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# Association of Vitamin D Receptor Taq I Polymorphism and Susceptibility to Oral Squamous Cell Carcinoma

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**Abstract.** *Background: It has been hypothesised that vitamin D receptor (VDR) gene polymorphisms may influence both the risk of cancer occurrence and prognosis. Materials and Methods: The distribution of VDR Taq I polymorphism in 64 patients with OSCC was determined by polymerase chain reaction based restriction fragment length polymorphism (RFLP) and compared with that of 87 healthy controls. Results: There was a significant difference in the distribution of VDR Taq I genotypes between OSCC patients and healthy controls. Patients with the VDR Tt genotype were found to be at significantly higher risk for OSCC than those with other genotypes (p=0.036). In particular, female OSCC patients were at higher risk (p<0.001) for oral cancer. Conclusion: These results suggest that the VDR Taq I polymorphism may be associated with susceptibility to OSCC. Female predilection of the OSCC risk in association with VDR gene polymorphism should also be investigated.*

Oral carcinoma is not a major cancer type and accounts for about 3% of all cancer cases (1), although it is one of the ten common causes of mortality in the Western world (2). Moreover, there has been no remarkable success documented in 5-year survival of oral carcinoma in the last 50 years (2). These findings divert researchers attention to aetiological factors of oral squamous cell carcinoma (OSCC). Aetiological factors of oral carcinoma, such as smoking (3), alcohol drinking (4), viral infection (5) and some genetic factors (6), have been well known for many years; however, not all, but only a small number of smokers and alcohol drinkers develop oral carcinoma. This dilemma supports the idea that genetic variations play an important role in oral cancer aetiology. Identification of the factors that would make an individual prone to OSCC is important in order to determine the at-risk subgroups and to start prevention programs.

Vitamin D has been associated with anti-tumour properties and a lower risk of several types of cancer, including head neck squamous cell carcinoma (7). Vitamin D is produced in the skin after exposure to UV radiation and may be obtained from dietary resources in low concentrations, or from supplements (8). Its action is mediated through the vitamin D receptor (VDR), which is expressed both in normal and neoplastic cells (9). The most biologically active form of vitamin D, 1,25-dihydroxyvitamin $D_3$ (1,25 $[OH]_2D_3$) is also the natural ligand for the vitamin D receptor (10, 11). The receptor has a crucial role in skeletal metabolism, calcium/phosphate balance and many other interactions with pathways of fibronectin, retinoid signalling and regulating the expression of p21 and p27 arresting cells (7, 10). The latter controls the proliferation of squamous cell carcinoma of the head and neck and therefore *VDR* polymorphism could be an important biomarker to assess the risk of OSCC.

The *VDR* gene, located on chromosome 12, contains several polymorphisms (10, 12). A series of common polymorphisms in the *VDR* gene were recently reported to be associated with both circulating levels of active vitamin D and *in vitro* measures of gene expression (13). Four major polymorphic sites have been described within the *VDR* gene. A polymorphic FokI site in exon 2 results in an alternative transcription initiation site, leading to a protein variant with three additional amino acids at the amino terminus (14). Polymorphic Apa1 site is present in intron 8, and a silent T to C substitution creates a Taq I restriction site in exon 9 (15).

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*Key Words:* Vitamin D receptor, polymorphism, oral squamous cell carcinoma.

Since vitamin D mediates its effect through VDR, known to be genetically polymorphic, this study investigated the association of the Taq I restriction site polymorphism of *VDR* gene in patients with OSCC.

## Materials and Methods

This study included 64 OSCC patients who were treated at İstanbul University (Department of Otorhinolaryngology, Institute of Oncology and Department of Oral Surgery and Medicine Clinics) between 2006 and 2008. The mean ages (±standard deviation) of OSCC patients were 55.28±13.58 years. Blood samples were collected from the patients before any treatment had been started (surgery and/or radiotherapy). The control group was selected from patients, who were treated for non-neoplastic diseases, attending the otolaryngology, oral medicine and surgery clinics of the same hospital. The mean age of the control group was 57.24±12.83 years. Written informed consent was taken from all groups. The distribution of *VDR* Taq I polymorphism in 64 patients with OSCC was determined by polymerase chain reaction-based RFLP and compared with that of 87 healthy controls.

*DNA isolation*. Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood by a salting-out procedure (16).

*Genotyping*. Polymorphic restriction fragment length polymorphism (RFLP) were genotyped using Taq I polymorphism in the 30 region of *VDR*. For Taq I polymorphism, the following primers (MBI Fermentas, Lithuania) were used to amplify the *VDR* gene: 50'–CAG AGC ATG GAC AGG GAG CAA G-30; 50–GCA ACT CCT CAT GGG CTG AGG TCT CA-30'. For detection of the TaqI RFLP, 50-100 ng genomic DNA was amplified with 1× polymerase chain reaction (PCR) buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer and Taq polymerase (MBI Fermentas) in a 25 ml reaction volume. The PCR conditions were as follows: Initial denaturation step of 94˚C for 4 min followed by 5 cycles of 94˚C for 45 s, 64˚C for 60 s and 72˚C for 2 min; and a further 25 cycles of 94˚C for 30 s, 64˚C for 30 s and 72˚C for 45 s. PCR products were digested with Taq I restriction enzyme (MBI Fermentas) at 65˚C and electrophoresed on 2% agarose gels and stained with ethidium bromide. Genotypes were determined as TT (490, 245 bp), Tt (490, 290, 245, 205 bp), or tt (290, 245, 205 bp) for Taq I polymorphism.

*Statistical analyses*. Statistical analyses, using SPSS version 11 (SPSS Inc., Chicago, IL, USA), included the chi-square test for genotype and allelic frequency comparison. Whenever an expected cell value was less than five, Fisher's exact test was used. The odds ratio (OR) and the 95% confidence interval (95% CI) were calculated as an estimate of the relative risk. A *p*-value of less than 0.05 was regarded as statistically significant.

## Results

The analysis included 64 OSCC cases and 87 cancer-free controls. Frequency distributions of select characteristics are summarised in Table I. The genotype distribution of *VDR* Taq I polymorphism was in agreement with the Hardy-Weinberg equilibrium among controls ($p$=0.320) but not among patients ($p$=0.030). There was a significant difference in the distribution of *VDR* Taq I genotypes in female OSCC patients and female controls but no significant differences were detected in males, nor in the total population (Table I). The allelic frequencies T and t in OSCC patients were 0.60:0.40 and those in normal individuals were 0.57: 0.43, respectively. Subjects with the *VDR* Tt genotype were found to be at significantly higher risk for OSCC than those with another genotype ($p$=0.036, OR=1.39, 95% CI=1.02–1.90). In contrast, the *VDR* tt genotype, which was associated with the prevention of cancer in previous studies, was found to be slightly higher in the control group compared to the OSCC patient group; however this difference was not statistically significant ($p$=0.060; OR=1.14, 95% CI=1.00-1.31). When the genotype distribution of the subjects was investigated according to gender, *VDR* Tt genotype was found in association with OSCC in females ($p$<0.001; OR=2.43, 95% CI=1.51-3.91). Moreover, a preventive role of VDR tt genotype was statistically significant for females ($p$=0.045, Fisher exact test; OR=1.33, 95% CI=1.07-1.65). These observations did not hold for the male subjects, neither for Tt ($p$=0.839; OR=0.96, 95% CI=0.64-1.43), nor for tt ($p$=0.809; OR=1.02, 95% CI=0.87-1.19) genotype distributions. Prognosis of the cancer may also be affected by genetic differences. Therefore, *VDR* genotype distribution according to TNM classification and histopathologic differentiation were analysed and summarised in Table II. Another remarkable finding was the T allele carriage of all tumours bigger than 4 cm diameter ($p$=0.074, Fisher exact test). There were no significant findings in the distribution of *VDR* Taq I polymorphism in OSCC patients according to nodal metastasis, differentiation and grade of the tumour.

OSCC patients were divided into two subgroups according to age. The first group consisted of patients aged 45 years or younger, while the second group consisted of patients older than 45 years of age. OSCC patient age as well as smoking status and family history of cancer did not affect the distribution of *VDR* Taq I polymorphism; alcohol consumption was associated with TT genotype. Patients with TT genotype who consumed alcohol were found to have 2.44 times higher risk for OSCC than those who did not consume alcohol ($p$=0.016; OR=2.44, 95% CI=1.17-5.07).

## Discussion

This case–control study demonstrated a statistically significant association between *VDR* Taq I heterozygous Tt genotype and increased risk of OSCC. Moreover, those Tt genotype carrying subjects had >2-fold increase of risk if they were females. *VDR* tt genotype, which was associated with prevention from cancer in previous studies (7), was found to be slightly higher in the control group than in

Table I. *Distribution of VDR gene Taq1 polymorphism in OSCC patients and controls.*

| | TT | Tt | tt | $p^a$ |
|---|---|---|---|---|
| OSCC n(%) | | | | |
| Male | 16 (37.2) | 22 (51.2) | 5 (11.6) | $p$=0.921 |
| Female | 3 (14.3) | 17 (81.0) | 1 (4.8) | $p$=0.002 |
| Total | 19 (29.7) | 39 (60.9) | 6 (9.4) | $p$=0.063 |
| Controls n(%) | | | | |
| Male | 15 (33.3) | 24 (53.3) | 6 (13.3) | |
| Female | 16 (38.1) | 14 (33.3) | 12 (28.6) | |
| Total | 31 (35.6) | 38 (43.7) | 18 (20.7) | |

[a]Two-sided chi-square test for genotype distribution.

Table II. *Distribution of VDR genotypes of the patient group according to TNM classification.*

| | TT N(%) | Tt N(%) | Tt N(%) |
|---|---|---|---|
| Tis | 1 (33.3) | 2 (66.7) | - |
| T1 | 7 (43.8) | 6 (37.5) | 3 (18.8) |
| T2 | 4 (20.0) | 13 (65.0) | 3 (15.0) |
| T3 | - | 8 (100.0) | - |
| T4 | 7 (41.2) | 10 (58.5) | - |
| N0 | 12 (33.3) | 21(58.3) | 3 (8.3) |
| N1 | 4 (33.3) | 6 (50.0) | 2 (16.7) |
| N2 | 3 (18.8) | 12 (75.0) | 1 (6.3) |
| N3 | - | - | - |
| Stage 0 | 1 (50.0) | 1 (50.0) | - |
| Stage I | 5 (41.7) | 5 (41.7) | 2 (16.7) |
| Stage II | 3 (27.3) | 7 (63.6) | 1 (9.1) |
| Stage III | 2 (13.3) | 11 (73.3) | 2 (13.3) |
| Stage IV | 8 (33.3) | 15 (62.5) | 1 (4.2) |
| Poor diff. | 1 (14.3) | 5 (71.5) | 1 (14.3) |
| Moderate diff. | 12 (36.4) | 17 (51.5) | 4 (12.1) |
| Well diff. | 5 (25.0) | 14 (70.0) | 1 (5.0) |

patients. An investigation of *VDR* Taq I genotypes through genders revealed that the *VDR* tt genotype was strongly associated with the protection from OSCC in females.

In many studies on various types of cancer, Taq I polymorphism was not found to be associated with the occurence or the prognosis of the disease (7-9, 17). In a study on lung cancer, TT genotype was found to be related with cancer susceptibility (18); however the majority of the cancer studies on VDR Taq I polymorphism have demonstrated its protective role, which is attributed to tt genotype (7-9).

Many studies in the literature have reported that the presence of variant t allele had a protective effect on some cancer types such as prostate (19) and breast (8) cancer. John *et al*. (17) reported that the Taq tt genotype was associated with reduced risk for prostate cancer. Similarly, Ma *et al*. (20) indicated that reduced risk was associated with *VDR* Taq I tt genotype but, conversely, only among men with low serum 25-OHD levels. Liu *et al*. (7) reported that Taq tt homozygous variants were associated with decreased head and neck cancer risk but the study did not indicate the gender specific distribution of the genotypes.

There were also contraversial reports about the preventive effect of the Taq t allele for cancer. Ogunkolade *et al*. (21) observed in their expression study that t allele was responsible for lower levels of both mRNA and protein, thus higher cancer risk. Doğan *et al*. (18) performed a case–control study on *VDR* polymorphism in lung cancer patients. They reported no protective effect of the t allele or tt genotype. Other studies have failed to find an association between the Taq I polymorphism and the risk of prostate cancer in Caucasian and/or Japanese populations (22-25). Ntais *et al*. (26) conducted a meta-analysis on the association between *VDR* Taq I polymorphism and the risk of prostate cancer and concluded that this gene polymorphism is unlikely to be a major determinant of the risk of prostate cancer.

Although in the present study no significant result could be found for homozygous TT or tt genotypes, the results indicate that individuals carrying the Tt genotype have an increased risk for the development of OSCC.

A recent large scale case–control study of colorectal cancer reported a protective effect of the *VDR* tt variant genotype (27). Lurie *et al*. (28) showed that Taq t allele carriers among Caucasian and Japanese women were at higher ovarian cancer risk, but this association was not statistically significant. It has been suggested VDR may also inhibit androgen receptor expression found in the majority of ovarian tumours and dihydrotestosterone has been shown to increase VDR expression in the ovary (29).

The present study also provided evidence that all tumours bigger than 4 cm in diameter carried the T allele. In contrast, Barrasso *et al*. (8) found no association with any of the alleles or genotypes of *VDR* Taq I polymorphism in tumour size of breast cancer patients. Liu *et al*. (7) did not state any information about the tumour size in their head and neck cancer study.

OSCC patient's age as well as smoking status and family history of cancer did not affect the distribution of *VDR* Taq I polymorphism; however, alcohol consumption was associated with the TT genotype. Patients with the TT genotype who consumed alcohol were found to have significantly higher risk for OSCC than those who did not consume alcohol. No association of any genotypes of Taq I polymorphism with smoking and alcohol consumption were found in head and neck (7) or breast (8) cancer studies. In a lung cancer study, the alcohol consumption of the patients

was not examined; however, smoking was found to be associated with TT genotype ($p$=0.012) (20).

No associations were also noted between the distribution of *VDR* Taq I polymorphisms and nodal metastasis, differentiation and grade of the tumour in OSCC patients. These findings are in agreement with the results of a breast cancer study (8).

Although the basis of the antitumour properties of vitamin D receptors have been described in the literature (30), the mechanisms which bind the *VDR* polymorphisms and alter the risk of cancer need to be explored. No published studies of *VDR* and OSCC exist in the literature and only a study by Liu *et al*. (7) from the U.S.A. with a large group of head and neck squamous cell carcinoma (HNSCC) patients included OSCC patients. The limited knowledge on OSCC and VDR gene polymorphism leads to the suggestion that the role of *VDR* gene polymorphism is mainly attributed to females. In the latter study by Liu *et al*. (7), a significant protective role of the *VDR* tt genotype against the risk of HNSCC was observed, but the genotype distribution among genders was not stated.

The biochemical evidence for a putative relationship of this polymorphism with VDR function remains unclear, because this polymorphism does not alter the amino acid sequence of the VDR protein, but may alter the level of *VDR* mRNA (18).

Published studies on the association of *VDR* polymorphism with susceptibility to cancer suggest that the impact of this genetic variation differs in various cancer types and also in different ethnicities. These differences also depend on the methodology with which *VDR* polymorphism was investigated (combined genotypes, haplotypes), sample size and serum levels of vitamin D. Therefore, it is clear why there are so many conflicting results for even the same type of cancer. This is the first study focusing exclusively on OSCC and provides some evidence that the *VDR* gene may influence susceptibility to OSCC.

## Conclusion

In summary, in this first case–control study on *VDR* Taq I polymorphism in OSCC patients, it is concluded that the *VDR* Tt genotype is found to be associated with an increased risk of OSCC compared to other homozygous genotypes. Furthermore, this association was observed to be stronger in female OSCC patients. These findings suggest that the *VDR* Tt genotype may be a risk factor for OSCC and also that the tt genotype has a protective role for OSCC, especially in females. However, additional work based on various populations is needed to confirm these findings and the relationship between vitamin D levels and OSCC risk should also be investigated. However, this hospital-based study may have introduced uncontrolled selection biases, and larger, population-based studies, particularly focusing on female populations, are needed to confirm these findings.

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