

The Role of Vitamin D Receptor Polymorphisms on Dental Caries

Dilsah Cogulu*/ Huseyin Onay **/ Yasemin Ozdemir ***/ Gulcin Itirli Aslan ****/
Ferda Ozkinay *****/ Cemal Eronat *****

Objective: To determine the association between the *ApaI*, *FokI*, *Cdx2* and *TaqI* polymorphisms of vitamin D receptor (*VDR*) gene in caries-active (high-moderate) and caries-free children. **Study design:** A hundred and fifty children (75 males, 75 females, mean age: 10.19 ± 1.61 years) were included in the study. The subjects were divided into three groups as high caries risk group (*DMFT*, *dft* > 4) (*n*=55), moderate caries risk group (*DMFT*, *dft* = 1-4) (*n*=57) and caries-free group (*n*=38). From each individual, blood samples were collected and DNA was extracted. The *VDR* gene was genotyped for the polymorphisms *ApaI*, *FokI*, *Cdx2* and *TaqI* using polymerase chain reaction and restriction fragment length polymorphism methods. All data were analyzed by chi-square test, Fisher's exact test and *t* test. **Results:** There was statistically significant difference in the frequency of *TaqI* genotypes (*tt*) between caries-active and caries-free children (*p*=0.029). No statistically significant differences were detected between *ApaI*, *FokI*, *Cdx2* genotypes and dental caries. **Conclusion:** In the future, *VDR* gene polymorphisms may be used as a marker for the identification of patients with high caries risk.

Key words: Vitamin D receptor, polymorphism, dental caries.

INTRODUCTION

Dental caries is a major oral health problem in most industrialized countries, affecting 60-90% of the population. Dental caries incidence is affected by host factors that may be related to the structure of dental enamel, immunologic response to cariogenic bacteria, or the composition of saliva.¹⁻⁵ Genetic variation of the host factors such as Vitamin D polymorphism may contribute to increased risks for dental caries.⁶

The Vitamin D endocrine system is central to the control of bone and calcium homeostasis. The active form of the vitamin D is 1,25-dihydroxyvitamin D (calcitriol), the circulating level of which is tightly regulated and acts through a specific receptor to mediate its genomic actions on almost every aspect of calcium homeostasis.⁷⁻⁹ Genetic alterations of the Vitamin D Receptor (*VDR*) gene could lead to important defects on gene activation, affecting calcium metabolism, cell proliferation, immune function, etc..., which could be explained by changes in the protein sequence.¹⁰⁻¹²

The *VDR* gene locus is found at 12q13.1 region in the human genome. It is about 10 kb and can generate several tissue-specific transcripts^{7,8,13} There are more than 200 polymorphisms of the *VDR* gene, including more commonly *ApaI*, *FokI*, *Cdx2* and *TaqI*.^{14,15}

There has been a lack of reports on the relationship between *VDR* gene polymorphisms and dental caries. Therefore, the aim of this study was to determine the association between the *ApaI*, *FokI*, *Cdx2* and *TaqI* polymorphisms of *VDR* gene in caries-active (high-moderate) and caries-free children

MATERIALS AND METHOD

A total of 350 children aged between 6-12 year-old were enrolled in the study. Ethical approval was obtained (06-12/3) and written informed consent was acquired from each parent. Children having any systemic disease were not included to the study.

Tooth-brushing habits (less than once, once, twice), daily dietary sugar exposures (0-2 times, >2 times) were recorded by a structured questionnaire.

*Dilsah Cogulu, DDS, PhD, Ege University, School of Dentistry, Department of Pedodontics, İzmir-Turkey.

**Huseyin Onay, MD, PhD, Ege University, School of Medicine, Department of Medical Genetics, İzmir-Turkey.

*** Yasemin Ozdemir, DDS, PhD, Private Dentist, İzmir-Turkey.

****Gulcin Itirli Aslan, PhD, Izmir University, School of Medicine, Department of Medical Biology, , İzmir-Turkey.

*****Ferda Ozkinay, MD, PhD, Ege University, School of Medicine, Department of Medical Genetics, İzmir-Turkey.

*****Cemal Eronat, DDS, PhD, Ege University, School of Dentistry, Department of Pedodontics, İzmir-Turkey.

Send all correspondence to
Dilsah Cogulu
Ege University School of Dentistry
Department of Pedodontics
35100 Bornova-Izmir, TURKEY
Phone: +90 232 3886431
Fax: +90 232 3880325
E-mail: dilsah.cogulu@ege.edu.tr

Dental examinations of the children were conducted by a pediatric dentist under natural light with the aid of a dental mirror and explorer. Dental plaque scores were recorded according to Silness & Loe index.¹⁶ The mesial, distal, lingual and buccal surfaces of all erupted teeth were scored by one observer on a scale of 0-3. The mean surface score per tooth was determined by dividing the total score of all surfaces by four. Children with the dental plaque score 3 were not included to the study. Among 350 children, 150 children (75 males, 75 females) with similar dental plaque scores, oral hygiene habit and sugar consumption were included to the study to eliminate the other risk factors of dental caries. The DMFT (D: decayed, M: missing, F: filled, T: tooth for permanent teeth) and dft (d: decayed, m: missing, f: filled, t: tooth for deciduous teeth) indices were recorded according to WHO criteria¹⁷ and all children were classified according to the DMFT scores¹⁸; Group-1: with high caries (dmft/DMFT>4, n=55), Group-2: with moderate caries (dmft/DMFT=1-4, n=57) and Group-3: caries-free (dmft/DMFT=0, n=38).

DNA extraction and genotyping

After obtaining an informed consent, 5 ml venous whole-blood specimens were collected in ethylenediamine tetraacetate (EDTA) tubes and stored at -20°C until processing. DNA (Deoxyribonucleic acid) was extracted from the blood samples by using QIAamp DNA Blood mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The *VDR* gene was genotyped for the polymorphisms ApaI, FokI, Cdx2 and TaqI using polymerase chain reaction (PCR). Following PCR reactions, all four polymorphisms (rs7975232-ApaI, rs10735810-FokI, rs11568820-Cdx2 and rs731236-TaqI) in the *VDR* gene were genotyped using restriction fragment length polymorphism (RFLP). Amplification was performed in a 20 ml reaction mixture containing genomic DNA (100-150 ng), 200 nM dNTPs (Deoxyribonucleotide) (MBI-Fermentas, USA), 2-8 pmol of each primer and 0.5 U of Taq DNA polymerase (MBI-Fermentas, USA) per tube in a gradient thermal Master cycle (Eppendorf, USA). Amplification reactions (a mechanism leading to multiple copies of a chromosomal region within a chromosome arm) were set up separately for ApaI (AA, Aa, aa), FokI (FF, Ff, ff), Cdx2 (GG, GA, AA) and TaqI (TT, Tt, tt) polymorphic sites of *VDR* gene using specific primers (Table-1). A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA.

Statistical analysis

Allele frequencies, genotype frequencies and carriage rates of the alleles in all groups were compared by using Fisher's exact test. The Hardy-Weinberg equilibrium at individual loci was assessed by χ^2 test using Statistical Package for the Social Sciences (SPSS) for Windows (version 15.0; SPSS Inc., Chicago, IL, USA) and clinical association was calculated by paired t test. A value $p < 0.05$ was considered to be statistically significant.

RESULTS

The mean age of the 150 (75 males and 75 females) children was 10.19 ± 1.61 years. The mean DMFT and dmft scores of all 150 children were; 1.35 ± 0.93 (Group-1: 2.67 ± 1.24 , Group-2: 0.26 ± 0.17 , Group-3: 0) and 3.67 ± 2.61 (Group-1: 5.76 ± 2.70 , Group-2: 0.36 ± 0.30 , Group-3: 0), respectively. The mean value of dental plaque score of all children was 1.12 ± 0.10 .

Among 350 children, 150 children with similar dental plaque scores, oral hygiene habit and sugar consumption were included to the study to eliminate the other risk factors of dental caries. No significant correlation was detected between DMFT scores and caries risk factors (chi-square test, $p > 0.05$). Tooth-brushing habit was regularly twice a day in only 26% (n=39) of these 150 children. Daily sugar consumption was over 2 times *per day* only in 20 % (n=30) of these 150 children. (Table-2).

Table-1. Specific primers and PCR condition

	Specific primers	PCR condition
Apa1	F: AGCATGGACAGGGAGCAA R: CCTGTGCCTTCTTCTCTATCC	15 cycles: 95°C (30 sec), 61.9°C (30 sec), 72°C (30 sec) 20 cycles: 95°C (30 sec), 54.9°C (30 sec), 72°C (30 sec)
Fok1	F: CCCTGGCACTGACTCTGCTC R: GGAAACACCTTGCTTCTTCTCC	30 cycles: 94°C (60 sec), 60°C (60sec), 72°C (60sec)
Cdx2	F: AGGCAGGAAGGAAGGAAGA R: CCTGGTTTGCTTTCATTGC	30 cycles: 95°C (3 min), 95°C (60 sec), 60°C (60 sec), 72°C (60 sec)
Taq1	F: CCTGTGCCTTCTTCTCTATCC R: AGCCTGAGTGCAGCATGA	30 cycles: 95°C (5 min), 95°C (45 sec), 54°C (45 sec), 72°C (45 sec)

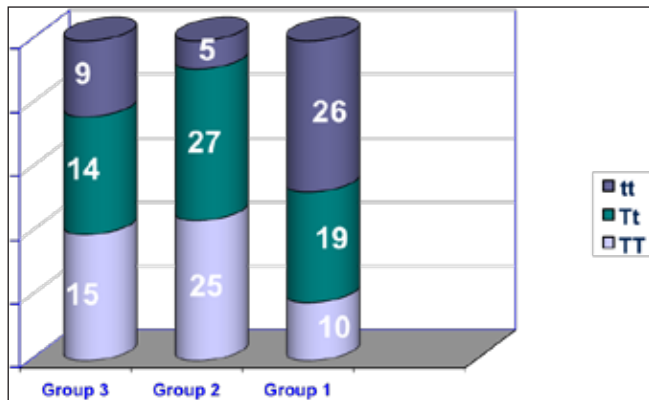
Apa1, Fok1, Cdx2 and Taq1: Studied Vitamin D Receptor polymorphisms
PCR: Polymerase chain reaction

Table-2. The distribution of caries risk factors according to the groups

	Tooth Brushing (n)			Sugar intake (n)		Dental plaque (n)		
	<1	1	≥2	0-2	>2	0	1	2
Group 1 (DMFT>4)	17	24	14	43	12	8	34	13
Group 2 (DMFT= 1-4)	20	23	14	47	10	7	36	14
Group 3 (DMFT = 0)	10	17	11	30	8	9	20	9

*No significant correlation was found between DMFT scores and caries risk factors ($p > 0.05$)

The allele and genotype frequency distribution and carriage rate of *VDR* TaqI polymorphism among patients was given in Figure-1. There was statistically significant difference in the frequency of TaqI genotypes (tt) between caries-active and caries-free children ($p=0.029$). No statistically significant differences were detected between ApaI, FokI and Cdx2 genotypes and dental caries ($p>0.05$).



DISCUSSION

Environmental and genetic factors play important roles in the mechanisms involved in the development of dental caries. It is generally accepted that dental plaque scores, oral hygiene habit and sugar consumption are primary etiological factors for dental caries.¹⁻⁵ At the beginning of the study, DMFT, dft scores and plaque index were recorded in 350 children and a structured questionnaire was designed. From these children 150 children with similar dental plaque scores, oral hygiene habit and sugar consumption were included to this study to eliminate the other risk factors of dental caries. No significant correlation was detected between DMFT scores and dental caries risk factors.

Studies on genes inducing susceptibility to dental caries have been carried out by various groups in different populations.¹⁹⁻²¹ The Vitamin D receptor (*VDR*) gene is a candidate gene for susceptibility to several diseases. *VDR* gene polymorphisms have found to be strongly associated with mineral density. Vitamin D is known to modulate calcium homeostasis and has a role in the regulation of electrolytes and blood pressure. There is now an increasing amount of evidence to show that 1,25 (OH)₂D₃, the most active metabolite of Vitamin D, regulates the immune response and possesses anti-inflammatory activity.²² However, only few data have been published indicating the relationship between *VDR* gene polymorphisms and dental caries.⁶ Therefore, we aimed to investigate the incidence of common *VDR* gene polymorphisms (ApaI, FokI, Cdx2 and TaqI) in dental caries.

Vitamin D has special roles in regulating the metabolisms of calcium and phosphorus, and in the immunosystem. The biologic function of vitamin D must be associated with its receptor. Studies demonstrated human *VDR* gene is localized in chromosome 12.^{13,23} It is clear that mutations in functionally critical areas of *VDR* gene can have profound effects on mineral metabolism and bone mineral density.²⁴

A comprehensive early study showed that vitamin D supplementation significantly reduced the incidence of caries in children.

The investigators also noted the substantially lower incidence rate of caries in summer compared to other seasons.²⁵ It has been shown that the incidence of caries in 12-14 year-old was correlated with the hours of sunshine.²⁶ A follow-up report showed that 6-8 year-old and 9-11 year-old similarly had caries rates inversely related to ultraviolet exposure.²⁷

Caries was strongly associated with bone mineral density and this likely reflected a caries-vitamin D association.²⁸ Year-round exposure to ultraviolet wavelengths was credited for preventing an increase in caries in school children.²⁹ Reduced exposure to ultraviolet light was blamed for increase in caries.^{30,31} Another study can be planned on the role of *VDR* polymorphism and mineral content of teeth.

A polymorphism is a genetic variant that appears in at least 1% of the population. These changes can occur in non-coding parts of the gene, so they would not be seen in the protein product. Changes in these regulatory parts of the gene would then affect the degree of expression of the gene, and thus the levels of the protein.¹⁰⁻¹² More than 200 polymorphisms of the *VDR* gene have been reported.^{14,15} Among them, most common polymorphisms (FokI, ApaI, Cdx2 and TaqI) have been studied in the present investigation. There was statistically significant difference in the frequency of TaqI genotypes (tt) between caries-active and caries-free children ($p=0.029$). No statistically significant differences were detected between ApaI, FokI, Cdx2 genotypes and dental caries ($p>0.05$). Based on the results of this study, in the future *VDR* gene polymorphisms may be used as a marker for the identification of a patient with high caries risk.

The only data that had been reported in the literature studied the relationship between BsmI and ApaI polymorphisms and dental caries. No significant relationship had been reported in this study.⁶ No significant relationship was also found between ApaI polymorphism and dental caries in the present study.

Further studies involving genetic linkage assessment are needed to evaluate the direct effect of these polymorphisms on dental caries; also, the studies should be carried out on a large cohort of population.

REFERENCES

- Shuler CF. Inherited risks for susceptibility to dental caries. *J Dent Educ* 65: 1038-45, 2001.
- Harris R, Nicoll AD, Adair PM, Pine CM. Risk factors for dental caries in young children: a systematic review of the literature. *Community Dent Health* 21: 71-85, 2004.
- McDonald RE, Avery DR, Stookey GK. Dental caries in the child and adolescent. In: *Dentistry for the Child and Adolescent*, Ed.: McDonald RE, Avery DR, St.Louis: Mosby Inc, 209-46, 2000.
- Milgrom P, Riedy CA, Weinstein P, Tanner ACR, Manibusan L, Bruss J. Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children. *Community Dent Oral Epidemiol* 28: 295-306, 2000.
- Balakrishnan M, Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. *Aust Dent J* 45: 235-45, 2000.
- Sengün A, Duran I, Erdal ME, Ozkaya M, Ozturk B, Ozer F. Vitamin D Receptor Gene Polymorphism is associated with Dental Caries. 81st Gen. Sess. Of Int. Assoc. for Dental Research, 2003.
- Morris KL, Zemel MB. 1,25-dihydroxyvitamin D3 modulation of adipocyte glucocorticoid function. *Obes Res* 13: 670-7, 2005.
- Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta* 371: 1-12, 2006.
- Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res* 13: 325-49, 1998.
- Margherita T. Cantorna. Session 2: Micronutrients and the immune system mechanism underlying the effect of vitamin D on the immune system. *Proceed Nutrition Soc* 69: 286-89, 2010.
- Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. *Faseb J*, 15: 2579-85, 2001.
- Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ et al. Cloning and expression of full-length cDNA encoding human vitamin D receptor. *Proc Natl Acad Sci* 85: 3294-8, 1988.
- McBeath EC, Zücker TF. The role of vitamin D in the control of dental caries in children. *J Nutr* 15: 547-64, 1938.
- Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *J Steroid Biochem Mol Biol*, 89-90: 187-93, 2004.
- Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 338: 143-56, 2004.
- Silness, J, Loe H. Periodontal Disease in Pregnancy.II. Correlation between Oral Hygiene and Periodontal Condition, *Acta Odontol Scand* 22: 121-35, 1964.
- World Health Organization. Oral Health surveys basic methods. 4th ed. Geneva World Health Organization, 1997.
- Oulis CJ, Berdouses ED (2009) Fissure sealant retention and caries development after resealing on first permanent molars of children with low, moderate and high caries risk. *Eur Archs Paediatr Dent* 10, 211-217.
- King JM, Pitter AFV, Edwards H. Some social predictors of caries experience. *Br Dent J* 155: 266-68, 1983.
- Ramos-Gomez FJ, Weintraub JA, Gansky SA, Hoover CI, Featherstone JD. Bacterial, behavioral and environmental factors associated with Early Childhood Caries. *J Clin Pediatr Dent* 26: 165-73, 2002.
- Chankanka O, Cavanaugh JE, Levy SM et al. Longitudinal associations between children's dental caries and risk factors. *Journal of Public Health Dentistry* 71: 289-300, 2011.
- Demay MB. Mechanism of vitamin D action. *Ann N Y Acad Sci* 68: 204-13, 2006.
- Faraco JH, Morrison NA, Baker A, Shine J, Frossard PM. Apa1 dimorphism at the human vitamin D receptor gene locus. *Nucl Acids Res* 11: 2150, 1989.
- Amano Y, Komiyama K, Makishima M. Vitamin D and periodontal disease. *J Oral Sci*, 51: 11-20, 2009.
- Lin NU, Malloy PJ, Sakati N, al-Ashwal A, Feldman D. A novel mutation in the deoxyribonucleic acid-binding domain of the vitamin D receptor causes hereditary 1,25-dihydroxyvitamin D-resistant rickets. *J Clin Endocrinol Metab* 81: 2564-69, 1996.
- East BR. Mean annual hours of sunshine and the incidence of dental caries, *Am J Pub Health*: 29:777-80,1939.
- Kaiser H, East BR. Relation of Dental Caries in Rural Children to Sex, Age and Environment. *Am J Dis Chil* 60:1289-303, 1940.
- Fabiani L, Mosca G, Giannini D, Giuliani AR, Farelo G, Marci MC, Ballatori E. Dental caries and bone mineral density: a cross sectional study. *Eur J Paediatr Dent* 7:67-72, 2006.
- Hargreaves JA, Thompson GW. Ultraviolet light and dental caries in children. *Caries Res* 23:389-92, 1989.
- Pini Prato G, Adorni Braccesi M, Silli U. Ultraviolet rays in the prevention of dental caries. *Riv Clin Pediatr* 82:259-67, 1969.
- Dantsig IN. Dental caries and ultraviolet deficiency (based on observations on naval personnel. *Stomatologia* 53:10-3, 1974.