Single-nucleotide polymorphism in Vitamin D receptor gene and its association with dental caries in children

Victoria Guru Aribam, Nalini Aswath, Arvind Ramanathan

Departments of Oral Medicine and Radiology and 1Human Genetics Research Laboratory, Sree Balaji Dental College and Hospital, BIHER University, Chennai, Tamil Nadu, India

ABSTRACT

Background: Though factors such as diet, oral hygiene, salivary flow and surface characteristics of the tooth enamel play a role in the causation of dental caries, genetic factors also contribute significantly in influencing the susceptibility or resistance to the occurrence of caries. Aims and Objectives: To analyse the relationship between a single nucleotide polymorphism in the vitamin D receptor gene TaqI locus and associate its presence with dental caries in children. Materials and Methods: A case control study was conducted among 120 children in the age group of 6-12 years. 60 cases with a DMFT of >3 and 60 controls with a DMFT of 0 were selected. Genomic DNA was extracted from salivary samples collected from the patients and presence of polymorphism was analyzed by PCR-RFLP. Results: Pearson chi square test and Fisher exact tests were used for comparison of differences in genotypes or allele frequencies between groups. No significant difference between the cases and control was observed among the different genotypes and the alleles. However, there was an inclination in the incidence of caries with the genotype ‘tt’ and ‘Tt’ when compared with the genotype ‘TT’. Conclusion: In this study done to analyse the SNP in vitamin D receptor gene and its association with dental caries in children indicates a higher caries risk for a patient with ‘t’ allele and ‘tt’ genotype.

KEYWORDS: Dental caries, single-nucleotide polymorphism, TaqI locus, Vitamin D receptor gene

Introduction

Dental caries can be defined as a complex, chronic, multifactorial disease and one of the most prevalent diseases in industrialized and developing countries. [1]
Regardless of all the etiological factors such as diet, oral hygiene, salivary flow, and surface characteristics of the tooth enamel, there are individuals who appear to be more susceptible to caries and those who are extremely resistant, which suggest that genetic factors also influence the development of caries.

In craniofacial development and maintenance of good oral health, Vitamin D plays a principal role and also affects enamel and dentin formation. During periods of primary and permanent tooth formation, events of malnutrition and Vitamin D deficiency can result in enamel hypoplasia and dental caries. Odontoblasts and ameloblasts are the target cells for Vitamin D function. Vitamin D receptor (VDR) deficiency influences odontoblast. ApaI (VDR 7975232 C > T), BsmI (VDR 1544410 A > G), FokI (VDR 2228570 C > T), and TaqI (VDR 7312736 T > C) are some of the polymorphisms of the VDR gene.

One of the most common known of the single-nucleotide polymorphisms (SNPs) is TaqI (rs731236) (T > C, chromosome position: 48238757). It is a synonymous polymorphism located on codon 352 (isoleucine) of exon 9 near the 3’ untranslated region of VDR gene on band 13 of long arm of chromosome12. Morphogenic abnormalities, matrix disorganization, and hypomineralization are the major features of the Vitamin D pathway disturbance during tooth formation. Due to its central role in bone and tooth formation, children and adolescents are particularly vulnerable to the clinical manifestations of insufficient Vitamin D. Therefore, the objective of this study is to analyze the relationship between a SNP in the VDR TaqI locus and associate its presence with dental caries in children.

Materials and Methods

A case-control study was conducted among 120 children aged between 6 and 12 years. The DMFT and dmft indices were recorded according to the WHO criteria (1997). Sixty cases with a DMFT of >3 and sixty controls with a DMFT of 0 were selected by purposive random sampling. Children with any systemic disease were excluded. Ethical approval was obtained from the institutional ethics committee, and written informed consent was acquired from each parent or guardian.

DNA Extraction

Salivary samples obtained from participants with or without caries were centrifuged at 10,000 rpm for 3 min at room temperature to pellet cells. The pelleted cells were washed once with phosphate-buffered saline pH 7.5, and suspended in 0.1 ml of cell lysis buffer (containing 0.1% sodium dodecyl sulfate (SDS), 25 mM ethylenediaminetetraacetic acid, 75 μg/100 μl Proteinase-K, and 200 mM Tris-Cl at pH 8 [Sigma-Aldrich, St. Louis, MO, USA]). These samples were then incubated at 57°C for 12 h with intermittent agitation. Subsequently, the 200 μl of 100 mM ammonium acetate was added to the cell lysates, mixed, and incubated at room temperature for 15 min. The samples were then centrifuged at 12,000 rpm for 15 min to precipitate protein fraction in cell lysate. Subsequently, one-sixth volume of isopropanol was added to all the samples, mixed, and centrifuged at 12,500 rpm for 15 min at room temperature to precipitate the genomic DNA.

Polymerase chain reaction

Primers designed to specifically amplify 493 base pair region of exon 9 of VDR gene containing the SNP rs731236 at codon 352 were used to amplify the SNP region. The following set of primers was used:

- VDRTaqF1: ATAGCAGAGCAGAGTTCCAAGC
- VDRTaqR1: GTCGGCTAGCTTCTGGATCATC.

Polymerase chain reaction (PCR) was performed on 100 ng of DNA samples under the following conditions: after an initial denaturation at 94°C for 5 min, the samples were amplified for 35 cycles with denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and primer extension at 72°C for 30 s. A final extension at 72°C for 5 min was performed before stopping the amplification reaction. Amplifications were performed in a 25 μl reaction and were confirmed for their specificity by running a 5 μl aliquot of the reaction in a 1.5% agarose gel. Most of the samples migrated at the expected size of 493 base pairs. A few samples, however, either did not show any amplification (marked by upward arrow) or showed nonspecific amplification (marked by downward arrow). While samples that showed nonspecific...
amplification were not included in the analysis, those that did not show amplification were reamplified with higher concentration of DNA. Figures 1 and 2 show gel images of PCR amplicons of DNA extracted from control and caries, respectively.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (PCR-RFLP) ANALYSIS AND DETECTING THE “TT,” “Tt,” AND “tt” POLYMORPHISM.

The amplification products of PCR were digested with Taq1 restriction enzyme in a 25 μl reaction volume at 65°C for 1 h in a thermal cycler. The digested products were analyzed by running a 10 μl aliquot in a 2% agarose gel at 50V for 30 min.

Based on the occurrence of the polymorphism(s), Taq1 restriction enzyme cuts the PCR amplicons into any one of the following manners:

1. If the PCR amplicon contains TCGA/TCGA (“tt” genotype), the Taq1 enzyme cuts the 493 base pair PCR amplicon into 382 base pair + 111 base pair fragments. Agarose gel electrophoresis of the Taq1-digested PCR amplicons is displayed as twin bands migrating in the gel.

2. If the PCR amplicon contains TCGA/TTGA (“Tt” genotype), the PCR amplicons containing TCGA will be cut into 382 base pair + 111 base pair fragments, whereas those PCR fragments containing TTGA will not be cut by the Taq1 enzyme. Agarose gel electrophoresis of the Taq1-digested PCR amplicons is displayed as triplet bands (493 base pair + 382 base pair + 111 base pair) migrating in the gel.

3. If the PCR amplicon contains TTGA/TTGA (“TT” genotype), the Taq1 enzyme does not cut the PCR amplicons. Agarose gel electrophoresis of the Taq1-digested PCR amplicons is displayed as a single band (493 base pair) migrating in the gel.

A representative of agarose gel electrophoresis of the digestion products of control and caries is shown in Figures 3 and 4, respectively.

Statistical analysis
The statistical analysis was done using IBM SPSS software version 24.0. The Pearson Chi-square test and Fisher’s exact tests were used for comparison of differences in genotypes or allele frequencies between groups. Odds ratio, confidence interval, and P value were calculated.

Results
The distribution of “TT,” “Tt,” and “tt” polymorphism in the sample was tabulated. The distribution of “TT,” “Tt,” and “tt” in the caries group was 36.6%, 41.6%,
and 21.6%, respectively, whereas in the normal group, the distribution of “TT,” “Tt,” and “tt” was 43.3%, 38.3%, and 18.3%, respectively, which clearly indicates that the distribution of “TT” genotype is more in normal control group than the caries group. The “Tt” and “tt” genotype distribution is more in the caries group as compared to the normal control group. Pearson’s Chi-square test revealed $P = 0.747$. When the distribution of the genotypes “TT,” “Tt,” and “tt” between the cases and controls was statistically analyzed, $P = 0.58$ for “TT” genotype, 0.85 for “Tt” genotype, and 0.82 for “tt” genotype were obtained. The odds ratio revealed that there was a 0.76 times lower chance for a caries person to have the genotype “TT,” 1.15 times higher chance for a caries person to have the genotype “Tt,” and 1.23 times higher chance for a caries person to have the genotype “tt” [Table 1].

When the “T” allele was compared among the case and the control groups, $P = 0.43$ was obtained. Although not statistically significant, we saw that there was 0.81 times lower chance of a caries person to have the “T” allele compared to a caries-free person having the same allele. When the “t” allele was analyzed between the case and control groups, there was 1.23 higher times higher chance of a caries person to have the “t” allele compared to a caries-free person having the same allele [Table 2].

### Discussion

Dental caries is a widely prevalent disease worldwide. According to the Global Oral Health Data Bank, the prevalence varies from 49% to 83% across different countries. It has shown to have a negative impact on the health-related quality of life irrespective of age.$^{9}$ While there is clear evidence that dental caries is multifactorial, there seems to be a contributory genetic component in the etiology of the disease.

Oral bone loss, periodontal disease, and loss of teeth have been associated with deficient concentrations of 25-hydroxyvitamin D (25(OH)D), and it has been shown that supplementation with Vitamin D improves clinical periodontal outcomes. Through the efforts of Mellanby et al., much of the early focus on the role of Vitamin D in caries occurred in the 1920s and 1930s. The beneficial effects of Vitamin D supplementation in reducing dental caries in children have been documented in various historical reports.$^{4}$

Caries was strongly associated with bone mineral density and this likely reflected a caries-Vitamin D association.$^{10}$

### Table 1: Vitamin D receptor genotype comparisons with cases and controls

| VDR genotypes | Number of persons having caries (%) | Number of persons not having caries (%) | Test value ($\chi^2$ or Fisher’s exact test) | $P$ | OR (95% CI) | $P$ for OR |
|---------------|------------------------------------|----------------------------------------|--------------------------------------------|-----|-------------|------------|
| TT Present    | 22 (45.8)                          | 26 (54.2)                              | 0.583                                      | 0.747 | -           | -          |
| TT Absent     | 38 (52.8)                          | 34 (47.2)                              | 0.556                                      | 0.576 | 0.757 (0.364-1.575) | 0.456 |
| Tt Present    | 25 (52.1)                          | 23 (47.9)                              | 0.139                                      | 0.852 | 1.149 (0.553-2.387) | 0.709 |
| Tt Absent     | 35 (48.6)                          | 37 (51.4)                              |                                            |      |              |            |
| tt Present    | 13 (54.2)                          | 11 (45.8)                              | 0.208                                      | 0.820 | 1.232 (0.502-3.022) | 0.648 |
| tt Absent     | 47 (49.0)                          | 49 (51.0)                              |                                            |      |              |            |

VDR=Vitamin D receptor, OR=Odds ratio, CI=Confidence interval
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Through actions on intestinal absorption, renal reabsorption, as well as through trapping and mobilization of calcium and phosphorus from mineralized tissues, the Vitamin D pathway systemically manages mineral homeostasis. Various studies using both animal models and human trials have shown that disruption of the Vitamin D pathway leads to inadequate levels of calcium and phosphorous in circulating plasma which decreases the mineralization of skeletal bones and also has a negative impact on the formation of teeth.[5]

The Taq I polymorphism that is characterized by a single-base transition (T > C) leading to a synonymous change at codon 352 in exon 9 belongs to the most studied variant. It creates a Taq I restriction site; resulting alleles are called “T” (Taq I site absent) and “t” (Taq I site present).[11]

Izakovicova Holla et al.[11] did a study where they found no significant differences in VDR Taq1 allele and genotype frequencies between caries-free and caries-affected children which is consistent with our result. Hu et al.[12] suggested that the “t” allele and “Tat” genotype might be risk factors of susceptibility to dental caries in adults. They suggested that heterozygous “Tat” genotype of the VDR Tami gene has elevated susceptibility to caries and added that “t” allele may be a disease susceptibility gene for caries. Cogulu et al.[10] showed that there was a statistically significant difference in the frequency of Taq1 genotypes (“tt”) between caries-active and caries-free children (P = 0.029).

Kong et al.[13] showed that VDR BsmI polymorphism was associated with the risk of deciduous tooth decay in Chinese children aged 4-7 years. Morrison et al. established that homozygotes (“tt” genotype) for a variant of the VDR are associated with decreased bone mineral density.[14]

In our study, there was no statistically significant difference between the different genotypes in case and control groups. However, when the comparison of the distribution of the various genotypes was statistically analyzed using Fisher’s exact test, the odds ratio revealed that individuals having the genotype “tt” and the allele “t” may be more susceptible to dental caries which may suggest that T allele has protective nature against caries. There was an inclination in the incidence of caries with the genotype “tt” and “Tt” when compared with the “TT” genotype.

Literature reveals that there is a strong association between the incidence of caries and bone mineral density. Homozygotes (tt genotype), a variant of the VDR, are associated with decreased bone mineral density.[14] This could explain the results of our study that the individuals having “tt” and “Tt” genotype and the “t” allele are more prone to dental caries as compared to the TT genotype and the T allele. Individuals with decreased bone mineral density will be more prone to dental caries. The limitation of this study was that we only focused on Taq1 locus of VDR gene, and the sample size was small.

### Conclusion

Although there were no statistically significant differences in the results of our study, there was an inclination in the incidence of caries with the genotype “tt” and “Tt” when compared with the “TT” genotype and the “t” allele as compared to the “T” allele. VDR gene polymorphism may be used as a biomarker for predicting the occurrence of dental caries. Individuals with the “t” allele and genotypes “tt” and “Tt” may be more prone to the occurrence of dental caries since it was established that the individuals having the “tt” genotype have decreased bone mineral density. Further studies on a large number of samples are required to arrive at a definite conclusion.

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### Conflicts of interest

There are no conflicts of interest.

### Table 2: Allele type comparisons with cases and controls

| Allele type | Number of persons having caries (%) | Number of persons not having caries (%) | Fisher’s exact test value | P | OR (95% CI) | P for OR |
|-------------|------------------------------------|----------------------------------------|--------------------------|---|-------------|---------|
| T           | Present 69 (47.9)                   | 75 (52.1)                              | 0.625                    | 0.429 | 0.812 (0.733-2.067) | 0.429 |
|             | Absent 51 (53.1)                    | 45 (46.9)                              |                          |     |             |         |
| t           | Present 51 (53.1)                   | 45 (46.9)                              | 0.625                    | 0.429 | 1.232 (0.734-2.067) | 0.429 |
|             | Absent 69 (47.9)                    | 75 (52.1)                              |                          |     |             |         |

OR=Odds ratio, CI=Confidence interval
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