Association of vitamin D receptor gene polymorphisms and periodontitis in a Taiwanese Han population

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Abstract  Background/purpose: Genetic polymorphisms in the vitamin D receptor (VDR) gene are related to the immune response and bone metabolism, both of which are implicated in the pathogenesis of periodontitis. This study was to investigate the association between VDR-gene polymorphisms and periodontitis among a Taiwanese Han ethnic population.

Materials and methods: Ninety-two aggressive periodontitis (AgP), 385 chronic periodontitis (CP) and 163 healthy controls (HC) were recruited from dental clinics. Demographic characteristics and possible confounding factors were obtained using a self-reported questionnaire. The VDR rs731236 (TaqI), rs7975232 (ApaI), rs1544410 (BsmI), and rs2228570 (FokI) polymorphisms were genotyped using PCR-RFLP methods. Statistical analyses were applied to determine the associations.

Results: The individual VDR polymorphisms were not associated with risk of AgP and CP. The f allele of rs2228570 was related to decreased risk for AgP. Subjects with TAbF (adjusted OR = 7.2, 95% CI = 3.2–7.2, p < 0.0001) or TAbf (adjusted OR = 0.17, 95% CI = 0.05–0.48, p = 0.002) combined polymorphisms were significantly associated with AgP. Subjects

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Introduction

The vitamin D endocrine system is associated with many biologic responses including bone metabolism, modulation of the immune system, regulation of cell proliferation and differentiation.\(^1\) The biological active form of vitamin D is an important immunomodulatory agent which activates monocytes, stimulates cell-mediated immunity, suppresses T-lymphocytes proliferation, reduce immunoglobulin production and cytokines synthesis. Vitamin D also can suppress proinflammatory responses, and enhance innate immunity, leading to a conversion of the Th1 immune response towards the Th2 profile.\(^2\) The biological effects of vitamin D are exerted via the vitamin D receptor (VDR), a transcription factor in the nuclear receptor superfamily. VDRs promote the function of vitamin D and regulate the expression of many genes.

The VDR gene has several polymorphisms. Associations between VDR polymorphisms and susceptibility to periodontitis have been conducted in different settings and ethnic backgrounds (Table 1). The results obtained so far are conflictive. Among Chinese subjects, BsmI, Apal and TaqI genotype distributions were not significantly different between generalized aggressive periodontitis (GAgP) and healthy control (HC), but the FF genotype of FokI polymorphism was overrepresented in GAgP.\(^3\) Two studies reported that the Tt genotype of TaqI polymorphism might be associated with the risk of developing early-onset periodontitis (EOP) among Chinese.\(^4\)\(^,\)\(^5\) TaqI polymorphism was not associated with the susceptibility to adult periodontitis among Chinese.\(^5\) Chinese subjects with the TT genotype and the T allele were more susceptible to severe CP, while BsmI, Apal and FokI polymorphisms were not associated with risk for severe CP.\(^6\)

At the present time, information on the existence of VDR polymorphisms is limited to areas in the gene studied by using restriction fragment length polymorphisms (RFLP). A number of the currently known VDR polymorphisms have been depicted.\(^7\) Among them BsmI-, Apal-, TaqI- and FokI-polymorphisms have been investigated in relation to periodontitis. It is very important to analyze all known VDR polymorphisms and their interrelationships, since they will interact with each other to determine VDR expression and activity.\(^8\) We were the first to examine the relationships between VDR BsmI, Apal, TaqI and FokI polymorphisms/ haplotypes and the risk of susceptibility to CP and AgP among Taiwanese Han individuals.

Materials and methods

Determination of the sample size

Genetic Power Calculator was utilized to estimate power calculation.\(^9\) This sample size including 92 AgP, 385 CP and 163 HC had 80% power to detect susceptibility with a genotypic relative risk \(\geq 1.5\) at a significance level of 0.05 for a SNP with a risk allele frequency \(\geq 0.36.\)^\(^1^0\)

Study subjects

This case control study consisted of 92 AgP patients (39 females, 53 males), 385 CP patients (170 females, 215 males), and 163 ethnically matched periodontal HC (86 females, 77 males). They were recruited from patients who visited the Department of Periodontics, Kaohsiung Medical University Hospital. All subjects had at least 18 teeth when they were enrolled in the study. Exclusion criteria included systemic diseases associated with destructive periodontal disease, such as diabetes mellitus, immunosuppression, human immunodeficiency virus infection and polymorphonuclear or monocyte defects determined via the questionnaire and medical history review. Subjects who had received professional prophylaxis or had taken antibiotics in the previous 6 months, were pregnant, were lactating, or were in need of antibiotic prophylaxis before periodontal treatment were also excluded from this study. The Institutional Review Board of Kaohsiung Medical University approved the study protocol. All subjects signed informed consents. The study subjects were diagnosed as having AgP, CP, and HC on the basis of clinical examinations of probing depth (PD) and attachment loss (AL), and radiographic patterns of alveolar bone destruction. The diagnostic criteria for AgP and CP were defined in accordance with the classification agreed on at the World Workshop for Periodontics and The American Academy of Periodontology.\(^1^1\) Subjects who had more than eight teeth with AL \(\geq 5\) mm and PD \(\geq 6\) mm, at least three affected teeth other than first molars and incisors, and the severity of periodontal tissue destruction was not consistent with the amounts of microbial deposits were diagnosed as AgP. CP was indicated in subjects \(> 35\) years of age, with AL \(\geq 5\) mm at more than one tooth, more than three sites with PD \(\geq 6\) mm, and lesions involving more than two teeth in each quadrant. The level of attachment loss must appear consistent with the plaque...
level or local contributing factors. HC was indicated by no evidence of AL/C21m and PDM or ethanol. The recruited healthy controls were older than 35 years so that they could not be too young to have periodontitis. Subjects who still had the smoking habit or had quit smoking within the last 6 months before enrolling in this study were designated as current smokers. Former smokers were subjects who had quit smoking for at least 6 months.

**Sample collection and DNA extraction**

Twenty milliliters of heparin-anticoagulated peripheral blood were collected from each study subject. Genomic DNA was extracted and concentration was determined by ultraviolet (UV) spectrophotometry.

**Genotyping of VDR genetic polymorphisms**

The rs731236 (TaqI), rs7975232 (Apal), rs1544410 (BsmI) and rs2228570 (FokI) VDR genetic polymorphisms were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers and PCR conditions used to identify these four VDR SNPs are listed in Table 2. The results of each genetic polymorphism were validated by performing repeat testing and direct sequencing on 20% of the subject pool selected by random sampling. No discrepancy was found.

**Table 1** Studies for the association of VDR genetic polymorphisms and susceptibility to periodontitis.

| Locus | Subjects | Disease phenotype | Risks | Reference |
|-------|----------|-------------------|-------|-----------|
| Apal  | Chinese  | 107 severe CP     | Apal, BsmI & FokI: NS | Wang et al. (2009) |
| BsmI  |         | 121 HC            | TaqI TT vs (Tt + tt) OR = 2.75, p = 0.02 |
| FokI  |         |                   | FokI FF vs (Ff + ff) OR = 2.90, p = 0.04 |
| TaqI  |         |                   |                   |
| Apal  | Chinese  | 51 GAgP           | Apal, BsmI & TaqI: NS | Li et al. (2008) |
| BsmI  |         | 53 HC             | FokI FF vs (Ff + ff) OR = 2.90, p = 0.04 |
| FokI  |         |                   |                   |
| TaqI  |         |                   |                   |
| Apal  | Turkish  | 72 CP             | NS                | Gunes et al. (2008) |
| BsmI  |         | 102 HC            |                   |
| TaqI  |         |                   |                   |
| Apal  | Japanese | 17 severe CP     | NS                | Naito et al. (2007) |
| BsmI  |         | 80 not-severe CP  |                   |

AgP: aggressive periodontitis; AP: adult periodontitis; CP: chronic periodontitis; EOP: early-onset periodontitis; L-EOP: localized early-onset periodontitis; G-EOP: generalized early-onset periodontitis; NS: no association.
Statistical analysis

Statistical analyses were performed using a JMP statistical software package (SAS Institute, Cary, NC, USA). Comparisons of descriptive statistics among the three groups were expressed as mean (± SD) and within group proportions. Differences in the demographic factors of frequency distribution and numeric data among the three groups were tested using the χ²-test and ANOVA test, respectively. The frequency distribution of the genotype and its allele frequencies in each group were compared by the χ²-test. Homozygote major allele carriers were used as the reference category. To increase statistical power for the less frequent variant, the rare homozygotes were combined with the heterozygotes assuming a dominant effect. γ² was calculated to estimate linkage disequilibrium for each pair of polymorphisms. Linkage disequilibrium was measured based on calculating D’ using Haploview 4.1. The haplotype frequencies were determined by PowerMarker software V3.25. The risk association of genotypes/haplotypes/combined polymorphisms and periodontitis was computed by logistic regression analysis and expressed as an odds ratio (OR) with a 95% confidence interval (CI). Multiple testing among groups was corrected by Bonferroni method. To control the possible confounding effects, age, gender, and smoking status were used as independent variables of multiple logistic regressions for adjustment.

Results

The same population had been recruited for our previous studies (Ho et al., 2008; Ho et al., 2010; Chou et al., 2011) to determine the susceptibility to periodontitis. Briefly, the demographic characteristics of the study subjects are presented in Table 3. The gender distribution was similar for these three groups. The mean age of the AgP patients was significantly lower than the CP patients and HC. The frequencies of smoking habit were significantly different between CP and HC (p = 0.001), and between AgP and HC (p = 0.042). There were more CP patients to be current smokers. The probing depth and clinical attachment level in AgP and CP were significantly higher than those in HC (p = 0.01).

The genotype frequencies of each of the VDR polymorphisms did not deviated from the Hardy–Weinberg equilibrium (p = 0.79, 0.99, 0.25, and 0.85 for rs731236, rs7975232, rs1544410, and rs2228570, respectively). In AgP patients, the genotype distribution of rs2228570 (FokI) was borderline-significantly different (p = 0.007) from the HC (Table 4). When the minor allele frequency of rs2228570 was compared between AgP and HC, the frequency of f allele was significantly lower in AgP (adjusted OR = 0.57, 95% CI = 0.39–0.82, p = 0.003). The frequencies of genotype and minor allele of the other SNPs were not significantly different between AgP and HC. In CP patients, logistic regression analysis showed the genotype distributions and the minor allele frequencies of the investigated four SNPs were not significantly different from the HC (Table 4).

The linkage disequilibrium coefficient (r²) and D’ value was measured to determine the linkage disequilibrium between these four VDR SNPs. The plot shown in Fig. 1 identified significant linkage disequilibrium between rs731236 and rs7975232, and between rs731236 and rs1544410. The linkage disequilibrium between rs2228570 and the other three SNPs was weak. VDR haplotype comprising rs731236 and rs7975232 did not demonstrate greater effect on risk for AgP and CP than the individual genotype (data not shown).

Fifteen different combined polymorphisms were identified after analyzing combinations of the genotype

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**Table 2** Primers and PCR conditions used for determination of the four VDR SNPs.

| SNP name | dbSNP   | PCR Primer | PCR condition                        |
|----------|---------|------------|--------------------------------------|
| SN1 TaqI| rs731236| F: 5-CAG AGC ATG GAC AGG GAG CAA-3  |
|          |         | R: 5-GCA ACT CCT CAT GGC TGA GGT CTC-3 |
|          |         | Denaturation at 95 °C for 1 min, 35 cycles at 95 °C for 1 min, 66 °C for 1 min, 72 °C for 2 min, final extension at 72 °C for 10 min |
|          |         |            | PCR products were digested with TaqI (5’ U/sample) at 65 °C for 4 h |
| SN2 Apal| rs7975232| F: 5-CAG AGC ATG GAC AGG GAG CAA-3  |
|          |         | R: 5-GCA ACT CCT CAT GGC TGA GGT CTC-3 |
|          |         | Denaturation at 95 °C for 1 min, 35 cycles at 95 °C for 1 min, 66 °C for 1 min, 72 °C for 2 min, final extension at 72 °C for 10 min |
|          |         |            | PCR products were digested with Apal (10’ U/sample) at 22 °C for 4 h |
| SN3 BsmI| rs1544410| F: 5-CAA CCA AGA CTA CAA GTC CCG GTT CATGTA-3 |
|          |         | R: 5-AAC CAG CGG GAA GAG TGC AAG GG-3 |
|          |         | Denaturation at 95 °C for 2 min, 35 cycles at 95 °C for 1 min, 63 °C for 1 min, 72 °C for 1.5 min, final extension at 72 °C for 7 min |
|          |         |            | PCR products were digested with BsmI at 65 °C overnight |
| SN4 FokI| rs2228570| F: 5-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3 |
|          |         | R: 5-AGC TGG CCC TGG CAC TGA CTC TGC TCT-3 |
|          |         | Denaturation at 95 °C for 3 min, 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, final extension at 72 °C for 5 min |
|          |         |            | PCR products were digested with FokI at 37 °C for 3 h |
frequencies for the 4 VDR polymorphisms. The AgP had 12-, CP had 13- and HC had 10 combined polymorphisms. We selected the combined polymorphisms of higher frequencies (Tabf, TabF, TAbF, and TAbf, ranging from 33% to 8%) for further analysis (Table 5). These four combined polymorphisms were not associated with risk for AgP. The Tabf combined polymorphism had a positive correlation with the risk for CP (adjusted OR $= 1.69$, 95% CI: 1.58–1.77).

### Table 3 The demographic characteristic of studied individuals.

|                | AgP n = 92 | CP n = 385 | HC n = 163 | p-Value<sup>a</sup> |
|----------------|------------|------------|------------|--------------------|
| Gender n (%)   |            |            |            |                    |
| Female         | 39 (42.4)  | 170 (44.2) | 86 (52.8)  | $p = 0.135$        |
| Male           | 53 (57.6)  | 215 (55.8) | 77 (47.2)  |                    |
| Age<sup>b</sup> (mean ± SD) | 37.7 ± 7.0 | 52.6 ± 7.8 | 51.1 ± 10.0 | $p < 0.0001$ |
| Smoking<sup>c</sup> n (%) |            |            |            |                    |
| Non            | 65 (70.7)  | 277 (72.0) | 137 (84.0) | $p = 0.002$        |
| Current        | 14 (15.2)  | 82 (21.3)  | 12 (7.4)   |                    |
| Former         | 13 (14.1)  | 26 (6.7)   | 14 (8.6)   |                    |
| PD (mm) (mean ± SD) | 3.4 ± 0.7  | 3.5 ± 0.4  | 2.4 ± 0.2  | $p < 0.01$        |
| CAL (mm) (mean ± SD) | 4.2 ± 0.3  | 4.3 ± 0.7  | 0.5 ± 0.2  | $p < 0.01$        |
| AgP: aggressive periodontitis; CP: chronic periodontitis; HC: healthy controls; PD: probing depth; CAL: clinical attachment level.

<sup>a</sup> Comparisons performed by $\chi^2$-test or ANOVA.

<sup>b</sup> Age: $p < 0.0001$ for AgP vs HC; not significant for CP vs HC.

<sup>c</sup> Smoking status: $p = 0.042$ for AgP vs HC; $p = 0.001$ for CP vs HC.

### Table 4 Genotype distribution of VDR SNPs in periodontitis patients and healthy controls.

| Genotype | HC n = 163 | AgP n = 92 | CP n = 385 | p-Value<sup>a</sup> |
|----------|------------|------------|------------|--------------------|
| rs731236 (TaqI) |          |            |            |                    |
| TT       | 152 (93.3) | 81 (88.0)  | 352 (91.4) | $p < 0.05$         |
| Tt       | 9 (5.5)    | 11 (12.0)  | 33 (8.6)   | 1.58 (0.74–3.39)   |
| tt       | 2 (1.2)    | 0 (0)      | 0 (0)      | 0.93 (0.47–1.75)   |
| MAF (%)  | 4.0        | 6.0        | 3.39       | 0.03               |
| Tt/ttc   | 11 (6.8)   | 11 (12.0)  | 33 (8.6)   | 1.43 (0.72–3.07)   |
| rs7975232 (Apol) |        |            |            |                    |
| aa       | 80 (49.1)  | 41 (44.6)  | 177 (46.0) | $p < 0.05$         |
| Aa       | 72 (44.2)  | 41 (44.6)  | 165 (42.9) | 1.04 (0.71–1.52)   |
| AA       | 11 (6.7)   | 10 (10.8)  | 43 (11.1)  | 1.77 (0.87–3.60)   |
| MAF (%)  | 28.8       | 33.2       | 32.6       | 0.59 (0.90–1.59)   |
| Aa/AAc   | 83 (50.9)  | 51 (55.4)  | 208 (54.0) | 1.13 (0.77–1.66)   |
| rs1544410 (BsmI) |      |            |            |                    |
| bb       | 115 (70.5) | 75 (81.5)  | 308 (80.0) | $p < 0.04$         |
| Bb       | 43 (26.4)  | 13 (14.1)  | 72 (18.7)  | 0.63 (0.40–0.97)   |
| BB       | 5 (3.1)    | 4 (4.4)    | 5 (1.3)    | 0.37 (0.11–1.31)   |
| MAF (%)  | 16.3       | 11.4       | 10.6       | 0.61 (0.42–0.90)   |
| Bb/BBc   | 48 (29.5)  | 17 (18.5)  | 77 (20.0)  | 0.58 (0.38–0.90)   |
| rs2228570 (FokI) |    |            |            |                    |
| FF       | 43 (26.4)  | 42 (45.6)  | 104 (27.0) | $p < 0.23$         |
| Ff       | 76 (46.6)  | 33 (35.9)  | 202 (52.5) | 1.10 (0.71–1.71)   |
| ff       | 44 (27.0)  | 17 (18.5)  | 79 (20.5)  | 0.74 (0.44–1.24)   |
| MAF (%)  | 50.3       | 36.4       | 46.8       | 0.87 (0.67–1.28)   |
| Ff/ffc   | 120 (73.6) | 50 (54.4)  | 281 (73.0) | 0.95 (0.62–1.45)   |

AgP: aggressive periodontitis; CP: chronic periodontitis; HC: healthy controls; MAF: minimal allelic frequency; OR: odds ratio; CI: confidence interval.

<sup>a</sup> Adjusted for gender, age and smoking status for logistic regression analysis.

<sup>b</sup> $p < 0.006$ was accepted as significant for multiple testing corrected by Bonferroni method.

<sup>c</sup> t carrier, A carrier, B carrier or f carrier versus each reference.
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Figure 1 Linkage disequilibrium plot of four SNPs in the VDR gene. The number in the square is the D’ value.

CI = 1.28–2.27, p = 0.0003). The TabF and TAfb combined polymorphisms had a negative correlation with the risk for CP (adjusted OR = 0.50, 95% CI = 0.33–0.75, p = 0.001 and adjusted OR = 0.36, 95% CI = 0.20–0.65, p = 0.001, respectively). The TabF combined polymorphism was not associated with the risk for CP. 

Table 5 also shows the individuals with one or two copies of TabF could have increased risk for AgP (adjusted OR = 7.19, 95% CI = 3.21–7.20, p < 0.0001); individuals with one or two copies of TabF could have increased risk for AgP (adjusted OR = 0.17, 95% CI = 0.05–0.48, p = 0.002). Subjects with one or two copies of TabF (adjusted OR = 2.58, 95% CI = 1.76–3.80, p < 0.0001), or TABF (adjusted OR = 4.42, 95% CI = 2.57–8.07, p < 0.0001) could have increased risk for the development of CP. Individuals with one or two copies of TabF (adjusted OR = 0.36, 95% CI = 0.24–0.53, p < 0.0001), or TabF (adjusted OR = 0.45, 95% CI = 0.28–0.73, p = 0.001) could have a decreased risk for the susceptibility to CP.

Discussion

Our results revealed that: (1) the individual polymorphism was not associated with susceptibility to AgP and CP; (2) the f allele of rs2228570 was associated with reduced risk for developing AgP. The studied individual VDR polymorphism was not related to risk for CP. The results differed from the report by Deng et al.16 which showed CP had a significantly lower frequency of bb genotype of BsmI, higher frequency of AA genotype of Apal, and TT genotype of Taql; but no significant difference in any genotype of FokI. We showed no association of VDR gene polymorphisms with AgP. It was in consistent with the results of Deng et al.16 Another meta-analysis indicated that mutant allele t of Taql locus may be a protective factor for CP, but not for AgP; the mutant allele F of the FokI locus appeared to be a risk factor for AgP rather than CP; BsmI and Apal polymorphisms were not associated with the susceptibility to periodontitis.17 We also found the f allele of FokI polymorphism was associated with reduced risk for AgP, but not for CP. In contrast to this recent meta-analysis, our study indicated that Taql was not related to the susceptibility to periodontitis. The allelic distribution of Taql polymorphism found in the present study was comparable to that reported in the published study for Chinese.5 However, Taql-RFLP was not associated with the susceptibility to AgP and CP in our sample. This differed from the previous association studies that showed Taql polymorphism was linked to EOP and CP susceptibility (Table 1). The carriage rate of VDR Taql t allele was quite different between different ethnic populations. The t allele was not associated with susceptibility to periodontitis in our subjects; however, this might be relative to the very low carriage rate of the t allele in Taiwanese.

The FokI SNP is a functional polymorphism in the start condon at the 5’ promotor region of the VDR gene. The stronger interaction of the F allele with transcription factor can translate stronger signaling messages for bone resorption and inflammation. These findings might explain why the FF genotype and F allele had positive associations with the risk for the susceptibility to periodontitis. To our knowledge, the biological effects of FokI-polymorphism on periodontal tissue remain unknown.

In a study about BsmI SNP effect on VDR level showed the B allele was associated with higher VDR levels.18 In contrast, other studies reported BsmI SNP did not affect either the VDR mRNA level19 or VDR function.20 The Taql genetic variant effects on the mRNA stability have been evaluated. So far, the results are conflictive. Individuals with tt genotype had significantly lower levels of both VDR mRNA expression and protein and stronger inflammatory responses. The b, a, and T alleles and baT haplotype were associated with lower VDR mRNA levels.21 The B, t and F allele of BsmI, Taql- and FokI-polymorphisms were highly responsive compared to b, T, and f alleles. VDR BB, tt, and FF genotypes and composite genotype BBAAtt, and haplotype BAatt are associated with the increased expression of VDR.22

Previous studies reported that the haplotype or combined VDR polymorphisms might affect VDR expression18,21,22 consequently might predict periodontitis. In Caucasians, the rs731236 is in strong linkage disequilibrium with the rs7975232 and rs1544410, while rs228570 site appears to be an independent marker. Our subjects demonstrated the same result of linkage disequilibrium analysis. The VDR haplotypes comprising rs731236 and rs7975232 did not associate with risk for AgP and CP, while the combined polymorphisms did demonstrate greater
effect on risk for CP than the individual genotypes. Carriage of Tabf was related to the increased risk of CP. TabF and TAbf were associated with decreased risk of CP. In conclusion, the present study used a candidate gene approach and replicated previous studies in different populations (Table 1). A future well-designed genetic study with large and sufficiently powered case-control samples to identify genetic variance in periodontitis is necessary.7,23 Nevertheless, our present investigation supports the potential effects of VDR polymorphisms on CP and AgP in a Taiwan population; however, the pattern of association was different between the two diseases. The f-allele of rs2228570 might be the genetic protective factor against the development of AgP.

Conflicts of interest

The authors have no conflicts interest relevant to this article.

References

1. Haussler MR, Jurutka PW, Hsieh JC, et al. New understanding of the molecular mechanism of receptor-mediated genomic actions of the vitamin D hormone. Bone 1995;17:335–85.
2. Overbergh L, Decallonne B, Waer M, et al. 1α,25-dihydroxyvitamin D3 induces an autoantigen-specific T-helper 1/T-helper 2 immune shift in NOD mice immunized with GAD65 (p524-543). Diabetes 2000;49:1301–7.

| Combined polymorphism | HC n = 163 | AgP n = 92 | CP n = 385 |
|------------------------|-----------|-----------|-----------|
|                        | n (%)     | n (%)     | n (%)     |
|                        | Adjusted OR | 95% CI | p-Value   | Adjusted OR | 95% CI | p-Value   |
| Tabf                   |           |           |           |
| –                      | 241 (73.9) | 139 (75.5) | 1 Reference | 482 (62.6) | 1 Reference |
| +                      | 85 (26.1)  | 45 (24.5)  | 0.92 | 0.60–1.39 | 0.69 | 1.69 | 1.28 | 0.0003 |
| No copy                | 94 (57.7)  | 52 (56.5)  | 1 Reference | 135 (35.1) | 1 Reference |
| 1 or 2 copies          | 69 (42.3)  | 40 (43.5)  | 1.22 | 0.63–2.37 | 0.56 | 2.58 | 1.76 | <0.0001 |
| TabF                   |           |           |           |
| –                      | 198 (60.7) | 111 (60.3) | 1 Reference | 564 (73.3) | 1 Reference |
| +                      | 128 (39.3) | 73 (39.7)  | 1.17 | 0.60–2.29 | 0.65 | 0.50 | 0.33 | 0.001 |
| No copy                | 51 (31.3)  | 40 (43.5)  | 1 Reference | 214 (55.6) | 1 Reference |
| 1 or 2 copies          | 112 (68.7) | 52 (56.5)  | 0.65 | 0.33–6.5 | 0.21 | 0.36 | 0.24 | <0.0001 |
| TAbF                   |           |           |           |
| –                      | 309 (94.8) | 147 (79.9) | 1 Reference | 642 (83.4) | 1 Reference |
| +                      | 17 (5.21)  | 37 (20.1)  | 14.97 | 1.69 | 0.03 | 348.1 | 0.50 | 0.33 | 0.144 |
| No copy                | 147 (90.2) | 61 (66.3)  | 1 Reference | 263 (68.3) | 1 Reference |
| 1 or 2 copies          | 16 (9.8)   | 31 (33.7)  | 7.19 | 3.21–7.20 | <0.0001 | 122 (31.7) | 4.42 | 2.57 | <0.0001 |
| TAbf                   |           |           |           |
| –                      | 283 (86.8) | 177 (96.2) | 1 Reference | 712 (92.5) | 1 Reference |
| +                      | 43 (13.2)  | 7 (3.8)    | 0.08 | 0.004 | 0.019 | 0.08 | 0.004 | 0.001 |
| No copy                | 123 (75.5) | 85 (92.4)  | 1 Reference | 335 (87.0) | 1 Reference |
| 1 or 2 copies          | 40 (24.5)  | 7 (7.6)    | 0.17 | 0.05–0.48 | 0.002 | 0.45 | 0.28 | 0.001 |

AgP: aggressive periodontitis; CP: chronic periodontitis; HC: healthy controls; OR: odds ratio; CI: confidence interval.

a The order of SNPs in the combined polymorphism is TaqI-, ApaI-, BsmI-, and FokI-polymorphism.
b Adjusted for gender, age and smoking status for logistic regression analysis.
c p < 0.0125 was accepted as significant for multiple testing corrected by Bonferroni method.
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3. Li S, Yang MH, Zeng CA, et al. Association of vitamin D receptor gene polymorphisms in Chinese patients with generalized aggressive periodontitis. J Periodont Res 2008;43:360–3.

4. Tachi Y, Shimpuku H, Nosaka Y, et al. Association of vitamin D receptor gene polymorphism with periodontal diseases in Japanese and Chinese. Nucleic Acids Res Suppl 2001;1:111–2.

5. Sun JL, Meng HX, Cao CF, et al. Relationship between vitamin D receptor gene polymorphism and periodontitis. J Periodont Res 2002;37:263–7.

6. Wang C, Zhao H, Xiao L, et al. Association between vitamin D receptor gene polymorphisms and severe chronic periodontitis in a Chinese population. J Periodontal 2009;80:603–8.

7. Vaithilingam RD, Safii SH, Baharuddin NA, et al. Moving into a new era of periodontal genetic studies: relevance of large case-control samples using severe phenotypes for genome-wide association studies. J Periodontal Res 2014;49:683–95.

8. Whitfield GK, Remus LS, Jurutka PW, et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol 2001;177:145–59.

9. Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–50.

10. Altshuler DM, Gibbs RA, Pe'ltonen L, et al. Integrating common and rare genetic variation in diverse human populations. Nature 2010;467:52–8.

11. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontal 1999;4:1–6.

12. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res 1976;3:2303–8.

13. Ho YP, Lin YC, Yang YH, Ho KY, Wu YM, Tsai CC. Cyclooxygenase-2 gene-765 single nucleotide polymorphism as a protective factor against periodontitis in Taiwanese. J Clin Periodontal 2008;35:1–8.

14. Ho YP, Yang YH, Ho KY, Wu YM, Tsai CC. The association of Fc-gamma receptor IIIb genetic polymorphism and susceptibility to periodontitis in Taiwanese individuals. J Clin Periodontal 2010;37:145–51.

15. Chou YH, Ho YP, Lin YC, et al. MASP-8 C→T genetic polymorphism is associated with the susceptibility to chronic and aggressive periodontitis in Taiwanese. J Clin Periodontal 2011;38:1078–84.

16. Deng H, Liu F, Pan Y, Jin X, Wang H, Cao J. BsmI, TaqI, Apal, and FokI polymorphisms in the vitamin D receptor gene and periodontitis: a meta-analysis of 15 studies including 1338 cases and 1302 controls. J Clin Periodontal 2011;38:199–207.

17. Chen LL, Li H, Zhang PP, Wang SM. Association between vitamin D receptor polymorphisms and periodontitis: a meta-analysis. J Clin Periodontal 2012;83:1095–103.

18. Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. Nature 1994;367:284–7.

19. Mocharla H, Butch AW, Pappas AA, et al. Quantification of vitamin D receptor mRNA by competitive polymerase chain reaction in PBMC: lack of correspondence with common allelic variants. J Bone Min Res 1997;12:726–33.

20. Gross C, Musiol IM, Eccleshall TR, Malloy PJ, Feldman D. Vitamin D receptor gene polymorphisms: analysis of ligand binding and hormone responsiveness in cultured skin fibroblasts. Biochem Biophys Res Commun 1998;242:467–73.

21. Carling T, Rastad J, Åkerström G, Westin G. Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors. J Clin Endocrinol Metabol 1998;83:2255–9.

22. Selvaraj P, Chandra G, Jawahar MS, Rani MV, Rajeshwari DN, Narayanan PR. Regulatory role of vitamin D receptor gene variants of BsmI, Apal, Taql, and FokI polymorphisms on macrophage phagocytosis and lymphoproliferative response to mycobacterium tuberculosis antigen in pulmonary tuberculosis. J Clin Immunol 2004;24:523–32.

23. Scandellari AS, Jepsen S, Loos BG. Periodontal genetics: a decade of genetic association studies mandates better study designs. J Clin Periodontal 2011;38:103–7.