Research Article

Association between Single Nucleotide Polymorphisms in Vitamin D Receptor Gene Polymorphisms and Permanent Tooth Caries Susceptibility to Permanent Tooth Caries in Chinese Adolescent

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Received 24 July 2017; Accepted 22 October 2017; Published 12 November 2017

Academic Editor: Hai-Feng Pan

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Purpose. Dental caries is a multifactorial infectious disease. In this study, we investigated whether single nucleotide polymorphisms (SNPs) in vitamin D receptor (VDR) gene were associated with susceptibility to permanent tooth caries in Chinese adolescents.

Method. A total of 200 dental caries patients and 200 healthy controls aged 12 years were genotyped for VDR gene polymorphisms using the PCR-restriction fragment length polymorphism (PCR-RFLP) assay. All of them were examined for their oral and dental status with the WHO criteria, and clinical information such as the Decayed Missing Filled Teeth Index (DMFT) was evaluated. Genomic DNA was extracted from the buccal epithelial cells. The four polymorphic SNPs (Bsm I, Taq I, Apa I, and Fok I) in VDR were assessed for both genotypic and phenotypic susceptibilities.

Results. Among the four examined VDR gene polymorphisms, the increased frequency of the CT and CC genotype of the Fok I VDR gene polymorphism was associated with dental caries in 12-year-old adolescent, compared with the controls ($X^2 = 17.813, p \leq 0.001$). Moreover, Fok I polymorphic allele C frequency was significantly increased in the dental caries cases, compared to the controls ($X^2 = 14.144, p \leq 0.001$, OR = 1.730, 95% CI = 1.299–2.303). However, the other three VDR gene polymorphisms (Bsm I, Taq I, and Apa I) showed no statistically significant differences in the caries groups compared with the controls. Conclusion. VDR-Fok I gene polymorphisms may be associated with susceptibility to permanent tooth caries in Chinese adolescent.

1. Introduction

Dental caries is a polyfactorial infectious disease involving interplay between environmental factors and multiple genetic factors. Despite more than 100 years of continued prevention of this disease, caries is still a major oral problem throughout the world, affecting 60%–90% of schoolchildren [1]. As the economy is booming and sugar consumption is increasing in China, caries is nowadays the most popular oral disease in China [2].

The microbiological and environmental factors causing dental caries have been extensively studied. However, cariogenic microbial and environmental exposures are not sufficient to explain susceptibility to caries. Host susceptibility is highlighted by potential genetic factors for caries risk. More than 20 candidate genes have been reported, including enamel formation genes, immune response genes, and genes related to saliva, taste, and others [3].

Enamel is a hard and highly mineralized substance covering tooth crown and protects the tooth as a barrier. There were potential associations between genes responsible for enamel formation and susceptibility to caries. Previous studies suggest that the variation in genes encoding enamelin, tuftelin, and amelogenin may be related to susceptibility to dental caries [4, 5]. Recently, gene-linkage analysis suggests that several enamel-forming related genes may contribute to susceptibility to caries in different ethnic populations.
including Korean [6], Brazilian [7], and Japanese [8]. In
contrast, Gasse et al. found that SNPs of in amelogenin
gene are not associated with susceptibility to caries [9].
No association between mannose-binding lectin (MBL) and
dental caries was proven in Chinese children [10] nor between
amelogenin X (AMELX) gene and caries susceptibility in
Polish children [11].

Vitamin D (Vit D) plays a significant role in enamel
mineralization [12] and the level of Vit D in serum is proved
to be associated with caries [13]. Vitamin D receptor (VDR)
is regarded as a mediator for the effect of Vit D related
biomineralization. VDR is involved in biomineralization
during mineralized tissue development, such as bone and
tooth enamel [14, 15]. It has been indicated that the VDR gene
has multiple polymorphisms, including the following four
single nucleotide polymorphisms (SNPs): Bsm I (rs1544410),
Taq I (rs731236), Apa I (rs7975232), and Fok I (rs10735810)
[16]. Studies have shown that VDR gene polymorphisms may
affect susceptibility to various diseases, such as osteoarthritis,
diabetes, cardiovascular disease, and tuberculosis [17], as well
as susceptibility to oral diseases, such as periodontitis [18] and
dental implant loss [19].

Concerning the incidence of dental caries, gene immune
deficiency and inflammation alterations may contribute to
host susceptibility and affect the course of caries [20], so
we speculated that VDR gene polymorphisms might have
relationship with dental caries. In the present study, four
SNPs of VDR, Bsm I (rs1544410), Taq I (rs731236), Apa I
(rs7975232), and Fok I (rs10735810), were genotyped with the
restriction fragment length polymorphism (RFLP) analysis
method, aiming to find association between dental caries and
VDR gene polymorphisms in Chinese adolescents.

2. Materials and Methods

2.1. Study Subjects. Our research was approved by the Ethics
Committee of Guangzhou Medical University. It was
performed under the guidelines of the World Medical Asso-
ciation Declaration of Helsinki. Informed consents were
acquired from all adolescents and their guardians.

Four hundred Chinese adolescents aged 12 were recruited
from the same city district primary schools for this study.
Twelve-year-old adolescents were chosen for their permanent
teeth, including second molars which had already erupted.
Only permanent teeth were considered when the deciduous
teeth still exist. Subjects were categorized into two groups
according to the Decayed Missing Filled Teeth (DMFT)
Index: caries-free (DMFT index = 0) and caries experience
(DMFT index > 0). Each group contained two hundred
members. Information about oral hygiene levels and habits
including bleeding on probing (BOP), Volpe Manhold Index
scores (VM-value), and brushing habit of each individual
were collected at the same time.

2.2. Sample Collection. Two dentists conducted the clinical
examinations. They used a periodontal probe and dental
mirror according to the criteria recommended by the WHO
guidelines. The diagnostic criterion of caries lesions was
determined by visual examination of tooth surfaces. Values of
Cohen’s kappa for agreement were 0.90 between two dentists.

Buccal epithelial cells were obtained by rubbing the buc-
cal mucosa with a swab. In accordance with the instructions
of manufacturer, genomic DNA was extracted with the use
of a commercial kit (Tiangen, Beijing, China) following the
instructions of the manufacturer. Extracted DNA was
aliquoted for each sample and stored at −20°C until PCR
amplification.

2.3. SNP Selection and Genotyping. The primers to amplify
the VDR gene were designed using Primer Premier 5 (Pre-
mi er Biosoft Inter, Palo Alto, USA) and synthesized by
Shanghai Chaoshi Co. (Shanghai, China). Four SNPs of VDR
gene were analyzed: Apa I (rs7975232, located at intron 8, in
the 3’ UTR), Bsm I (rs1544410, located at intron 8, in the 3’
UTR), Taq I (rs731236, located at intron 9, in the 3’ UTR),
and Fok I (rs10735810, located at intron 2, at the first start
codon). Genotyping was performed by the restriction frag-
ment length polymorphism (RFLP) technique [21]. Table 1
presents the primers and their amplifying fragment lengths.

2.4. Statistical Analysis. Data for this study were analyzed
with Statistical Package for the Social Sciences (SPSS) 18.0
statistical software (USA). A Hardy–Weinberg equilibrium
assessment was performed. The difference in proportion
of gender, age, and oral hygiene status between the caries
group and the caries-free controls was compared by Chi-
square test. The significance of allele, genotype frequency, and
allele carriage rate differences between two groups were also
evaluated by Chi-square test. The results were analyzed with
95% confidence interval (CI). Haploview4.2 (http://www
.broadinstitute.org/haplovie w) was used to carry out linkage
disequilibrium analysis (LD) and haplotype analysis. The
values of LD were calculated with D’ and displayed with
confidence bounds. The significance of different haplotypes
was offered by permutation tests. The p value < 0.05 was
selected to define statistical significance.

3. Results

The distribution of gender and age in caries group and
caries-free group was equiposed. The female to male ratio
was 1:0.89 and 1:1.04 for healthy controls and dental caries
adolescents, respectively. The mean age of caries-free group
and dental caries adolescents was 12.24 ± 0.30 years and
12.20 ± 0.40 years, respectively. The gender and mean age
between the two subjects was not significantly different
(p value > 0.05). The data of BOP, VM-value, brushing
time, and frequency of the two analyzed groups showed no
significant difference (p value > 0.05). Table 2 illustrates the
demographic characteristics of the analyzed groups.

Table 3 shows the distribution of genotypes and allele
frequencies for Bsm I (rs1544410), Taq I (rs731236), Apa
I (rs7975232), and Fok I (rs10735810) SNPs of VDR gene
in the two analyzed groups. The results were fitted to a
Hardy–Weinberg equilibrium (p value > 0.49).

Significant differences were detected in the frequencies of
Fok I genotype between the caries group and the caries-free
group. In particular, the frequency of Fok I’s TT genotype was
### Table 1: The primers of vitamin D receptor (VDR) and band site of genotype.

| SNP       | Alleles | Primers                                                                 | Fragment size (bp) | Digested fragment length (bp) |
|-----------|---------|--------------------------------------------------------------------------|--------------------|-------------------------------|
| Bsm I     | G/A     | 5'-ATACCTACTTTGCTGGTTTG-3' 5'-AGCCCATCTCCATTTGCTTG-3'                    | 512                | AA: 512                        |
|           |         |                                                                          |                    | AG: 512, 315, 197             |
|           |         |                                                                          |                    | GG: 315, 197                  |
| Taq I     | T/C     | 5'-AGCAGAGCAGAGTTCAAGACAGA-3' 5'-ATCTTGGCATAGACAGCTGGCT-3'                | 345                | CC: 345                        |
|           |         |                                                                          |                    | CT: 345, 260, 85              |
|           |         |                                                                          |                    | TT: 260, 85                   |
| Apa I     | C/A     |                                                                              | 740                | AA: 740                        |
|           |         |                                                                          |                    | AC: 740, 535, 205             |
|           |         |                                                                          |                    | CC: 535, 205                  |
| Fok I     | C/T     | 5'-AGCTGGCCCTGGCACTTGGCTGGCT-3' 5'-ATGGAAACACCTTGCTTTCTCTCCCTC-3'          | 265                | CC: 265                        |
|           |         |                                                                          |                    | CT: 265, 196, 69              |
|           |         |                                                                          |                    | TT: 196, 69                   |

### Table 2: Demographic characteristics of the study subjects.

|                   | Cases (n = 200) | Controls (n = 200) | \(X^2\) | \(p\) value |
|-------------------|----------------|--------------------|---------|-------------|
| Mean age          | 12.20 ± 0.40   | 12.24 ± 0.30       | 0.640   | 0.424       |
| Gender (%)        |                |                    |         |             |
| Female            | 98 (49)        | 106 (53)           |         |             |
| Male              | 102 (51)       | 94 (47)            |         |             |
| BOP (+)           | 28 (14.0)      | 23 (11.5)          | 0.562   | 0.454       |
| VM-value (+)      | 41 (20.5)      | 36 (18.0)          | 0.402   | 0.526       |
| Brushing time     |                |                    |         |             |
| <3 min            | 171 (85.5)     | 165 (82.5)         | 0.700   | 0.413       |
| ≥3 min            | 29 (14.5)      | 35 (17.5)          |         |             |
| Brushing frequency|                |                    |         |             |
| <2 times a day    | 95 (47.5)      | 91 (45.5)          | 0.161   | 0.688       |
| ≥2 times a day    | 105 (52.5)     | 109 (54.5)         |         |             |

Note: BOP: bleeding on probing; VM-value: Volpe Manhold Index scores.

Obviously lower in the caries experience group than that in the controls (9% versus 24.5%, resp.). We also found a lower carriagrate rate of allele T (57% in the caries group versus 67.5% in the controls, \(p\) value = 0.03) and a higher carriagrate rate of allele C (91% versus 75.5%, \(p\) value ≤ 0.001) in the caries group, compared to the caries-free controls. The odds ratio of the carriage of allele C reached 3.281, which showed a strong relationship between allele C of Fok I and caries group (OR > 3). We found that the frequency of allele C was markedly higher, while the frequency of allele T was significantly lower in the caries cases compared to the caries-free controls.

By contrast, for the other three VDR gene SNPS (Bsm I, Taq I, and Apa I), the genotypes and allele frequencies (G/A, T/C, and C/A, resp.) showed no statistically significant differences between the caries group and the control group. All these four SNPs showed strong evidence of recombination when we performed LD analysis on data of caries and caries-free group or caries-free group only. The linkage between Taq I and Bsm I reached an uninformative status, an intermediate state from strong recombination to strong linkage disequilibrium, when LD analysis was carried out on data of caries group only (Figure 1). Haplotypes of these four SNPs were presented in an order based on the physical location of these four SNPs. The haplotypes TCGT and TAAC showed a significant difference between the cases and controls since their \(p\) values were far less than 0.05. But when we put these data into 999-time permutation tests, only haplotype TCGT and SNP Fok I still had significant difference between the cases and controls (Table 4).

### 4. Discussion

Dental caries is a polyfactorial infectious disease involving interplay between environmental factors and multiple genetic factors. To rule out the effects of environmental factors in these two groups we compared, we collected information about oral hygiene levels and oral habits of each individual in cases and controls. There were no significant differences between case group and control group among these several factors (Table 2). This result offered support to the hypothesis that the differences of caries experience between cases and controls were possibly associated with genetic factors.

VDR gene polymorphisms are important factors for normal enamel development [12] and have been shown to
Table 3: Summary of allele and genotype frequencies in 12-year-old adolescents.

| VDR gene polymorphisms (rs number) | Total | Caries experience (n = 200) | Caries-free (n = 200) | X² | p value | OR (95% CI) | Power (%) |
|-------------------------------------|-------|-----------------------------|-----------------------|----|---------|-------------|-----------|
| **Bsm I (rs1544410)**              |       |                             |                       |    |         |             |           |
| AA                                 | 0     | 0                           | 0                     | 0.448 | 0.503 | 1.097 (0.707–2.026) | 9.09 |
| AG                                 | 67 (16.75) | 36 (18)                 | 31 (15.5)             |    |         |             |           |
| GG                                 | 333 (83.25) | 164 (82)                  | 169 (84.5)            |    |         |             |           |
| Carriage of allele A               |       |                             |                       |    |         |             |           |
| A                                  | 67 (8.375) | 36 (9)                     | 31 (7.75)             | 0.407 | 0.523 | 1.177 (0.713–1.944) | 9.8  |
| G                                  | 733 (91.625) | 364 (91)                  | 369 (92.25)           |    |         |             |           |
| **Taq I (rs731236)**               |       |                             |                       |    |         |             |           |
| CC                                 | 329 (82.25) | 171 (85.5)                | 158 (79)              | 2.894 | 0.089 |             |           |
| CT                                 | 71 (17.75) | 29 (14.5)                 | 42 (21)               |    |         |             |           |
| TT                                 | 0     | 0                           | 0                     |    |         |             |           |
| Carriage of allele T               |       |                             |                       |    |         |             |           |
| C                                  | 729 (91.125) | 371 (92.75)               | 358 (89.5)            | 2.612 | 0.106 | 1.501 (0.915–2.462) | 47.5 |
| T                                  | 71 (8.875) | 29 (7.25)                 | 42 (10.5)             |    |         |             |           |
| **Apa I (rs7975232)**              |       |                             |                       |    |         |             |           |
| AA                                 | 57 (14.25) | 33 (16.5)                 | 24 (12)               | 2.898 | 0.235 |             |           |
| AC                                 | 164 (41) | 85 (42.5)                 | 79 (39.5)             |    |         |             |           |
| CC                                 | 179 (44.75) | 82 (41.0)                 | 97 (48.5)             |    |         |             |           |
| Carriage of allele A               |       |                             |                       |    |         |             |           |
| A                                  | 278 (34.75) | 151 (37.5)                | 127 (31.75)           | 3.175 | 0.075 | 1.304 (0.974–1.745) | 43.1 |
| C                                  | 522 (65.25) | 249 (62.25)               | 273 (68.25)           |    |         |             |           |
| **Fok I (rs10735810)**             |       |                             |                       |    |         |             |           |
| CC                                 | 151 (37.75) | 86 (43.0)                 | 65 (32.5)             | 17.813 | 0.000 |             |           |
| CT                                 | 182 (45.5) | 96 (48.0)                 | 86 (43.0)             |    |         |             |           |
| TT                                 | 67 (16.75) | 18 (9)                    | 49 (24.5)             |    |         |             |           |
| Carriage of allele C               |       |                             |                       |    |         |             |           |
| C                                  | 484 (60.5) | 268 (67)                  | 216 (54)              | 17.30 | 0.000 | 1.730 (1.299–2.303) | 97.1 |
| T                                  | 316 (39.5) | 132 (33)                  | 184 (46)              |    |         |             |           |

Four SNPs of VDR, Bsm I (rs1544410), Taq I (rs731236), Apa I (rs7975232), and Fok I (rs10735810) were genotyped by the restriction fragment length polymorphisms analysis method in our study. Previous studies suggested that the distribution of VDR polymorphisms could have different patterns in different ethnicity [21, 23–26]. In this study, we failed to find any incidence of AA genotype for Bsm I and TT genotype for Taq I in Chinese adolescents. The most common genotype for each of the polymorphisms was GG for Bsm I (83.25%), CC for Taq I (82.25%), CC for Apa I (44.75%), and CT for Fok I (45.5%). The general distribution of VDR gene polymorphisms in our study showed similar pattern with previous study of Chinese population [21] but was different to those on African Americans [24], Turks [25], and Jordanians [26]. These consistency and inconsistence of our results with previous studies presented extra evidences to the existence of distribution specificity of VDR gene polymorphisms based on ethnicity and also suggested that the population we collected in this study could represent characteristics of Chinese to some extent.

Our findings showed that the susceptibility of Chinese adolescents to permanent tooth caries was associated with the genotype frequency of Fok I. The frequency of allele C contribute to susceptibility to various immune diseases [22]. Variations in human VDR gene lead to phenotypically diverse inherited enamel malformations [15]. Considering the role of the VDR gene in enamel formation, we hypothesized a possible contribution between VDR gene polymorphisms and dental caries. Our results support the hypothesis that susceptibility of Chinese adolescent to caries of permanent teeth was associated with the genotype frequency of the Fok I SNP.
Table 4: Haplotype analysis and permutation tests.

(a)

| Haplotype | Freq. | Case, control ratio counts | Case, control frequencies | $X^2$ | $p$ value |
|-----------|-------|----------------------------|---------------------------|-------|-----------|
| TCGC      | 0.309 | 132.7:267.3, 114.7:285.3   | 0.332, 0.287              | 1.901 | 0.168     |
| TCGT      | 0.25  | 80.3:319.7, 119.5:280.5    | 0.201, 0.299              | 10.259| 0.0014    |
| TAGC      | 0.197 | 89.7:310.3, 67.9:332.1     | 0.224, 0.170              | 3.758 | 0.0525    |
| TAGT      | 0.088 | 35.0:365.0, 35.4:364.6     | 0.087, 0.088              | 0.003 | 0.9596    |
| TCAT      | 0.05  | 21.9:378.1, 18.4:381.6     | 0.055, 0.046              | 0.312 | 0.5764    |
| CAGT      | 0.03  | 12.1:387.9, 12.1:387.9     | 0.030, 0.030              | 0     | 0.9992    |
| CCGC      | 0.018 | 7.6:392.4, 4.5:393.5       | 0.019, 0.016              | 0.088 | 0.7669    |
| CCGT      | 0.017 | 4.1:395.9, 9.2:390.8       | 0.010, 0.023              | 1.979 | 0.1595    |
| TAAC      | 0.013 | 9.4:390.6, 1.2:398.8       | 0.023, 0.003              | 6.391 | 0.0115    |

(b) #999 permutations performed

| Name   | $X^2$ | Permutation | $p$ value |
|--------|-------|-------------|-----------|
| TCGT   | 10.259| 0.016       |           |
| Fok I (rs10735810) | 8.544 | 0.026 | |
| TAAC   | 6.391 | 0.0581      |           |
| TAGC   | 3.758 | 0.3033      |           |
| Apa I (rs7975232) | 3.175 | 0.4765 | |
| Taq I (rs731236) | 2.612 | 0.6056 | |
| CCGT   | 1.979 | 0.7217      |           |
| TCGC   | 1.901 | 0.7598      |           |
| Bsm I (rs1544410) | 0.407 | 0.998 | |
| TCAT   | 0.312 | 0.998 | |
| TAGT   | 0.003 | 1 | |
| CAGT   | 0     | 1 | |
| CCGC   | 0.088 | 1 | |

was obviously higher in cases compared with the caries-free controls. The odds ratio of carriage of allele C reached 3.281, suggesting that allele C seems to be a risk factor of caries experience. The increased frequency of genotypes CT and CC were also found in caries group, while the genotype TT and allele T frequency were found to be significantly reduced in the caries group compared with the caries-free controls. These findings indicated that the allele T of Fok I appeared to be a protective factor for the caries experience, as the odd ratio and 95%CI of carriage rate of allele T was 0.638 (0.425–0.959) <1. In contrast, for the other three VDR genes (Apa I, Bsm I, and Taq I), the genotypes and allele frequencies (C/A, G/A, and T/C, resp.) showed no statistically remarkable differences between the caries group and the control group.

It is notable that the minor allele frequency (MAF) of Bsm I and Taq I was extremely low in CHB base on HapMap project since MAF of Bsm I was 0.022 and Taq I was 0.011 (http://hapmap.ncbi.nlm.nih.gov/). This could explain why we failed to find any incidence of AA genotype for Bsm I and TT genotype for Taq I. These results also suggested that the number of samples needed to finally confirm the association between Bsm I, Taq I and caries susceptibility could be hundred times of the sample amount in our study. HapMap project data showed that Apa I had a much higher MAF (0.321), but the power on Apa I in our data merely reached 43.1%, which means that our data on Apa I had greater possibility of being a false negative result, while power on Fok I reached a 97.1% strong level (Table 3). Apa I, Bsm I, and Taq I could have potential association with caries susceptibility, but our data cannot offer a positive support to this hypothesis.

A recent study of dental caries in northwestern Chinese population indicated that Taq I could act as a caries risk factor in middle-age adults [27], which seemed to be a challenge to our data. But firstly, the power on Taq I in our data was not strong enough to reject false negative. On the other hand, China is a multinational country with 55 ethnic minorities and the majority Han people, and many of the ethnic minorities are living in relatively remote areas of China such as the southwest and northwest of China. So, the detailed composition of northwestern Chinese population could be much more complex than ours. This might partially explain the inconsistency.

In LD analysis, all these four SNPs showed very strong evidence of recombination except for Taq I and Bsm I in caries group data. However, the linkage of Taq I and Bsm I in caries group still did not reach the level of strong LD. Haplotype analysis and further permutation tests revealed that haplotypes TCGT and Fok I each were more likely to have association with exposure factor, while the haplotype TCGT...
Figure 1: LD analysis of SNPs in caries and caries-free group. LD plot of SNPs Taq I (rs731236), Apa I (rs7975232), Bsm I (rs1544410), and Fok I (rs10735810) in caries and caries-free group (a), caries-free group only (b), and caries group only (c). All these four SNPs showed strong recombination of each other (block in white) in both caries and caries-free group (a) and caries-free group only (b), while linkage of Taq I (rs731236) and Bsm I (rs1544410) reached a higher level (block in gray, $D^r = 1$) in caries group only.

C variant exists; it might produce VDR protein of different sizes [28, 30]. This was consistent with our haplotype analysis result as the protective haplotype TCGT had T of Fok I not C. However, 3'UTR was also related to mRNA including the mRNA localization and stability and also the translation efficiency [31].

This study is the first to analyze the association between VDR gene polymorphisms and the susceptibility of Chinese school adolescents to caries in permanent teeth. Our findings suggest that Fok I could be a potential risk factor for caries in this specific population group. This finding may contribute to our knowledge of dental caries in terms of etiology and treatment course.

Ethical Approval

This study was approved by the Ethics Committee of Guangzhou Medical University (Resolution nos. 2013-03, 2014-05, 2015-11, and 2015-09) and is in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent

The guardians of all adolescents included in the study gave their written consent for clinical examination and oral swab collection.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Our research was supported by Grant-in-Aid from Guangdong Provincial Department of Science and Technology (Grant nos. 2014-807 and 2015-110-51), Health and Family Planning Commission of Guangdong Province (Grant no. A2016506), and the Bureau of Health of the Guangzhou Municipality (Grant no. 20161A010092).

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