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Vitamin D receptor polymorphism (−1056 Taq-I) interacts with smoking for the presence and progression of periodontitis

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Abstract

Aim: The aim of this analysis was to investigate the relationship between a vitamin D receptor (VDR) polymorphism and the diagnosis and progression of periodontitis.

Material and Methods: Data were derived from two different studies, including 231 subjects with healthy periodontium, 224 aggressive periodontitis and 79 chronic periodontitis (CP) patients in a case–control investigation. Sixty-one of these CP patients also took part in an observational study with a 1-year follow-up, in which progression of periodontitis was determined at the subject level. All 534 subjects provided a blood sample from which genomic DNA was extracted to study VDR – 1056 TaqI polymorphism.

Results: The interaction between smoking and VDR polymorphism was associated with the diagnosis of periodontitis in Caucasians [$p = 0.001$, odds ratio (OR) = 1.33, 95% confidence intervals (CI) = 1.12–1.57] and all subjects ($p = 0.033$, OR = 1.60, 95% CI = 1.04–2.48). In the longitudinal study, subjects were divided into two clusters at 1 year according to the median number of progressing sites (Δcumulative attachment loss > 2 mm). Logistic regression analysis revealed that the interaction between VDR Taq-I polymorphism and smoking showed limited evidence of association with the ''severe progression'' cluster ($p = 0.033$, OR = 15.24, 95% CI = 1.24–187.42).

Conclusions: Vitamin D receptor Taq-I TT polymorphism was moderately associated with both the presence and the progression of periodontitis in smokers, while no association was detected in non-smoking individuals. VDR genetic factors may interact with smoking in the pathogenesis of periodontitis.

Key words: genetic polymorphisms; periodontitis; progression; smoking; vitamin D receptor

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Conflict of interest and source of funding statement

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Periodontitis is a chronic disease believed to have a complex multifactorial aetiopathogenesis. Microbiological factors interact with genetic and environmental factors (such as smoking) to determine disease onset and progression. In this context, the interactions between genes and between genes and environmental factors might be of major importance. Ultimately, the susceptibility to periodontitis may be determined by a cumulative individual high-susceptibility profile model, given by a combination of multiple susceptibility genotypes (Kinane & Hart 2003). Among putative genetic risk factors, a restriction fragment length polymorphism (RFLP) of the vitamin D receptor (VDR) gene has been associated previously with various infections and pathologies (Bellamy et al. 1999), including periodontitis, in different populations (Tachi et al. 2003, de Brito et al. 2004, Brett et al. 2005, Park et al.

2006). Its mechanism of action is unclear and maybe a function of linkage disequilibrium with a functional polymorphism elsewhere in the VDR gene.

Genetic factors may play a role not only in the onset but also in the progression of periodontitis. Some attempts have been made in the past to identify site-based risk predictors for periodontal disease progression, with contradictory results (Lindhe et al. 1989, Papapanou & Wennstrom 1990, Ship & Beck 1996, Machtei et al. 1997). Consistent with a combined genetic–environmental pathogenesis, results from these studies show that a small number of subjects seem to have a very high rate of progression, which accounts for most of the progressing sites detected in the study populations (Heitz-Mayfield 2005). In this context, genetic and environmental factors (such as smoking) may interact in increasing an individual's susceptibility to periodontitis.

Here, we report the results of two studies with the ultimate objective of investigating the association between genetic and environmental factors in periodontitis: the first study had a case–control design, while the second had a longitudinal design. The specific aims were to (i) investigate the association between a VDR polymorphism and the diagnosis and/or progression of periodontitis and (ii) to assess the role of cigarette smoking as an effect modifier in this association.

Material and Methods

Study subjects

This analysis includes a total of 534 subjects, selected among patients referred to the Eastman Dental Hospital, University College London, by general dental practitioners. Based on the current classifications (Armitage 1999), 224 individuals had been diagnosed with aggressive periodontitis (AgP), 79 had been diagnosed with chronic periodontitis (CP) and 231 had been recruited from periodontal disease-free patients referred to other departments of the hospital (Oral Surgery, Conservation and Endodontic). All the patients gave written informed consent. The studies had been reviewed and approved by the Eastman/UCLH joint ethics committee.

Inclusion criteria for controls

The control subject sample has been described previously (Nibali et al. 2006). Subjects registered with the UK National Health Service and attending other Departments of the EDH were screened for inclusion. Only patients with a minimum age of 25 years were enrolled. Volunteers with specific known genetic diseases or a history of periodontal disease or tooth loss due to periodontal disease were not included. A single examiner (L. N.) performed a basic screening periodontal examination on these subjects using the Periodontal Screening and Recording index and reference to the existing radiographs taken for the clinical problem that resulted in their referral. In the event of detecting codes 3, 4 or * in any sextant, further investigation was performed. This consisted of pocket depth and recession (REC) estimates using a UNC-15 probe (Hu-Friedy, Leimen, Germany) and further radiographic investigation consisting of either a panoramic view or individual periapicals. Subjects were excluded if they presented with at least one site with probing pocket depth (PPD) and cumulative attachment loss (CAL) $\geqslant 4$ mm or radiographic evidence of bone loss.

For both cases and controls, smoking status and ethnic origin were self-reported. Subjects were divided into Caucasians, Blacks (including Black-Africans and Afro-Caribbean), Asians and Others (including mixed).

Inclusion criteria for AgP patients

The AgP subject sample has been described previously (Nibali et al. 2006). The diagnosis of AgP was based on the 1999 Consensus Classification of Periodontal Diseases (Armitage 1999). Our diagnostic criteria took into consideration only clinical, and not laboratory, evidence. We classified patients as having AgP when we had evidence of:

- Healthy status, except from the presence of periodontitis (e.g. all subjects with diabetes were excluded).
- Rapid attachment loss and bone destruction, proven by radiographs obtained a few years apart. When this was not possible, severe disease at a young age was used, with patients <35 at the time of the initial diagnosis.
- Familial aggregation. We tried to ascertain the familial aggregation by means of a specific questionnaire and, when possible, by examining first-degree relatives. However, patients showing clear clinical signs of AgP but without a positive family history were still included (Llorente & Griffiths 2006).

All the patients with a suspected diagnosis of AgP were examined by a single experienced clinician (G. S. G.). Full-mouth measures of PPD, REC [measured as distance from the cement–enamel junction (CEJ) to the free gingival margin (FGM)] and lifetime cumulative attachment loss (LCAL, measured either as a direct measurement of CEJ to the base of the pocket or as a calculation of PPD+REC) were obtained at six sites per tooth. Appropriate dental radiographs were also obtained for each patient.

Inclusion criteria for CP patients

Inclusion criteria were (i) diagnosis of chronic periodontitis (Lang et al. 1999), with a minimum of 16 evaluable teeth with at least four molars present, and a minimum of four sites with PPD $\geqslant 5$ and $\geqslant 2$ mm of CAL in different quadrants, and (ii) age 30–75 years. Exclusion criteria were (i) more than two sites with PPD $\geqslant 8$ mm or attachment loss $\geqslant 10$ mm; (ii) systemic diseases, such as hepatitis, liver dysfunction, cancer or cardiovascular diseases, diabetes; (iii) history of alcohol or drug abuse, as assessed by the examining clinician; (iv) a course of systemic antibiotic within 2 weeks before baseline; (v) currently on immunosuppressive therapy; (vi) pregnancy; and (vii) severe dental disease other than periodontal disease.

All CP patients were examined by a single examiner (F. D. A.). Sixty-one of these patients were entered in a longitudinal study with a 1-year follow-up. At the baseline visit, patients were given standard sodium fluoride toothpaste and encouraged to use it, refraining from using different toothpastes or mouthwashes. Patients were instructed to brush their teeth in their usual manner and routine. A prophylactic session was also performed at baseline and 6 months. During these sessions, strictly supragingival polishing was performed. Full-mouth periodontal examination was performed at each visit by the same examiner (F. D. A.). Single pass, whole-mouth measures of PPD, REC, CAL, and bleeding on probing (positive or negative, as assessed 15 s after probing) were collected using a manual,

incremental UNC-15 periodontal probe. Six sites were measured for each natural tooth, one each at the mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual sites encircling the tooth. The measurements were made at the corresponding contact points or their equivalent in case of a missing tooth, and at the midpoint of buccal and lingual surfaces.

Intra-examiner repeatability

A total of 10 subjects with a complete range (mild to severe) of periodontal disease were recruited. The examiner (F. D. A.) measured full-mouth PPD and CEJ–FGM distance for all 10 subjects using a manual incremental UNC-15 probe in the same manner as described above. Repeated measures were taken the same day with a minimum 15 min. of separation. The examiner had no access to the patients' first measurement. The examiner showed 99% agreement for measures of CAL within 2 mm of difference; the intra-class correlation for CAL was 0.92, with a standard deviation between the two measures equal to 1.05.

Definition of progression

In order to minimize the risk of including false positives, >2 mm ΔCAL (baseline CAL $-$ CAL at 6 months or 1 year) was chosen as a threshold to define progressing sites. This choice was based on the intra-examiner calibration [corresponding to approximately three standard deviations (SD) of the measures of the intra-examiner reproducibility] and on published data (Oringer et al. 1998).

Patient protection

During the longitudinal study, whenever disease progression (ΔCAL >2 mm) was detected or if a periodontal abscess was diagnosed, the tooth or teeth affected were treated with subgingival instrumentation and exited from the study. After the 1-year visit, all the patients received full-mouth subgingival instrumentation as needed.

Definition of smoking categories

All subjects were divided into three categories according to self-reported smoking history: (i) non-smokers (subjects who had never smoked), (ii) former smokers and (iii) current smokers. In the longitudinal study, former smokers and never smokers were grouped together, as no effect on periodontal progression was expected in subjects who had given up smoking.

Genetic analysis

A blood sample was collected at the baseline visit via venipuncture and stored at -70°C until the genetic analysis was performed. DNA was extracted from leucocytes using the Nucleon® BACC2 kit (Nucleon Bioscience, Coatbridge, UK) according to the manufacturer's instructions. RFLPs were analysed blindly for VDR -1056 TaqI polymorphism by the polymerase chain reaction as described previously (Brett et al. 2005).

Statistical analysis

All the data were entered in a computer file, proofed for entry errors and analysed with a statistical package (SPSS version 11.5). Continuous variables are reported as means $\pm$ SD. Comparisons of continuous and categorical data between groups were analysed with ANOVA and χ^2 test, respectively. The α value was set at 0.05. A binary-independent variable (periodontitis or healthy control) was used to investigate the association between VDR genotypes and the diagnosis of periodontitis. Sub-analyses were performed between localized AgP (LAgP), generalized AgP (GAgP), CP and controls. Multiple logistic regression analysis adjusting for known confounders (age, gender, ethnicity and smoking) was performed to detect differences in genotype frequency. Because of the risk of finding spurious associations (Pritchard & Rosenberg 1999), separate analyses were performed in smokers and non-smokers, and in Caucasians. In the longitudinal study, two progression clusters were arbitrarily identified based on the number of sites showing progression (above or below the median). Logistic regression analysis was performed to investigate associations between demographic and genetic factors and progression cluster. Age, gender, smoking and ethnicity were entered in the model as

Table 1. Demographic data

	LAgP ($n=57$)	GAgP ($n=167$)	CP ($n=79$)	Total periodontitis ($n=301$)	Healthy ($n=231$)	Comparisons between total periodontitis and healthy (p)
Age	25.6 ± 8.4	31.4 ± 6.0	46.7 ± 8.7	34.3 ± 10.6	38.4 ± 12.2	<0.001
Gender (female)	37 (64.9%)	108 (64.7%)	60 (75.9%)	205 (67.7%)	132 (57.1%)	0.015
Ethnicity						
Caucasian	24 (42.1%)	88 (52.7%)	58 (73.4%)	170 (56.1%)	144 (62.3%)	0.513
Black	14 (24.6%)	45 (26.9%)	6 (7.6%)	65 (21.5%)	45 (19.5%)	
Asian	13 (22.8%)	21 (12.6%)	14 (17.7%)	48 (15.8%)	29 (12.6%)	
Other	6 (10.5%)	13 (7.8%)	1 (1.3%)	20 (6.6%)	13 (5.6%)	
Smoking						
Never	39 (68.7%)	79 (47.3%)	32 (40.5%)	150 (49.5%)	135 (58.4%)	0.063
Former	10 (17.5%)	42 (25.1%)	25 (31.6%)	77 (25.4%)	41 (17.7%)	
Current	8 (14.0%)	46 (27.6%)	22 (27.9%)	76 (25.1%)	55 (23.8%)	
VDR						
TT	25 (43.9%)	70 (41.9%)	39 (49.4%)	134 (44.2%)	90 (39.0%)	0.126
Tt	23 (40.4%)	74 (44.3%)	32 (40.5%)	129 (42.6%)	96 (41.6%)	
tt	9 (15.8%)	23 (13.8%)	8 (10.1%)	40 (13.2%)	45 (19.5%)	

Continuous variables are reported as means ± SD. Comparisons of continuous and categorical data between groups were analysed with ANOVA and χ^2 test, respectively.

CP, chronic periodontitis; GAgP, generalized aggressive periodontitis; LAgP, localized aggressive periodontitis; SD, standard deviation; VAD, vitamin D receptor.

Table 2. Demographic data in Caucasians

	LAgP ($n = 24$)	GAgP ($n = 88$)	CP ($n = 58$)	Total periodontitis ($n = 170$)	Healthy ($n = 140$)	Comparisons between total periodontitis and healthy (p)
Age	25.6 ± 8.4	31.4 ± 6.0	46.7 ± 8.7	34.3 ± 10.6	38.4 ± 12.2	< 0.001
Gender (female)	16 (66.7%)	60 (68.2%)	40 (69.0%)	116 (68.2%)	74 (51.4%)	0.003
Smoking						
Never	12 (50.0%)	26 (29.5%)	15 (25.9%)	53 (31.2%)	135 (58.4%)	<0.001
Former	6 (25.0%)	31 (35.2%)	23 (39.7%)	60 (35.3%)	41 (17.7%)	
Current	6 (25.0%)	31 (35.2%)	20 (34.5%)	57 (33.5%)	55 (23.8%)	
VDR						
TT	11 (45.8%)	33 (37.5%)	27 (46.6%)	71 (41.8%)	53 (36.8%)	0.215
Tt	11 (45.8%)	43 (48.9%)	24 (41.4%)	78 (45.9%)	63 (43.8%)	
tt	2 (8.3%)	12 (13.6%)	7 (12.1%)	21 (12.4%)	28 (19.4%)	

Continuous variables are reported as means ± SD. Comparisons of continuous and categorical data between groups were analysed with ANOVA and χ^2 test, respectively.

CP, chronic periodontitis; GAgP, generalized aggressive periodontitis; LAgP, localized aggressive periodontitis; SD, standard deviation; VAD, vitamin D receptor.

confounders, regardless of their significance. An analysis of the interaction between genetic and demographic and environmental factors (Kleinbaum et al. 1982) was also performed for all polymorphisms. Both Wald's and likelihood-ratio tests were performed for the interactions in the presence of small sample sizes, for which the latter is considered to be more appropriate (Agresti 1996).

Results

Case–control study

The demographic characteristics of all the patients who took part in the study and of the Caucasian subgroup are reported in Tables 1 and 2. Higher percentages of females and smokers (past or current) were detected in the patient group compared with the controls. The ethnic distributions between groups were similar, with a slightly higher percentage of Caucasians among controls. VDR genotype distributions satisfied the criteria for Hardy–Weinberg equilibrium in each study group. No statistically significant differences in genotype distributions were observed in all subjects and in the Caucasian subgroup.

In Caucasians, logistic regression analysis revealed that the interaction between VDR genotypes/smoking was significantly associated with the diagnosis of periodontitis [$p = 0.001$, odds ratio (OR) = 1.33, 95% confidence intervals (CI) = 1.12–1.57, adjusted for age and gender].

When subjects of all ethnicities were analysed, logistic regression analysis confirmed the association between the VDR genotypes/smoking interaction and the diagnosis of periodontitis ($p = 0.033$, OR = 1.60, 95% CI = 1.04–2.48, adjusted for age, gender and ethnicity). In particular, when only smokers (current and former) were taken into account ($n = 153$ periodontitis patients and 96 controls), VDR genotypes showed a clear association with periodontitis ($p = 0.025$, OR = 2.43, 95% CI = 1.12–5.27, adjusted for age, gender and ethnicity). Figure 1 shows VDR genotypes in periodontitis patients and controls divided by their smoking status. While in non-smokers the genotype distribution between controls and patients is very similar, among smokers there is an evident enrichment of T allele presence in the periodontitis group (mainly seen as an increase in T homozygosity).

Longitudinal study

One hundred and fifty-one sites in 52 patients exhibited progression, 78 sites at the 6-month evaluation and 73 sites at

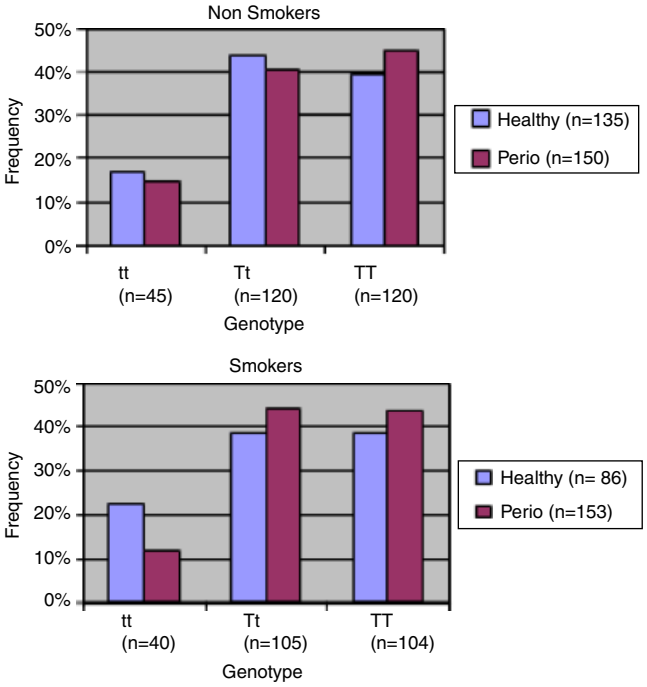

Fig. 1. Presence of periodontitis in non-smokers and smokers by their vitamin D receptor Taq-I genotypes. Interaction VDR genotypes/smoking association with periodontitis: $p = 0.033$, OR = 1.60, 95% CI = 1.04 – 2.48.

1 year, while the remaining nine patients presented with no progression (Table 3). Sixty of the progressing sites were molar sites, 44 pre-molars and 47 anteriors. Two clusters of patients were identified, 27 patients belonging to the first category (severe progression, minimum of three ''losing sites'' per subject) and 34 to the second category (mild progression, <3 progressing sites). The genotype distributions in relation to progression clusters are reported in Table 4. None of the demographic and environmental factors analysed (age, gender, ethnicity, smoking) showed significant associations with the progression cluster.

VDR TT polymorphism was not associated with disease progression in the bivariate analysis, but its interaction with smoking was associated with the severe progression cluster, independent of age, gender and ethnicity differences (Wald's test $p = 0.033$ OR = 15.24, 95% CI = 1.24–187.42; likelihood ratio test $p = 0.010$). The association of VDR genotype with disease progression was not observed in the non-smoker group, but was remarkable in the current smoking group. Indeed, while VDR heterozygous subjects showed only a moderate, T homozygous subjects presented a stronger association with periodontitis progression (OR = 1.49 and 22.78, respectively) (Fig. 2).

Eight patients accounted for one third of all losing sites (total sites, 49). Six of these patients, two of whom were smokers, were T homozygous.

Table 3. Number of progressing sites per patient

Number of progressing sites	Number (%) of patients	Cumulative (%)
0	9 (14.8%)	14.8
1	13 (21.3%)	36.1
2	12 (19.7%)	55.7
3	11 (18.0%)	73.8
4	8 (13.1%)	86.9
5	3 (4.9%)	91.8
6	2 (3.3%)	95.1
7	2 (3.3%)	98.4
8	1 (1.6%)	100
Total	61	

The number of progressing sites per patients with the correspondent frequency is reported. Sites that exhibited at least 3 mm of attachment loss from baseline were considered as progressing sites. A threshold of three progressing sites per patient is considered in order to identify two clusters of patients (mild or severe progression).

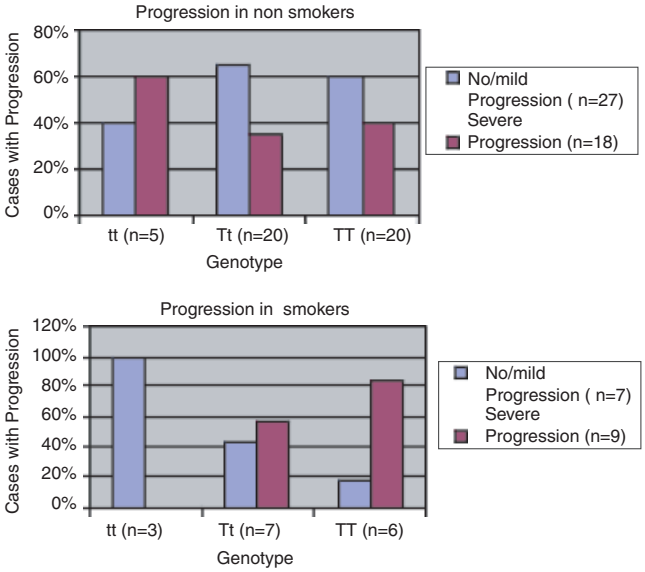

Fig. 2. Periodontitis progression in non-smokers and smokers by their vitamin D receptor Taq-I genotypes. Interaction VDR genotypes/smoking association with progression: $p = 0.033$, OR = 15.24, 95% CI = 1.24–187.42.

Table 4. Demographic and clinical data on progression

	Total	Progression		Comparison between groups (p)
		no/mild	severe	
Number of patients	61	34	27	–
Age				
Median ± interquartile range	50.0 ± 12.0	50.0 ± 13.8	52.0 ± 12.0	0.356
Mean ± SD	49.1 ± 8.2	48.2 ± 8.7	50.4 ± 7.7	
Gender				
Male	17 (27.9%)	10 (29.4%)	7 (25.9%)	1.000
Female	44 (72.1%)	24 (70.6%)	20 (74.1%)	
Ethnicity				
Caucasians	46 (75.4%)	24 (70.6%)	22 (81.5%)	0.572
No Caucasians	15 (24.6%)	10 (29.4%)	5 (18.5%)	
Smoking				
Current smokers	16 (26.2%)	7 (20.6%)	9 (33.3%)	0.380
No smokers	45 (73.8%)	27 (79.4%)	18 (66.7%)	
VDR Taq-I				
tt	8	5 (14.7%)	3 (11.1%)	0.729
Tt	27	16 (47.1%)	11 (40.7%)	
TT	26	13 (38.2%)	13 (48.1%)	

Patients are divided in two clusters at 1 year, according to number of progressing sites. Demographic and clinical data are presented. Continuous variables are reported as means ± SD. Comparisons of continuous and categorical data between groups were analysed with ANOVA and χ^2 test, respectively.

SD, standard deviation; VDR, vitamin D receptor.

Discussion

The results of this study suggest that a VDR polymorphism is associated with periodontitis in smokers. While VDR Taq-I polymorphism independently was not associated with the presence and progression of periodontitis, its interaction with smoking was associated with the presence of periodontitis and with progression of CP. This association was stronger in the Caucasians ethnic subgroup.

The VDR is a nuclear receptor that binds to the active form of vitamin D and thereby modulates gene expression by interacting with the promoter region of the target genes. The vitamin D system is involved in several endocrine pathways such as calcium metabolism (Uitterlinden et al. 2002), immune modulation and regulation of cell growth, differentiation and induction of apoptosis of a variety of cells, such as osteoblasts, keratinocites, T-cells and cancer cells (Hayes et al. 2003, Welsh et al. 2003, Mullin & Dobs 2007). In particular, weakened VDR responses have been linked with intensified T-helper type 1 (Th1) actions and Th2 suppression (Mullin & Dobs 2007). Some VDR polymorphisms have been associated with circulating levels of active vitamin D and in vitro measures of gene expression (Morrison et al. 1992). However, this study focused on an RFLP of the VDR gene, called Taq-I, present in the coding sequence (exon 9) but ''silent'': its nucleotide variation (T to C) does not change the amino acid sequence of the encoded protein. Nonetheless, it has been widely studied, because it may be in linkage disequilibrium with an unknown functional sequence elsewhere in the gene.

Recently, VDR polymorphisms, in association with poor vitamin D nutrition, have been linked to susceptibility to chronic mycobacterial infections. Moreover, VDR gene polymorphisms have been found to be associated with osteoporosis, osteoarthritis, hyperparathyroidism, cancer and infection susceptibility (e.g. to tuberculosis and hepatitis B), but with conflicting results (Bellamy et al. 1999). The VDR Taq-I TT genotype was found to be associated with chronic periodontitis in a Japanese population (Tachi et al. 2003) and in Caucasians (Brett et al. 2005). This finding has not been supported by other studies in Japanese populations (Yoshihara et al. 2001 and Sun et al. 2002). On the other hand, the t allele was found to be associated with LAgP in a Caucasian population (Hennig et al. 1999) and with CP in a Brazilian population (de Brito et al. 2004). No association was detected between the same Taq-I polymorphism and progression over 23 years in a group of healthy or mild periodontitis Japanese subjects, while a significant result was observed for the VDR Apa-I AA genotype (Inagaki et al. 2003). However, none of these studies presented subanalysis in smokers and non-smokers. The discrepancies in the results of previous studies with respect to VDR genotypes may be due to different ethnicities of the populations studied and the heterogeneous study inclusion criteria.

Smoking is thought to have an important modifying effect on periodontitis pathogenesis (Palmer et al. 2005). Possible mechanisms of action include effects on the immune and inflammatory processes, on neutrophil function and cell-mediated and humoral immunity. Smoking also affects bone metabolism, as smokers have been reported to have reduced bone mineral content compared with non-smokers (Haber et al. 1993). Smoking and other environmental factors have been shown to interact with genetic factors in the pathogenesis of a variety of diseases, such as stroke (Romero 2007), diabetes (Barroso 2005) and metabolic syndrome (Iannucci et al. 2007).

Considering that VDR and smoking may work along the same pathogenetic pathways, speculations about the VDR–smoking interaction in periodontitis pathogenesis may involve a synergistic effect on immune response (such as on the Th1/Th2 axis) and/or bone metabolism. However, we have to acknowledge the limitations of the analyses presented here, such as the relatively small sample size (especially in the longitudinal study) and potential residual confounding factors (ethnic, socio-economic, microbiological factors), with the risk of finding spurious associations (highlighted by the wide confidence intervals of some of the associations detected).

Our results confirm that a small subset of subjects exhibited a high rate of progression of chronic periodontitis, with a number of progressing sites in few subjects accounting for a large proportion of the total. This confirms the importance of a patient-centred approach in order to understand the factors associated with the development and progression of periodontal disease (Heitz-Mayfield 2005).

These study analyses highlight the importance of genetic markers in periodontitis pathogenesis. Genetically determined host factors, especially in association with environmental factors, may play a role in the onset and development of periodontitis. The ability to determine risk predictors for attachment loss may provide the possibility to adopt different treatment strategies and maintenance regimes, depending on the individual risk of developing disease progression. However, because of the previously mentioned limitations of these analyses, these results have to be considered cautiously. Larger studies should be conducted in order to confirm the importance of our findings, and in particular of the interaction between VDR/smoking, in relation to disease pathogenesis. If the clinical effect of this interaction is confirmed, further primary and secondary prevention plans for VDR TT-positive smokers may be envisaged.

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Clinical Relevance

Scientific rationale for the study: Genetic and environmental factors are thought to affect the onset and progression of periodontitis.

Principal findings: The interaction between smoking and a VDR was associated with the diagnosis and progression of periodontitis.

Practical implications: The effect of smoking on the pathogenesis of periodontitis may be increased in subjects with a specific VDR genotype. If confirmed, this might have implications for the periodontal management of these patients.