. The frequencies of genotypes between study groups were compared using Genepop v3.3 genetic software and Instat statistical package.

Results: No differences were observed among the groups concerning the distributions of genotypes under investigation.

Conclusions: Carriage rates of the polymorphisms under investigation in systemically healthy subjects of Greek origin are well within the range reported for Caucasians but these polymorphisms cannot discriminate between non-periodontitis and periodontitis (chronic or aggressive) cases.

Key words: *COL1A1*; Greek; *IL1A*; *IL1B*; *IL1RN*; periodontitis; SNP; *TNFA*

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During the last few years, various reports in the literature refer to possible genetic markers for periodontal disease initiation and progression as reviewed in a recent paper by Loos et al. (2005). Up to now, the majority of these reports have investigated polymorphisms in the genes encoding components of the host–response system such as pro-inflammatory cytokines. The *IL1* cluster of genes and in particular the ''composite genotype'' (allele 2 at *IL1A* −889 or +4845 and *IL1B* +3954), as described by Kornman et al. (1997), is the most extensively studied in relation to frequencies of distribution in populations of different ethnic origin, periodontal disease severity, microbial parameters and outcome of various treatment modalities (Ehmke et al. 1999, McDevitt et al. 2000, Socransky et al. 2000, Cullinan et al. 2001, Laine et al. 2001, Papapanou et al. 2001, Rogers et al. 2002, Christgau et al. 2003, Jepsen et al. 2003). The original observation of Kornman et al. (1997) in non-smokers has, however, been challenged in more recent reports (Meisel et al. 2003) and in non-Caucasian populations (Armitage et al. 2000, Anusaksathien et al. 2003) and, in addition, it is suggested that the biological basis of this relationship, i.e. the overproduction of IL-1β in carriers of allele 2, as shown by Pociot et al. (1992), appears to require further documentation (Mark et al. 2000, Dominici et al. 2002, Taylor et al. 2004). The intron 2 of the *IL1RN* gene, which encodes the interleukin-1 receptor antagonist (IL-1 ra), contains a sequence with a variable number of tandem repeats of 86 bp (VNTRs), resulting in five different alleles. The relationship of these alleles (especially carriage of allele 2) with periodontal disease has not been as extensively reviewed as the ''composite genotype'' (Laine et al. 2001, Meisel et al. 2002), although a number of reports suggest a complex allele-dependent regulating effect of this gene on IL-1 production (Santtila et al. 1998, Vamvakopoulos et al. 2002).

Polymorphisms in genes encoding other important immunological molecules, such as TNF-α, also appear as promising candidates for genetic markers of periodontitis. Although the same caveat of limited data in the literature concerning the exact biological impact of these polymorphisms applies for *TNFA* (Galbraith et al. 1998, 1999, Hajeer & Hutchinson, 2001), a number of studies in populations of different origins have

investigated their relationship with susceptibility or severity of periodontal disease as reviewed by Loos et al. (2005).

Few data in the literature refer to frequencies of cytokine encoding gene polymorphisms in populations of Greek origin (Costeas et al. 2003) or their possible correlations to periodontal disease initiation or progression (Sakellari et al. 2003).

Type 1 collagen, the major protein of bone, is encoded by the *COL1A1* and the *COL1A2* genes. A single-nucleotide polymorphism (SNP) affecting a binding site for the transcription factor Sp1 in the *COL1A1* gene was identified in 1996 (Grant et al. 1996). The polymorphic Sp1 site lies within the first intron of the *COL1A1* gene in a region known to participate in the regulation of collagen transcription. Functional studies in osteoblasts have shown that collagen produced from carriers of this G to T polymorphism, defined as an ''s'' allele, has an increased ratio of a1 (1) protein relative to a2 (2) and in addition the yield strength of bone, bone mass and bone quality appear to be affected in individuals with this genetic variant (Mann et al. 2001). This *COL1A1* Sp1-binding site polymorphism has been extensively studied as a candidate gene for osteoporosis, and meta-analysis studies have shown that carriage of the ''s'' allele is associated with prevalent osteoporotic fractures and BMD in Caucasian, but not in Chinese subjects (Lei et al. 2003, Mann & Ralston 2003). Overall, the data suggest that the *COL1A1* Sp1 polymorphism is a functional variant that has adverse effects on bone composition and therefore might be of interest as a susceptibility marker for periodontal disease, although no such data are currently available.

The aim of the present study was to investigate the frequencies and the correlation between four SNPs (*IL1A* +3954, *IL1B*+4845, *TNFA* −308 and *COL1A1* Sp1) and a VNTR (*IL1RN* intron 2), alone or in combination, and periodontal conditions in subjects of Greek origin.

Material and Methods

Subject sample and clinical recordings

In all, 192 patients and personnel of the Department of Periodontology, Preventive Dentistry and Implant Biology, Dental School, Aristotle University of Thessaloniki, Greece, volunteered for the present study. All participants were in good general health. The parents of all the subjects who participated in the present study were of Greek origin and care was taken to include age-matched subjects in the two groups. Data for participants are presented in Table 1.

Subjects were stratified according to their periodontal status as follows: subjects were considered as non-periodontitis cases when they displayed no probing depth (PD) or probing attachment level (PAL) >3 mm. Subjects were considered as periodontitis cases (chronic or aggressive) according to the analytical criteria of the American Academy of Periodontology (Armitage, 1999). Only generalized chronic and aggressive periodontitis subjects were included in the present study. Subjects, even at young ages not reporting familial aggregation of the disease, were not classified as aggressive periodontitis cases and not included in the present study.

Clinical data were recorded at all teeth present in the dentition. The following parameters were recorded at six sites for each tooth (disto-, mid- and mesiobuccal, mesio-, mid- and distolingual): PD, PAL and bleeding on probing (BOP).

All measurements were performed by one calibrated examiner (D. S.) using a manual probe (Hu-Friedy, Chicago, IL, USA). Smoking habits were also recorded.

The study was conducted according to the protocol outlined by the Research Committee, Aristotle University of Thessaloniki, Greece, was approved by the Ethical Committee of the School of Dentistry, and all participants signed an informed consent form.

Blood collection

Blood was collected from the finger by puncture with a lancet directly onto a DNase- and RNase-free paper card (IsoCode Card®, Schleicher and Schuell, Keene, NH, USA). The cards were baked at 80°C for 20 min., placed in a sealed storage bag with a desiccant and stored at room temperature away from light, according to the manufacturer's instructions.

Genotyping

All blood samples were coded and processed without the operators knowing the name and clinical status of the individuals. DNA was extracted from the IsoCode cards according to the manufacturer's instructions. The extracted DNA was analysed for five polymorphisms by the polymerase chain reaction (PCR) using the primers and conditions described in detail in the literature (Kornman et al. 1997, Galbraith et al. 1999, Walker et al. 2000, Efstathiadou et al. 2001, Laine et al. 2001).

Statistical analysis

Hardy–Weinberg equilibrium in the studied groups was tested for genotypic frequencies by a χ^2 test with one degree of freedom. Group differentiation was examined using the Genepop v3.3 (Raymond & Rousset 1995) genetic software.

The possible association of genotypic frequencies between non-periodontitis subjects, chronic and aggressive periodontitis subjects separate or combined was examined using a χ^2 test with one degree of freedom and statistical significance at the 95% level.

Two approaches were followed for the statistical analysis. The first referred to the genotypic comparison between non-periodontitis and periodontitis subjects (chronic and aggressive combined), while the second to the genotypic comparison between non-periodontitis and either chronic

Table 1. Clinical and demographic data of participants

Group	Total number	Male (%)	Female (%)	Age (mean ± SD)	Smokers (%)	Probing depth (mm)	Probing attachment level (mm)	Bleeding on probing
Non-periodontitis	90	44 (48.89)	46 (51.11)	43.86 ± 1.2	48 (53.33)	1.82 ± 0.5	1.96 ± 0.55	0.32 ± 0.23
Chronic periodontitis	56	27 (48.21)	29 (51.79)	50.70 ± 9.21	27 (48.21)	3.48 ± 0.87	4.35 ± 1.19	0.67 ± 0.35
Aggressive periodontitis	46	20 (43.48)	26 (56.52)	42.95 ± 5.94	24 (52.17)	3.76 ± 1.08	4.45 ± 1.13	0.68 ± 0.37
Periodontitis (combined)	102	47 (46.08)	55 (53.92)	46.82 ± 7.57	51 (46.08)	3.62 ± 0.9	4.4 ± 1.16	0.67 ± 0.36

or aggressive periodontitis subjects, separately.

Results

The clinical and demographic data of participants are displayed in Table 1. Care was taken to include age-matched subjects and a similar representation of both sexes and smoking habits.

The whole sample, as well as each group separately, was in Hardy–Weinberg equilibrium (χ^2 test, df = 1, p values >0.05). The distribution of genotypes in all studied groups is displayed in Table 2. The distribution of composite genotypes of *IL1A* and *IL1B*, as well as of *IL1A*, *IL1B* and *IL1RN* in all studied groups is displayed in Table 3.

According to the first approach, genotypes in all three *IL* loci studied, separately (Table 2), or as composite genotypes (Table 3) did not differentiate the two groups statistically significantly (χ^2 test, df = 1, p values >0.05).

Genotypes for *TNFA* −308 and *COL1A1* Sp1 also did not differentiate the two groups statistically significantly (χ^2 test, df = 1, p values >0.05).

In order to examine whether there is a possible correlation between the non-periodontitis subjects and a specific periodontitis condition (chronic or aggressive), the second approach was followed. According to this, genotypes in all three *IL* loci studied, either separately (Table 2), or as composite genotypes of *IL1A* and *IL1B*, as well as of *IL1A*, *IL1B* and *IL1RN* (Table 3), did not differentiate statistically significant the non-periodontitis either from chronic or aggressive periodontitis subjects (χ^2 test, df = 1, p values >0.05).

Genotypes for *TNFA* −308 and *COL1A1* Sp1 also did not differentiate the non-periodontitis either from chronic or aggressive periodontitis subjects statistically significantly (χ^2 test, df = 1, p values >0.05).

Discussion

In the present study, we investigated the prevalences of four SNPs (*IL1A*+3954, *IL1B*+4845, *TNFA* −308 and *COL1A1* Sp1) and a VNTR (*IL1RN* intron 2), alone, or as the ''composite genotype'' described by Kornman et al. and the combined carriage of the rare alleles at *IL1A*, *IL1B* and *IL1RN*, in subjects of Greek origin and their correlation with periodontal conditions. Data from the present, as well as our previous study (Sakellari et al. 2003), are among the few in the literature referring to subjects of Greek origin especially concerning polymorphisms of cytokine-encoding genes. These combined data deriving from systemically healthy individuals demonstrate that overall the prevalence of these SNPs is within the ranges reported for Caucasian populations (Table 2).

Table 2. Distribution of genotypes in the four groups

Genotype distribution		Non-periodontitis (%), $n = 90$	Chronic periodontitis (%), $n = 56$	Aggressive periodontitis (%), $n = 46$	Periodontitis (chronic and aggressive) (%), $n = 102$
IL1A+4845	A1A1	51	46	50	48
	A1A2	39	38	28	33
	A2A2	10	16	22	19
IL1B+3954	A1A1	56	59	56	58
	A1A2	39	32	35	33
	A2A2	5	9	9	9
IL1RN VNTRs	A1A1	62	48	54	51
	A1A2	22	38	22	30
	A1A3	6	7	9	8
	A2A2	10	7	11	9
	A2A3	0	0	2	1
	A2A4	0	0	2	1
TNFA-308	GG	73	84	74	79
	GA	27	14	22	18
	AA	0	2	4	3
COL1A1 Sp1	SS	52	61	52	57
	Ss	45	37	46	41
	ss	3	2	2	2

No statistical differences were observed at all comparisons (χ^2 test, $p>0.05$). n, number of individuals analysed in each group.

A caveat from the present study is the fact that when stratifying and comparing participants by combining the presence of periodontitis, carriage of the disease-associated alleles at all investigated loci and smoking status (smoker or non-smoker), the subject samples of our subgroups ranged between 7 (for *TNFA*) and 29 (for *IL1A*) subjects each and therefore this analysis was not included, due to the limited number, which would lead to erroneous statistical conclusions.

No differences were observed between the two groups concerning any of the investigated polymorphisms in the *IL1* cluster either alone (Table 2) or as ''composite'' haplotypes (Table 3).

Data from the present study add to the contradictory reports by several authors in various populations concerning the contributory effect of the *IL1A* and *IL1B* ''composite genotype'' to chronic periodontitis initiation or progression. In addition, no differences were observed between non-periodontitis and aggressive periodontitis cases, in agreement, with findings in populations of Caucasian, African-American, Hispanic and Japanese origin (Diehl et al. 1999, Parkhill et al. 2000, Walker et al. 2000, Hodge et al. 2001, Tai et al. 2002, Gonzales et al. 2003), although a positive relationship has been observed in males of Chinese origin (Li et al. 2004) and subjects of Chilean origin (Quappe et al. 2004). According to the findings of the present study, carriage of the ''rare'' alleles at the *IL1RN* locus or the combined carriage of the ''rare'' alleles at all three *IL1*-investigated loci could not discriminate between non-periodontitis and periodontitis (chronic, aggressive or combined) cases (Tables 2 and 3). According to reports in the literature referring to *IL1RN* polymorphisms, the combined carriage of allele 1 of the *IL1B*+3954 and allele 1 of the *IL1RN* was found to be associated with EOP (early onset) periodontitis (Parkhill et al. 2000) in Caucasians, while carriage of the polymorphic alleles (2, 3, 4 and 5) was found to correlate with AgP in subjects of Japanese origin (Tai et al. 2002), but not of Chinese origin (Li et al. 2004). The most extensively studied SNP at the *TNFA* locus, in Caucasian populations, is the G to A transition at the −308 position in the promoter region. Although the reported frequencies of detection of the A allele are higher than the ones in the present study (range 19–29%), no correlation

Table 3. Distribution of the ''composite'' genotypes of interleukin loci in the four groups

Genotype distribution		Non-periodontitis (%) $n = 90$	Chronic periodontitis (%) $n = 56$	Aggressive periodontitis (%) $n = 46$	Periodontitis (combined chronic and aggressive, %) $n = 102$
IL1A and *IL1B*	Positive	30	34	35	34
	Negative	70	66	65	66
IL1A, *IL1B*	Positive	7	13	9	11
and *IL1 RN*	Negative	93	87	91	89

No statistical differences were observed at all comparisons (χ^2 test, $p > 0.05$). *n*, number of individuals analysed in each group.

was observed between carriage of this allele and periodontal disease (Kornman et al. 1997, Fassmann et al. 2003, Folwaczny et al. 2004, Donati et al. 2005). In addition, other SNPs or microsatellites of the *TNFA* gene investigated in Caucasian populations could not be documented as susceptibility or severity factors in periodontal disease (Galbraith et al. 1998, Kinane et al. 1999, Craandijk et al. 2002). In contrast, this SNP is rarely detected in Japanese subjects (0.2–2%) and therefore does not appear to be a good marker of predisposition to periodontitis in subjects of this origin (Endo et al. 2001, Soga et al. 2003).

In the present study, we also investigated the possible correlation of an SNP, defined as an ''s'' allele, affecting a binding site for the transcription factor Sp1 in the *COL1A1* gene to periodontal conditions. In a recent report by Suzuki et al. (2004) in Japanese subjects, 310 SNPs in 125 genes encoding both inflammatory and structural factors of periodontal disease were investigated. These authors reported a positive correlation between aggressive periodontitis and SNPs in the *COL1A1*, *COL4A1* and IL-6 signal transducer (*IL6ST*) genes, but the *COL1A1* SNP in their study was located in an intron and does not cause an amino acid substitution and therefore according to the authors' suggestions, could either interfere in protein expression or be in linkage disequilibrium with causative polymorphisms. Although, according to our data, no differences were observed among the groups concerning the distributions and frequencies of the *COL1A1* Sp1 genotypes, as already suggested in Suzuki et al. (2004), polymorphisms in genes encoding structural and functional factors of periodontal tissues appear to require further investigation.

Data deriving from the present and other studies similar in subject sample and number of investigated genomic markers should be interpreted with caution. The large-scale approach and family linkage analysis or gene signal expression also appear promising and might be meaningful in studying the periodontitis–genetic association.

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Clinical Relevance

Scientific rationale for the study: Few data are available in the literature on the prevalence of various gene polymorphisms and their relation to periodontal conditions in subjects of Greek origin.

Principal findings: No significant associations were observed between four SNPs (*IL1A*+3954, *IL1B*+4845, *TNFA* −308, *COL1A1* Sp1), a VNTR (*IL1RN* intron 2) and periodontally distinct conditions in 192 healthy subjects of Greek origin.

Clinical implications: The periodontitis–gene association at the SNP level requires further documentation before being introduced in clinical practice.