

ORIGINAL ARTICLE

Polymorphisms in the interleukin-10 gene and chronic periodontitis in patients with atherosclerotic and aortic aneurysmal vascular diseases

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Background: Chronic periodontitis (CP), atherosclerotic and aortic aneurysmal vascular diseases (VD) are chronic inflammatory conditions with multifactorial etiologies, including involvement of predisposing genetic factors. In a previous study, polymorphisms in the gene for the anti-inflammatory interleukin-1 receptor antagonist were associated with CP in patients with VD.

Objective: This study investigates whether polymorphisms in the gene for the anti-inflammatory interleukin-10 (IL10) could be related to CP in the same manner.

Methods: Seventy-two patients with VD of whom 35 had CP were genotyped for single nucleotide polymorphisms (SNPs) in the IL10 –592 (rs1800872), –819 (rs1800871), and –1,082 (rs1800896) gene by Taqman rtPCR method and by DNA sequencing.

Results: The C alleles and C/C genotypes of IL10 –592 and IL10 –819 frequencies were significantly higher, while the frequencies of the IL10 –592 (C/A) and IL10 –819 (C/T) heterozygote genotypes were significantly lower in the VD group with CP compared to those without CP. The IL10 haplotype ATA frequency (–1,082, –819, –592) showed a trend to a significant difference between the two groups indicating protection against CP.

Conclusions: Taken together, our findings suggest an independent association of genetic polymorphisms in the IL-10 gene locus with CP in patients with VD. Development of CP and the implications on vascular disease emphasize the importance of early detection and adequate treatment of periodontitis among these patients.

Keywords: *IL10 SNP; periodontal disease; vascular disease*

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Chronic periodontitis (CP) and atherosclerotic and aortic aneurysmal vascular diseases (VD) are highly prevalent chronic inflammatory conditions. They cause tooth loss, ischemic stroke, myocardial infarction, and aneurysmal rupture and have therefore considerable consequences and cost, both at individual and societal levels (1–3).

These diseases are complex and multifactorial and share common risk factors such as age, smoking, and diabetes mellitus (4, 5). However, an association between CP and atherosclerotic vascular conditions has been reported, independent of confounding factors, and the impact of CP on systemic health is suggested to be an additional risk factor for initiation and progression of VD (5–7).

Familial and twin studies have shown genetic predisposition to periodontal diseases and VD (3, 8, 9). Due to the chronic inflammatory characteristics and similarities in risk factors, it has been suggested that a shared genetic risk profile can partly underlie the association found in the literature. Candidate-gene association studies have identified shared genetic susceptibility loci (10–12), but also specific genetic patterns of predisposition for periodontal and atherosclerotic VD (4, 10, 11, 13, 14).

The interleukin-1 receptor antagonist (IL-1Ra encoded by the IL1RN gene) acts as an anti-inflammatory cytokine by binding to cellular interleukin-1 (IL-1) receptors (15). In previous studies, we investigated the frequency of polymorphisms in the IL-1 gene cluster in patients with

VD and could observe an association between a variable number of tandem repeat (VNTR) in intron 2 of the IL1RN gene and CP among patients with VD (16).

IL-10 is another anti-inflammatory cytokine with important immunoregulatory functions. IL-10 is mainly produced by monocytes, macrophages, and CD4+ Th2 cells, but may be produced by almost all leukocytes, and exhibits a wide range of biological effects. Primarily, IL-10 inhibits the release of pro-inflammatory mediators such as tumor necrosis factor alpha (TNF- α), IL-1 β , IL-6, and IL-8 from monocytes/macrophages and inhibits the proliferation and the cytokine synthesis of activated CD4+ T cells. Further, IL-10 constrains antigen presentation of monocytes/macrophages by reducing the cell surface expression of major histocompatibility complex class II (MHC II), co-stimulating, and adhesion molecules (17). Additionally, it enhances the production of anti-inflammatory mediators such as IL-1 receptor antagonist (18). It also has important actions on B-cells by preventing apoptosis, enhancing proliferation and differentiation, increasing MHC II expression, and facilitating immunoglobulin class switching (17, 19).

Higher levels of IL-10 mRNA are expressed in gingival biopsies from patients with CP as compared with healthy control biopsies (20). In murine models, IL-10 knockout mice were found to be highly susceptible to severe alveolar bone loss as compared with wild-type mice (21). In addition to the inhibition of inflammation in periodontal disease, IL-10 may moderate tissue destruction by modulating matrix metalloproteinase (MMPs) and osteoclastogenesis through regulation of the receptor activator of nuclear factor-kappaB ligand (RANKL), colony-stimulating factor-1 (CSF-1), and osteoprotegerin (OPG) expression (22, 23). Furthermore, in an experimental periodontal model, IL-10 levels in periodontal tissue were associated with higher expression of tissue inhibitors of metalloproteinases (TIMPs) and inhibitor of osteoclastogenesis OPG, and lower expression of MMPs and RANKL, and with lower cellular infiltration in periodontal tissues and alveolar bone loss (24).

It is estimated that 50–80% of the differences in the IL-10 production can be explained by genetic factors (25). The single nucleotide polymorphisms (SNPs) in the promoter region of interleukin-10 (IL10) gene at positions –592, –819 and –1,082 are associated with altered IL-10 levels (26–28), and several studies have investigated the association between these polymorphisms and periodontal diseases (9, 25, 29), but with inconsistent results.

CP can, through systemic changes, cause an additional burden in the pathogenesis of VD. Severe periodontal disease is associated with endothelial dysfunction (measured by impaired flow-mediated dilation) (30), and in a recent prospective study, improvement in periodontal status was correlated with decreased progression of carotid

artery media thickness at 3-year follow-up (7). These findings emphasize the importance of early identification of patient populations that can benefit from periodontal intervention to prevent progression of VD. Previous studies have reported association between polymorphisms in genes for IL-1 (10), IL-1Ra (16), and TNF- α (14) with CP in patients with atherosclerotic and aneurysmal VD. The functional IL-10 SNPs have not been investigated for CP in patients with vascular conditions. Thus, this study investigates the potential role of polymorphisms in the IL10 promoter region for independent association with CP in Scandinavian patients with severe vascular disease in the major arteries.

Material and methods

Participants

The study included a total of 67 Norwegian, 3 Swedish, and 2 Danish VD patients. The patients were recruited at the Department of Vascular Surgery, Oslo University Hospital, Aker, and were seeking surgical treatment for abdominal aorta aneurism repair, carotid or femoral endarterectomy, or percutaneous transluminal angioplasty of the femoral arteries.

Clinical oral examinations were performed at the hospital ward to determine the periodontal status of the patient. The patients were categorized into two groups. The first group (VD with CP) was made up of patients undergoing treatment for VD and diagnosed with CP ($n=35$, mean age = 67.9, SD = 6.9, range: 51.3–85.0). The second group (VD without CP) was made up of patients undergoing treatment for VD who were assessed without CP ($n=37$, mean age = 71.1, SD = 7.8, range: 55.4–84.8). Demographic data of the study groups are shown in Table 1.

The patients' medical records were obtained for general health information. Ethnicity and smoking behavior were self-reported. Written informed consent was obtained from all participants; the study was approved by the Regional Ethical Committee (REK Sør, NO. 08/322b) and was in accordance with the Helsinki declaration of 1975, as revised in 2008. The study has been registered at ClinicalTrials.gov (ID. NCT01358630).

Inclusion criteria

The patients in both groups were diagnosed and treated according to standard procedures at the Department of Vascular Surgery, Oslo University Hospital. Diagnosis of CP was based on the classification system of the American Academy of Periodontology, established in 1999 at the International Workshop for Classification of Periodontal Diseases and Conditions (31). Clinical periodontal status was assessed by a trained dentist (Armingohar) as previously described (16).

Table 1. Demographic data of the patients with atherosclerotic and aortic aneurysmal vascular diseases (VD) with (w/) and without (wo/) chronic periodontitis (CP)

	VD w/CP	VD wo/CP
Age	<i>n</i> = 35	<i>n</i> = 37
Mean ± SD	67.9 ± 6.9	71.1 ± 7.8
Median (min–max)	67.6 (51.3–85.0)	71.2 (55.4–84.8)
Gender (<i>n</i>)		
Male	29	30
Female	6	7
Teeth (<i>n</i>)		
Mean ± SD	18.5 ± 7.4	24.9 ± 2.3
Median (min–max)	20 (4–28)	25 (20–29)
Periodontal pocket probing depth (<i>n</i> ≥ 5 mm)		
Mean ± SD	15.6 ± 10.9	0.7 ± 0.99
Median (min–max)	11 (4–55)	0 (0–3)
Diabetes (<i>n</i>)	3	13
Smoking (<i>n</i>)		
Non-smokers	1	9
Current or previously smokers	34	28
Pack-year Mean ± SD	37.0 ± 19.6	25.1 ± 14.5
Pack-year Median (min–max)	34.7 (4.9–88.0)	23.6 (3.1–51.3)
Body mass index (kg/m ²)		
Mean ± SD	26.4 ± 4.2	27.0 ± 4.5
Median (min–max)	26.4 (14.2–35.3)	26.5 (18.3–37.0)

Samples and DNA isolation

Vascular biopsies and saliva samples were collected for extraction of genomic DNA. The vascular biopsies were collected under surgical treatment from the walls of aneurysms and during excision of intravascular plaques in carotid or common femoral arteries.

Saliva samples were collected with the Oragene™ DNA Self-Collection Kit (Genotek, Ottawa, Ontario, Canada) according to the manufacturer's instructions. Extraction of genomic DNA was performed using the Masterpure Complete DNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) as previously described (16).

Analyses of DNA polymorphisms

SNP allele discrimination assays were performed by the real-time polymerase chain reaction (PCR) amplification method. The alleles of IL10 at –592 (rs 1800872) and –1,082 (rs1800896), were detected by 5' nuclease allelic discrimination Taqman method using Assays by Design/Demand (Applied Biosystems, Foster City, CA, USA) with locus-specific forward and reverse primers and two allele-specific oligonucleotide probes for each SNP of interest (Table 2), according to manufacturer's instructions.

The alleles of IL10 –819 (rs1800871) were detected by 5' nuclease allelic discrimination method using locus-specific forward and reverse primers and two allele-specific oligonucleotide probes for each SNP of interest (Table 2; Biochemistry Department, University of Oslo, Oslo Research Park) as described previously (32). The probes for each allele (listed in Table 2) were labeled with FAM or VIC at the 5' end, and non-fluorescent quencher or 'black-hole' quencher at the 3' end. The experiments were performed in the Mx-3005p (Agilent Technologies Inc., USA) using the TaqMan Universal PCR Master Mix (Applied Biosystems), along with approximately 20 ng DNA template.

To confirm the results of the Taqman genotyping, part of the IL-10 promoter region covering the SNP loci of interest was PCR amplified and sequenced. PCR reactions were performed with forward primer 5'-ACAAATC CAAGACAACACTACTAAG-3' and reverse primer 5'-ATGAATACCCAAGACTTCTCCTTGCTA-3' (Sigma-Aldrich, MO, USA), and OneTaq 2X Master Mix (New England Biolabs, Beverly, MA, USA) at an annealing temperature of 55°C. The PCR products were purified by Exosap-IT (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. Purified amplicons were sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and the forward and reverse primers. Sequence reactions were run on an ABI Prism 3730 DNA analyzer (Applied Biosystems). Sequence trimming, quality check and alignment were performed with the Sequencher 5.0 program (Gene Codes Corp., Ann Arbor, MI).

Statistical analyses

The statistical analyses were performed using the SPSS software (IBM SPSS Statistic version19) and JavaStat (<http://statpages.org/>). Chi-square and Fisher's exact tests were used for univariate comparisons of allelic and genotypic frequencies of the SNP loci between the groups. Binary logistic regression was used to adjust the genotypic differences for the effect of age, gender, body mass index (BMI), diabetes, and smoking status. The genotype analyses were performed by comparing each genotype frequency to the pooled total of the other groups. The assembly and prediction of haplotypes for the IL10 SNP cluster were done by the software 'Phase' (33). All loci were in Hardy–Weinberg equilibrium. A statistically significant difference was defined when *p* was <0.05.

Results

Genotyping was successful in all but two patients, who were excluded from further analysis.

In Table 3, allelic and genotypic frequencies of the SNPs IL10 –592, –819 and –1,082 are listed.

Using chi-square tests, a significant difference in allelic frequencies was found between the two study groups

Table 2. Primer and probe sequences used to detect nucleotide polymorphisms in this study

Gene	Location	Polymorphism	Primers/Probes
IL10	Promoter	SNP -592 C > A (rs1800872)	VIC/FAM: CTTTCCAGAGACTGGCTTCCTACAG [G/T] ACAGGCGG GGTCACAGGATGTGTTT
IL10	Promoter	SNP -819 C > T (rs1800871)	Fw: 5' GGCACACTGGTGTACCCTTGTA 3' Rv: 5' CATGACCCCTACCGTCTCTATTTT 3' FAM: 5' AGGCACAGAGATATTACAT 3' VIC: 5' AGGCACAGAGATGTTACAT 3'
IL10	Promoter	SNP -1,082 A > G (rs1800896)	VIC/FAM: TCCTCTTACCTATCCCTACTTCCCC [T/C] TCCCAAAGA AGCCTTAGTAGTGTG

SNP, single nucleotide polymorphism; Fw, forward primer; Rv, reverse primer.
Taqman probes are listed with the polymorphic bases in bold.

regarding IL10 -592 ($p=0.03$, OR = 2.23, 95% CI = 1.07–4.68) and IL10 -819 ($p=0.047$, OR = 2.1, 95% CI = 1.003–4.42) with the C allele being more frequent in the VD group with CP.

The frequencies of the IL10 -592 genotype 1/1 (C/C) showed a significant difference (chi-square, $p=0.01$, OR = 3.53, 95% CI = 1.33–9.31) comparing the group of VD patients with CP ($n=22$) with the group of VD patient without CP ($n=12$). After adjustment for the risk factors age, gender, BMI, diabetes and smoking status by logistic regression, this difference was significant ($p=0.02$, OR = 4.0, 95% CI = 1.28–12.35), indicating a significant association of the IL10 -592 C/C genotype with risk for CP among VD patients.

The heterozygote IL10 -592 genotype 1/2 (C/A) frequencies displayed a statistical significant difference comparing the two groups of patients (chi-square, $p=0.02$, OR = 0.31, 95% CI = 0.12–0.82), being lower in the VD patients with CP ($n=11$) compared to those without CP ($n=22$). This difference was significant ($p=0.02$, OR = 0.26, 95% CI = 0.08–0.82) in the logistic regression model, indicating a protection against periodontitis among VD patients with the IL10 -592 1/2 genotype.

The IL10 -592 genotype was significantly associated with CP in an overall analysis of the individual genotypes using chi-square ($p=0.03$). This association remained borderline significant in a logistic regression model ($p=0.05$) (results not shown).

The IL10 -819 genotype 1/1 (C/C) showed a significantly different frequency between the two groups (chi-square, $p=0.02$, OR = 3.12, 95% CI = 1.19–8.2), being higher in the VD with CP group ($n=22$) than in the VD without CP group ($n=13$). When adjusted for the risk by logistic regression, this difference remained significant ($p=0.02$, OR = 3.69, 95% CI = 1.2–11.4), indicating a possible association of the IL10 -819 C/C genotype with risk for CP among VD patients.

The frequencies of the heterozygote IL10 -819 genotype 1/2 (C/T) displayed statistical significant difference

comparing the VD patients with CP group ($n=11$) with those without CP ($n=21$) (chi-square, $p=0.03$, OR = 0.35, 95% CI = 0.13–0.92). This difference was significant in the logistic regression model ($p=0.03$, OR = 0.28, 95% CI = 0.09–0.88) suggesting protection against CP among VD patients with the IL10 -819 1/2 genotype.

No significant allelic or genotypic frequency differences in the IL10 -1,082 SNP were detected comparing the VD patients with and without CP ($p > 0.05$).

Haplotype analysis using Phase software predicted four different haplotypes as shown in Table 4. The predicted IL10 haplotype ATA (-1,082, -819, -592) designated as 1-2-2 showed a borderline significant difference comparing the two patient groups (chi-square, $p=0.047$, OR = 0.47, 95% CI = 0.21–1.06), possibly indicating protection against periodontitis compared to the other haplotypes.

In the logistic regression analysis no statistically significant correlations were found between the risk factors age, gender, BMI, diabetes and smoking status.

The results from SNP genotyping using real-time PCR amplification method and by DNA sequencing were in agreement.

Discussion

CP has been associated with VD and is suggested to be an additional risk factor for cardiovascular diseases independent of confounding factors (5, 6). Polymorphisms in inflammatory genes have been investigated for association with periodontal and cardiovascular disease susceptibility, and both shared (10–12) and distinct genetic associations (4, 10, 11, 13, 14) have been identified.

Here, we investigated whether polymorphisms in the IL-10 gene promoter region can be associated to CP among patients with VD. Three functional SNPs in the promoter region of the IL10 at positions -1,082 (A > G), -819 (C > T) and -592 (C > A) are reported to have a regulatory function on promoter activity (26), and are associated with altered IL-10 production (28, 34). These SNPs

Table 3. Allele and genotype frequencies within the IL10 gene locus in patients with atherosclerotic and aortic aneurysmal vascular disease (VD) with (w/) or without (wo/) chronic periodontitis (CP)

		Allele frequency			
		VD w/CP	VD wo/CP	<i>p</i> (VD w/CP vs. VD wo/CP) (chi-square)	
IL10 ^a	SNP –1082 rs1800896	<i>n</i> = 70	<i>n</i> = 74		
1	A	0.53 (37) ^c	0.64 (47)	0.2	
2	G	0.47 (33)	0.36 (27)		
IL10 ^a	SNP –819 rs1800871	<i>n</i> = 70	<i>n</i> = 74		
1	C	0.79 (55)	0.64 (47)	0.047^d	
2	T	0.21 (15)	0.36 (27)		
IL10 ^a	SNP –592 rs1800872	<i>n</i> = 70	<i>n</i> = 74		
1	C	0.79 (55)	0.62 (46)	0.03^e	
2	A	0.21 (15)	0.38 (28)		
		VD w/CP	VD wo/CP	<i>p</i> (VD w/CP vs. VD wo/CP) (chi-square)	<i>p</i> (VD w/CP vs. VD wo/CP) (logistic regression model)
IL10 ^b	SNP –1082 rs1800896	<i>n</i> = 35	<i>n</i> = 37		
1/1	A/A	0.26 (9) ^c	0.35 (13)	0.39	0.23
1/2	A/G	0.54 (19)	0.57 (21)	0.83	0.94
2/2	G/G	0.20 (7)	0.08 (3)	0.18	0.14
IL10 ^b	SNP –819 rs1800871	<i>n</i> = 35	<i>n</i> = 37		
1/1	C/C	0.63 (22)	0.35 (13)	0.02^f	0.02^g
1/2	C/T	0.31 (11)	0.56 (21)	0.03^h	0.03ⁱ
2/2	T/T	0.06 (2)	0.08 (3)	1	0.8
IL10 ^b	SNP –592 rs1800872	<i>n</i> = 35	<i>n</i> = 37		
1/1	C/C	0.63 (22)	0.32 (12)	0.01^j	0.02^k
1/2	C/A	0.31 (11)	0.59 (22)	0.02^l	0.02^m
2/2	A/A	0.06 (2)	0.08 (3)	1	0.8

SNP, single nucleotide polymorphism.

^aAllele designation (1 = more frequent, major allele; 2 = less frequent, minor allele).

^bGenotype designations.

^cNumber of alleles and genotypes in parentheses.

^dOR = 0.52, 95% CI = 1.003–4.42.

^eOR = 2.23, 95% CI = 1.07–4.68.

^fOR = 3.12, 95% CI = 1.19–8.2.

^gOR = 3.69, 95% CI = 1.2–11.4.

^hOR = 0.35, 95% CI = 0.13–0.92.

ⁱOR = 0.28, 95% CI = 0.09–0.88.

^jOR = 3.53, 95% CI = 1.33–9.31.

^kOR = 4.0, 95% CI = 1.28–12.35.

^lOR = 0.31, 95% CI = 0.12–0.82.

^mOR = 0.26, 95% CI = 0.08–0.82.

In the binary logistic regression model the genotypic differences were adjusted for the effect of age, gender, body mass index, diabetes, and smoking status.

have been investigated for association with CP susceptibility, but with inconsistent results (9, 29). Ethnic differences in IL10 allele and genotype frequencies may be a reason for the discrepancy in reported results; hence only Scandinavian patients were included in the present study (35).

The IL10 SNP at position –592 (C > A) is located in a negative regulatory region, the –819 (C > T) polymorphism may affect an estrogen receptor element (34), while the IL10 –1,082 (A > G) SNP lies within a putative e-twenty-six ETS like-transcription binding site and may influence transcriptional activity (27). Larsson et al.

Table 4. Estimated IL10 locus haplotype frequencies in patients with atherosclerotic and aortic aneurysmal vascular disease (VD) with (w/) or without (wo/) chronic periodontitis (CP)^a

Haplotype designations		VD w/CP <i>n</i> = (70)		VD wo/CP <i>n</i> = (74)		<i>P</i> (chi-square)	OR (95% CI)
		Frequency group	(<i>n</i>)	Frequency group	(<i>n</i>)		
211	G-C-C	0.471	(33)	0.365	(27)	0.195	
111	A-C-C	0.314	(22)	0.257	(19)	0.445	
122	A-T-A	0.214	(15)	0.365	(27)	0.047	OR = 0.47 (CI = 0.21–1.06)
112	A-C-A	0	(0)	0.014	(1)	1	

^aEstimation of population frequencies of predicted haplotypes.

^bIL10 (−1,082), IL10 (−819), and IL10 (−592) haplotype designation.

^cNucleotide designation of listed SNP allele numbers.

showed that the transcription factor Sp1 only bound to the G allele at the −1,082 position in the IL-10 promoter region resulting in higher IL-10 and Sp1 mRNA expression for GG genotypes in B-cells (28). The three SNPs are in close linkage disequilibrium, with three main preference haplotypes (−1,082, −819, −592): GCC, ACC and ATA (26, 34). The GCC haplotype is associated with high, the ATA haplotype with low and the ACC with an intermediate IL-10 production (36, 37).

Claudino et al. have shown in their study that both IL10 −592 CA and AA genotypes were more frequent in patients with CP, and that these genotypes were associated with disease severity and lower levels of IL-10, tissue inhibitor of metalloproteinase-3 (TIMP-3), and the inhibitor of osteoclastogenesis OPG mRNA expression in diseased periodontal tissues (22). Cullinan et al. investigated the possible association between IL-10 gene polymorphisms and periodontal disease progression in a prospective longitudinal study in an Australian population. They reported that individuals having either the ATA/ACC or the ACC/ACC genotype experienced less periodontal disease progression after 5 years compared to individuals with other genotypes (38).

In our patients, the putative high IL-10 producer alleles (−819 C and −592 C) and genotypes (−819 C/C and −592 C/C) were associated with CP, while the low producer ATA haplotype showed a trend to protection against CP. In addition, the G allele and G/G genotype of the IL −1,082 were more frequent among CP patients, but this difference did not reach a significant level comparing the two groups. In contrast to our study, the alleles IL10 −819 T and −592 A, which are connected with low IL-10 levels, have been associated with risk for CP in other Caucasian populations (29, 39). Similar to our study, the high IL10 producer G allele and GG genotype of IL −1,082 have been found to be associated with CP in a Swedish population (40). While in other studies, the low producer A allele of −1,082 has also been associated with risk for CP (41, 42). These previous findings

compared to our findings, suggest that studying populations of similar ethnicity show more consistency and reproducibility. This could be due to increase of IL10 genetic effect size in the Scandinavian (Nordic) population(s) from natural selection and/or variable exposures to environmental and other factors affecting immunocompetence in CP and VD.

It is plausible that IL-10 can reduce clearance of infectious pathogens by suppressing the protective innate and adaptive immune responses, and therefore is involved in the persistence of bacteria and chronic infections (19, 43). Murine models of infectious diseases have shown that even normal levels of IL-10 tend to limit the efficiency of immune responses against pathogens, and that the resistance to infection can be improved by reducing IL-10 levels (44). Additionally, IL-10 enhances the release of anti-inflammatory mediators such as IL-1 receptor antagonist (18). In a previous study, we found a significantly higher frequency of the allele 1 and a higher frequency of allele 2 of the IL1RN VNTR among CP patients with VD (16). Association studies have shown that homozygous carriers of allele 2 of the IL-1Ra gene have a longer and more severe pro-inflammatory immune response than persons with other IL1RN genotypes (15). Thus, it is conceivable that the carriage of allele 2 can be beneficial in combating microbial challenges in the gingival sulcus. Indeed, allele 2 carriers have shown reduced vaginal colonization by mycoplasma and resistance to human cytomegalovirus and Epstein-Barr virus (15). Together, these findings indicate that excessive down-regulation of the pro-inflammatory mechanisms could result in reduced efficiency of anti-pathogenic immune responses in periodontal disease. The genotype of CP patients in this study and our previous study (16), associated with excessive anti-inflammatory responses and potentially reduced pathogen clearance, can therefore be detrimental in periodontal disease susceptibility.

The number of patients in the present study was limited because it is difficult to recruit patients with life-threatening

VD, being prepared for major surgery. Sample size is associated with the power of statistical tests. *Post hoc* power calculations showed that the power of our tests was low. The consequence is an increase in the risk for type II error, the possibility that potential differences between groups might not be detected. Yet, we have found a statistically significant difference between the study groups regarding the IL10 -819 and -592 independent of shared risk factors, indicating that in persons with VD, genotypes of inflammatory factors within the IL-10 gene cluster are associated with CP.

Animal studies and *in vitro* studies on human aortic endothelial cells have shown anti-atherogenic properties of IL-10 (45, 46). Genotype and haplotype variants within the IL10 gene have been associated with atherosclerotic diseases, but with discrepant results (47, 48). Our findings, however, suggest that these gene variants still can have a stronger association to CP than VD.

The development of CP may constitute additional burden in the pathogenesis of VD by causing sustained low-graded systemic inflammation and bacterial dissemination in the circulation (49). In this study, we have identified genetic markers that are specifically associated with CP within a group of patients with VD. Information about these genetic components or predictive biomarkers may help to identify subgroup of VD patients who are at risk to develop CP, and selectively target them for effective preventive CP interventions.

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