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Analysis of the association between polymorphisms in the vitamin D receptor (*VDR*) gene and dental caries in a Chinese population

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ABSTRACT. Environmental influences on the development and progression of dental caries are well known; however, there is little evidence of a genetic component imparting susceptibility to dental caries. The aim of this study was to investigate the relationship between a single nucleotide polymorphism in the vitamin D receptor *Taq*I locus and dental caries susceptibility in a Chinese population. This case-control study was conducted with a case group (264 patients with dental caries from northwestern China) and a control group (219 individuals without dental caries or systemic disease from the same area). DNA was extracted from the peripheral venous blood of the study participants; the distribution of *Taq*I locus genotypes and allele frequencies was determined via polymerase chain reaction-restriction fragment length polymorphism. The data obtained were statistically analyzed using the

Hardy-Weinberg equilibrium and Chi-square test. The frequency of the Tt genotype in the case group (14.0%) was significantly higher than that in the control group (4.3%), as determined using the genotype TT as the reference. The risk of dental caries was increased 3.8-fold in individuals with the heterozygous Tt genotype compared to that in the individuals with the TT genotype. The proportion of the 't' allele in the case group (7.0%) and the control group (2.1%) was observed to be significantly different [P = 0.0003; OR = 3.592, confidence interval 95% (1.790-7.208)]. Our results therefore suggested that the allele 't' might be a genetic factor determining dental caries susceptibility in individuals from the northwest of China.

Key words: Vitamin D receptor; Gene polymorphism; Dental caries; Case-control study

INTRODUCTION

Dental caries is a common oral disease that affects a large number of people worldwide (Petersen, 2003); only cancer and cardiovascular disease have been known to exert a greater impact on human health. Many researchers are convinced that genetic host susceptibility might be involved in the development of dental caries in addition to the traditional quartet of susceptibility factors (bacteria, sugar, tooth tissue, and time) (Azevedo et al., 2010; Ozturk et al., 2010; Ohta et al., 2015).

The vitamin D receptor (VDR) is a nuclear biological macromolecule that mediates the biological activity of the major metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which includes maintenance of the calcium- and phosphate balance and regulation of bone metabolism, cell differentiation, and immune response (Valdivielso and Fernandez, 2006). Vitamin D plays a major role in tooth formation, especially in the development of the enamel and dentin (Berdal et al., 1987), and controls the expression of the classical $1,25(OH)_2D_3$ target genes and dental proteins *in vivo* (Papagerakis et al., 2002). The teeth of vitamin-D-deficient rats have been reported to display marked variations and abnormalities in morphogenesis and cell differentiation (Berdal et al., 1987). Furthermore, defects in the enamel and dentin have been related to hypocalcemia and hypophosphatemia, respectively, suggesting the additional role of mineral homeostasis in the tooth (Nikiforuk and Fraser, 1981). Calcification of the enamel and dentin ensured that the teeth were less prone to osteoporosis and bone demineralization, which in turn may be related to the biological characteristics of the VDR (Zhang et al., 2009). However, nutritional vitamin D deficiency or *VDR* gene mutation may lead to impaired functioning of the vitamin D pathway (Wharton and Bishop, 2003).

The human *VDR* gene is located on chromosome 12; four common polymorphisms of this gene (*Taq*I, *Apa*I, *Bsm*I, and *Fok*I) have been previously investigated in relation to various infectious diseases and susceptibility to diabetes (Lemos et al., 2008), osteoporosis (Uysal et al., 2008), and tuberculosis (Wilkinson et al., 2000; Selvaraj et al., 2008). However, no link has been purported between this gene and caries susceptibility.

Here, we have presented a case-control study investigating the association between *VDR* gene polymorphism and caries susceptibility in individuals with a similar socio-econom-

ic and cultural background and access to dental care (in their respective communities) in the northwest of China.

MATERIAL AND METHODS

Study populations

All participants were permanent residents of northwestern China, and underwent their routine health examination in October 2010; the participants were recruited with the help of the Chinese Ethnic Affairs Commission. The recruited individuals were diagnosed according to the decayed, missing, and filled teeth (DMFT) index, and divided into two groups based on caries experience. The case group contained 264 people with caries experience (DMFT ≥ 1), while the control group comprised of 219 individuals with no previous caries experience (DMFT = 0) (Table 1).

All individuals were subjected to an oral and dental examination by two associate professors with uniform training to ensure standardization of the inspections. The DMFT score was allotted according to guidelines of the World Health Organization (1997). This study was approved by the Ethics Committee of the Northwest University for Nationalities, and informed consent was obtained from all participating individuals.

Table 1. Demographic characteristics of sample groups.

Category	Case group (DMFT ≥ 1)	Control group (DMFT = 0)
Total	264	219
Male	145	100
Female	119	119
Age range	35-67	30-65
Mean age	50	53

DMFT: decayed, missing, and filled teeth index.

Sample collection and DNA extraction

Two milliliters of blood was obtained from the median cubital vein of all individuals, and stored in vials containing ethylene diamine tetraacetic acid (EDTA) anticoagulant at -70°C. DNA was extracted from EDTA-anti-coagulated peripheral blood using a standard proteinase K/phenol-chloroform organic extraction method (Cristina et al., 2012). The solvent-extracted DNA was dissolved in Tris-EDTA buffer and stored at -20°C before use.

Genotyping

The *VDRTaq*I(T/C) gene polymorphisms were genotyped by polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP). The primers were designed as described in the literature (Lorentzon et al., 2000), and synthetized by Sangon Biological Engineering Technology Services Co., Ltd. (Shanghai, China). Upstream primer: 5'-CAGAGCATGGACAGGGAGCAA-3'. Downstream primer: 5'-GCAACTCCTCATGGCT GAGGTCTC-3'. The PCR system contained the template DNA (100 ng), 2.5 µL 10X buffer (Geneomaga, Denver, CO, USA), 1.5 µL 25 mM $MgCl_2$, 0.25 µL Taq DNA polymerase (Geneomaga), 0.2 µL 20 mM dNTP (Geneomaga), 20 mM 1 µL of each upstream and downstream

primers, and deionized water, to a final volume of 25 µL. The PCR conditions were set as follows: three cycles of denaturation (95°C, 2 min), renaturation (65°C, 90 s), and extension (72°C, 1 min), followed by 32 cycles of denaturation (94°C, 1 min), refolding (65°C, 1 min), and extension (72°C, 1 min), and a final single cycle of extension (72°C, 10 min).

The PCR products (10 µL) were digested with the restriction enzyme, *Taq*1 (MBI Fermentas, Vilnius, Lithuania), at 65°C for 16 h. The restriction digestion products were resolved on a 2% agarose gel, stained with ethidium bromide, and observed under ultraviolet light. The PCR-RFLP results were further confirmed when identical results were obtained for randomly repeated analyses of 30% of the samples.

Statistical analysis

The Hardy-Weinberg equilibrium was assessed for the *VDRTaq*I genotypes. Genotypic and allelic frequencies of *VDRTaq*I gene variants were calculated in two groups, with the odds ratio (OR) and 95% confidence interval (95%CI) representing the relative risk analysis. Data analysis was performed on the SPSS 17.0 software platform (SPSS, Chicago, IL, USA). A chi-square P value of less than 0.05 ($P < 0.05$) was considered to be statistically significant.

RESULTS

*VDRTaq*I gene polymorphism analysis

All genotypes were in the range of the Hardy-Weinberg equilibrium. The frequencies of the *VDR* TT and Tt genotypes in the case group were 86.0 and 14.0%, respectively, while those in the control group were 95.7 and 4.3%, respectively. *VDR*-tt was not observed in either group.

The frequency of the Tt genotype in the case group (14.0%) was significantly higher than that in the control group (4.3%), as determined using the TT genotype as a reference. The difference between these results was statistically significant ($P = 0.0002$). The risk of developing dental caries in individuals with Tt was increased by 3.8 times compared to individuals with TT. The proportion of the allele 't' was 7.0% in the case group, which was significantly higher than that in the control group (2.1%), a difference that was also found to be statistically significant ($P = 0.0003$) (Table 2).

Table 2. Frequency of *VDRTaq*I SNP genotypes and alleles in the case and control groups.

Genotype/allele	Case group [N (%)] (N = 264/528)	Control group [N (%)] (N = 219/438)	P (χ^2)	OR (95%CI)
TT	227 (86.0)	210 (95.7)	0.0002 (13.63)	3.80 (1.79-8.07)
Tt	37 (14.0)	9 (4.3)		
T	491 (93.0)	429 (97.9)	0.0003 (12.95)	3.59 (1.79-7.21)
T	37 (7.0)	9 (2.1)		

N = number of genotypes/alleles; P values were obtained from the chi-squared test; OR = odds ratio; CI = confidence interval.

Frequency of *VDRTaq*I SNP genotypes by gender

No significant differences were observed between the male and female individuals following gender-based stratification with regard to the frequency of TT and Tt genotypes (P = 0.115; using the TT genotype as the reference). Similarly, no statistically significant gender-dependent differences were observed between the two alleles (P = 0.133) (Table 3).

Table 3. Frequency of *VDRTaq*I SNP genotypes and alleles by gender.

Genotype/allele	Male [N (%)] (N = 245/490)	Female [N (%)] (N = 239/478)	P (χ^2)	OR (95%CI)
TT	210 (85.7)	192 (80.3)	0.115 (2.488)	0.681 (0.422-1.100)
Tt	35 (14.3)	47 (19.7)		
T	455 (92.9)	431 (90.2)	0.133 (2.258)	0.705 (0.447-1.114)
T	35 (7.1)	47 (9.8)		

N = number of genotypes/alleles; P values were obtained from the chi-squared test; OR = odds ratio; CI = confidence interval.

DISCUSSION

A review investigating twins (Shuler, 2001) and reports of families and animal breeding (Hunt, 1944; Klein, 1946) have, together with genomics (Shelling and Ferguson, 2007), indicated dental caries to have an important genetic component. Indeed, 40-65% of caries risk has been attributed to this factor (Bretz et al., 2005). Recent studies have confirmed that the caries susceptibility of a patient may be determined by their DNA (Burgner et al., 2006; Patir et al., 2008; Peres et al., 2010; Werneck et al., 2010; Kang et al., 2011; **S**tanley et al., 2014), in addition to the well-known environmental factors involved in caries risk such as bacteria, diet, oral hygiene, and host factors (Lenander-Lumikari and Loimaranta, 2000; Nariyama et al., 2004; Zero, 2004).

Many years of long-term clinical observation have confirmed that high enamel calcification and density renders the teeth difficult to destroy; in addition, calcium deficiency in children has also been known to lead to a higher rate of caries than in healthy children, and also possibly to a (more) rapid decline following the onset of caries. Finally, vitamin D is known to be critical to the maintenance of a constant relationship between calcium and phosphate ions, which are key factors in the strengthening and protection of teeth. The starting point of dental caries is demineralization of the dental hard tissues, that is, the dissolution of calcium from the hydroxyapatite crystals that form the backbone of enamel (Brito et al., 2004). Ninety nine percent of the total bodily calcium has been estimated to exist in the bones and teeth. Vitamin D is a key hormone regulating the calcium metabolism, and promotes the deposition of calcium onto the teeth and bones. It also plays a key role in many physiological activities, including cell growth and differentiation, and immune and cardiovascular function. However, the activity of its receptor is influenced by polymorphisms in the *VDR* gene (Valdivielso and Fernandez, 2006), located in chromosome 12q13 and encoding a nuclear receptor-transcription factor. Some studies have suggested that such polymorphisms in the coding region may affect the receptor protein by altering its expression level and/or modulating its affinity for vitamin D, which in turn modulates its calcium homeostasis and metabolism (Brito et al., 2004). Polymorphisms in the *VDR* gene may also affect the expression of osteoblast genes (such as osteocalcin) via the trans-acting regulation of these genes (Morrison et al., 1992), and has even been

implicated in susceptibility to pulmonary tuberculosis (Liu et al., 2004; Sharma et al., 2011).

Here, we have conducted a preliminary study into the relationship between *VDRTaq*I gene polymorphisms and dental caries susceptibility in a Chinese population. The frequency of the Tt genotype was observed to be higher in individuals with caries than in those without caries; the "t" allele was found to be significantly more common in the case group than in the control group. These results suggest significant correlation between the *VDRTaq*I gene polymorphism and caries risk in these populations; in addition, the "t" allele was considered a caries susceptibility marker. We discovered no evidence of the tt genotype in this study, which was concordant with the findings of Liu et al. (2004), but contrary to those observed by Bellamy and Hill (1998). These differences may reflect the geographic and ethnic variations.

To our knowledge, this is the first study reporting an association between *VDRTaq*I gene polymorphisms and dental caries, although a link between this polymorphism and periodontal disease has been reported (Brito et al., 2004). We have provided a starting point for the genotyping of caries susceptibility genes in the Chinese population; however, dental caries is likely to be influenced by multiple genetic factors. Further investigation will be required to delineate the true role of the *VDR* gene in caries incidence, and its interactions with the environment and other susceptibility genes. Furthermore, polymorphisms in other *VDR* gene loci that were not investigated in this study may be linked to the incidence of dental caries; however, but a single locus study lacks the statistical power for the proper analysis of these links. A study jointly analyzing multiple sites in the *VDR* gene and/or other susceptibility genes and constructing haplotypes could greatly increase the statistical power of these investigations, and allow important associations to be definitively demonstrated for this complex genetic disease.

In summary, it could be concluded that, individuals belonging to the northwestern Chinese population expressing the heterozygous Tt genotype of the *VDR Taq*I gene have elevated susceptibility to caries. The "t" allele may be a disease susceptibility gene for caries; however, further research into this and other putative susceptibility genes is required to delineate variations in caries experience in different genetic groups.

Conflicts of interest

The authors declare no conflict of interest.

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