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# Investigation Between MTHFR A1298C polymorphism and Oral Squamous Cell Carcinoma Risk in Turkish Population

## Türk Toplumunda MTHFR A1298C Polimorfizmi ile Oral Sküamoz Hücre Karsinom Gelişim Riski Arasındaki İlişkinin Araştırılması

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## ABSTRACT

**Objective:** Oral squamous cell carcinoma (OSCC) with its poor survival rates and rising number of incidences arises through several etiological factors including environmental, genetic and epigenetic alterations. Several studies have established an association between cancer susceptibility and polymorphisms of methylenetetrahydrofolate reductase (MTHFR), the key enzyme involved in folate metabolism and, therefore in DNA synthesis, methylation and repair. The aim of the present study was to establish any association between MTHFR A1298C variants and alcohol and/or tobacco consumption, gender or age in respect to clinical histopathological parameters, and the risk of OSCC development in the Turkish population.

**Material and Method:** MTHFR A1298C genotyping in 107 OSCC patients and 107 cancer-free healthy controls was performed using the PCR-RFLP method.

**Results:** The study groups were age-matched with higher frequencies in the male gender. In the patients group, the distribution of MTHFR A1298C variants was not significant. Smoking was not found to be a risk factor: in non-smokers the frequency of the MTHFR A1298 allele was higher than the 1298C allele, and the A1298 allele carriers possessed moderately or well differentiated tumors with a diameter of <4 cm. However, these associations were not detected in smokers.

**Conclusion:** The present study alone did not demonstrate any association between the MTHFR A1298C polymorphism and the risk of OSCC in the Turkish population, however the prognosis of OSCC may be influenced by MTHFR A1298C variants.

**Keywords:** OSCC, MTHFR, polymorphism, gene, oral cancer

## ÖZ

**Amaç:** Düşük yaşam süresi ile karakterize olan ve çevresel faktörlerin yanı sıra genetik ve epigenetik değişimlerin katkılarının bulunduğu oral sküamoz hücre karsinomu (OSHK) gün geçtikçe yükselen insidansıyla dikkati çekmektedir. Günümüze kadar, folat metabolizmasının önemli enzimlerinden biri olan ve dolayısıyla DNA sentezi, metilasyonu ve tamir mekanizmalarında rol oynayan metilen tetrahidrofolat redüktaz (MTHFR) enzimine ait genetik varyantlar ile kanser yatkınlığı arasındaki ilişkinin araştırıldığı birçok çalışma yapılmıştır. Çalışmamızda, Türk popülasyonundaki MTHFR A1298C varyantları ile alkol ve/veya sigara kullanımı, cinsiyet, yaş veya klinik parametreler esasında oral sküamoz hücre karsinomu gelişim riskinin araştırılması hedeflenmiştir.

**Gereç ve Yöntem:** MTHFR varyantları PCR-RFLP yöntemleri kullanılarak aynı yaş grubundaki 107 hasta ile 107 sağlıklı bireyde analiz edilmiştir.

**Bulgular:** Hasta ve kontrol grubu arasında MTHFR A1298C genotip ve allel dağılımı bakımdan anlamlı bir fark elde edilmemiştir. Sigara-içmeyen hastalarda, A1298 allel frekansı mutant allele göre yüksek olup, bu alleli taşıyanlarda tümör çapının 4cm'den büyük olduğu ve bu tümörlerin orta veya iyi diferansiasyona sahip olduğu tespit edilmiştir. Ancak bu durum sigara içen hastalarda tespit edilmemiştir.

**Sonuç:** Çalışmamız sonucunda MTHFR A1298C polimorfizminin hastalık gelişiminde bir risk faktörü olmadığı, ancak hastalığın prognozunda özellikte mutant varyantın etkili olabileceği kanaatine varılmıştır.

**Anahtar Kelimeler:** OSHK, MTHFR, polimorfizm, gen, ağız kanseri

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a common neoplasia of the oral cavity with a poor survival rate. The predominant risk factors are alcohol and tobacco consumption, however gender, age, viral human papillomavirus (HPV) infections, chronic inflammation and dietary/lifestyle factors such as poor oral hygiene and low intake of cereals, vege-

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tables and fruits have important contributory roles for OSCC carcinogenesis. Moreover, epidemiological studies have shown that genetic factors have the potential to affect individual susceptibility to OCSS (1, 2).

In a number of studies, folate metabolism and related genes - which particularly takes place in biotransformation pathways in particular - have been linked with cancer susceptibility by altered intracellular S-adenosyl-methionine (SAM, the universal methyl donor) levels that affect methylation processes, and a distorted availability of nucleotides for DNA synthesis and DNA repair (3-7).

It is well-known that one of the essential enzymes in folate metabolism is methylenetetrahydrofolate reductase (MTHFR), the enzyme responsible for producing the circulating form of folate and the carbon donor for the re-methylation of homocysteine to methionine. MTHFR irreversibly catalyzes the reduction of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-MTHF and provides a methyl group for SAM biosynthesis which are all crucial for DNA synthesis, methylation as well as DNA repair mechanisms (5, 8). Thus, polymorphisms of the MTHFR gene may cause the dysregulation of folate metabolism which results in abnormal cell proliferation and DNA hypo-methylation which may all lead to a predisposition towards carcinogenesis. Furthermore, while some studies have reported the association of MTHFR polymorphisms with several cancer types (9-12), others have indicated a reduced risk of cancer by folate intake (13, 14).

Two common polymorphisms that alter enzyme activity have been described for the MTHFR gene: MTHFR C677T (rs1801133) which causes alanine substitution to valine amino acid in exon 4, and MTHFR A1298C (rs1801131) in exon 7 that results in another amino acid residue change from glutamate to alanine. In our previous study, the individual effects of MTHFRC677T polymorphism on OSCC prognosis may not have been significant, however, it was found that possessing the wild type T allele in conjunction with smoking and/or alcohol consumption increased the risk of OSCC (15). The aim of the present study was to investigate the association between MTHFR A1298C variants and alcohol and/or tobacco consumption, gender or age in respect to clinical histopathological parameters, and the risk of OSCC development in the Turkish population.

## MATERIAL AND METHOD

### Patient Selection and Clinical Investigation

107 OSCC patients diagnosed and recruited by the School of Dentistry, Department of Oral Surgery and Medicine in İstanbul University and 107 healthy volunteers as controls participated in this study. The patient groups were all newly diagnosed with clinical-histological parameters (such as tumour classification, invasion, differentiation and nodal status) and were scored according to the tumour-node-metastasis (TNM) classification system and were confirmed by pathologic examination. All patients who had previously undergone chemotherapy, radiotherapy, and surgery were excluded from the study. The control group was made up of healthy subjects with no symptoms of cancer or any kind of cancer history in their families.

All participants in the study provided written consent prior to their inclusion in the study. This study protocol was approved by the Helsinki Declaration and blood samples were collected only when written informed consent had been obtained. The study protocol was approved by the Ethical Committee of the İstanbul School of Medicine and the Research Fund of İstanbul University and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

### Polymerase Chain Reaction (PCR)-Based Detection of *MTHFR* A1298C Genotyping

Peripheral blood specimens from each participant were collected in tubes containing EDTA and genomic DNA samples were extracted from whole blood using the salting out procedure (16). The DNA samples were amplificated by polymerase chain reaction with locus-specific primers as shown in Table 1. After amplification, *MTHFR*A1298C (rs1801131) polymorphism was detected by cutting the PCR product with the restriction endonuclease MboII (New England BioLabs, U.K.) as previously reported (12, 17). The PCR product and restriction pattern of MTHFR rs1801131 locus are also shown in Table 1.

### Statistical Analyses

Statistical analysis was performed using the Statistical Package for Social Sciences software package version 11.5 (SPSS Inc., Chicago, IL, USA). The mean values of the clinical parameters between the patients and control groups were compared using the unpaired Student's t-test and expressed as mean±SD. Differences in the distribution of genotypes and alleles between patients and controls were tested using the Chi-square statistic. The Hardy-Weinberg equilibrium was tested for all polymorphisms. Allele frequencies were estimated using gene counting methods. A univariate analysis was performed to compare the distribution of age, sex and several independent factors with the frequencies of MTHFR A1298C alleles and genotypes. Values of $p<0.05$ were considered statistically significant.

## RESULTS

### Clinical Investigation

The general characteristics of the study groups are summarised in Table 2. While the study groups were age-matched, the frequency of the male gender was higher both in the control and patients group. Lower levels of body mass index, BMI (24.53±4.66 versus (vs) 26.43±2.99; p=0.012) and higher frequencies of first-degree family history (36.9% vs 9.8%; p=0.001) were detected in the patients group. The smoking status was not different between the study groups (p>0.05). 35.0% of patients smoked more than 20 cigarettes per day, and the ratio of never smoked/consumed cases to at least one time smoked/consumed cases were 35.9% vs 64.1% in the patient group ($p$=0.063) (Table 1). The demographic characteristics of the patients group are shown in Table 3. Moderately and well differentiated tumors were detected in higher ratios than poorly differentiated tumors (88.3%→11.7%). In addition, nodes were mostly smaller than 3 cm (82.2%→17.8%). Moreover, 35.0% pa-

**Table 1.** The locus-specific PCR primers and restriction pattern of MTHFR rs1801131

| MTHFR rs1801131 primers | PCR product | Restriction pattern of MboII | |
|---|---|---|---|
| 5′ – AAG GAG GAG CTG CTG AAG ATG – 3′ | 237 bp | A allele | 182, 28, 27 bp |
| 5′ – CTT TGC CAT GTC CAC AGC ATG – 3′ | | C allele | 210, 27 bp |

bp: base pairs

**Table 2.** General characteristics of the study group

| | Control group (n=107) | Patient group (n=107) | p |
|---|---|---|---|
| Age (years) | 59.02±9.57 | 56.11±13.75 | 0.216 |
| Gender (n) | | | |
| Female / Male | 14/93 | 35/72 | 0.001 |
| BMI | 26.43±2.99 | 24.53±4.66 | 0.012 |
| Smoking (%) (+/-) | 26.8/73.2 | 63.1/36.9 | p<0.001 |
| Smoking Status (%) | | | |
| > 20 /day | - | 35.0 | 0.302 |
| Never / Consumpted + consumpting | 18.2/81.8 | 35.9/64.1 | 0.063 |
| Family history (%) | | | |
| First degree (+/-) | 9.8/90.2 | 36.9/63.1 | 0.001 |
| Second degree (+/-) | 5.9/94.1 | 20.0/80.0 | 0.151 |

BMI: body mass index; n: number of individuals; parametric results are shown as mean ± SD; % results were calculated by Chi-square test. p<0.05 denoted statistical significance

tients had mechanical trauma, 64.1% patients had a prosthesis and 12.6% patients had an occupational risk for the development of OSCC (Table 3).

### Distribution of *MTHFR*A1298C Genotypes

The distributions of genotypes and alleles of *MTHFR*A1298C are shown in Table 4. No significant deviation from Hardy-Weinberg Equilibrium (HWE) was observed for the *MTHFR* A1298C polymorphism in the control group, while a significant deviation from HWE was observed for this polymorphism in the patient group. If only the genotype distribution of the patient group shows a deviation from HWE, this may provide additional support for an association of the marker locus with the disease in question. However, deviation from HWE can also be attributed to the small size of the sample group (18-20) (Table 4).

### Association of the *MTHFR*A1298C Genotypes with Clinical/ Demographic Parameters

In Table 5 the combined effect of *MTHFR*A1298C genotypes and smoking or family history are presented. Interestingly, it was found that significance was only detected in second degree family history (Table 4). As seen in Table 6, no statistical association was found between BMI and *MTHFR* A1298C variants in the study groups. The relation between tumor grade, diameter, differentiation or nodal status and MTHFR A1298C polymorphism is shown in Table 7. There were no differences in tumor grade or nodal status. However, it was found that the frequency of moderately and well differentiated tumors were higher in A1298 allele carriers (AA+AC genotypes) than the CC genotype (p=0.008). Furthermore, the frequency of tumors possessing diameters smaller than 4 cm was higher in AA genotype than in 1298C allele (CC+AC genotypes) carriers, and the frequency of tumors possessing diameters greater than 4 cm was higher in 1298C allele (CC+AC genotypes) carriers than in AA genotype (p=0.054). In Table 8 the effects of both smoking and MTHFR variants on the clinical parameters of patients are shown, and it was detected that the frequency of moderately and well differentiated tumors were significantly higher in A1298 allele (AA+AC genotypes) carrier non-smoker patients than those with CC genotype carriers (90.9% vs. 9.1%; p=0.017).

## DISCUSSION

Oral squamous cell carcinoma, the most common form of head and neck cancers, has unsatisfactory survival rates of five years - even when targeted with new treatment strategies. As previously described, there are many risk factors that affect development, prognosis, survival and even tendency of OSCC

| **Table 3.** Demographic profile of the patients group | |
|---|---|
| Tumor Grade (%) | |
| Early (Grade 1+2) | 46.5 |
| Late (Grade 3+4) | 53.5 |
| Differentiation Status (%) | |
| Moderately+Well | 88.3 |
| Poorly | 11.7 |
| Tumor diameter (%) | |
| < 4 cm | 62.4 |
| > 4 cm | 37.6 |
| Node metastasis (%) | |
| Presence | 34.7 |
| Absence | 65.3 |
| Node status (%) | |
| > 3 cm (n2 + n3) | 17.8 |
| < 3 cm (n0 + n1) | 82.2 |
| Mechanical trauma (%) | 35.0 |
| Occupational risk (%) | 12.6 |
| Prothesis (%) | 64.1 |
| Chi-square test was used to compare the related variants in OSCC patients group. p<0.05 denoted statistical significance | |

including genetic polymorphisms such as *MTHFR*A1298C and C677T. In our previous study, any individual effect of *MTHFR* C677T polymorphism was detected on OSCC prognosis, however with smoking and/or alcohol consumption the OSCC risk was increased by T allele (15). In addition, Cao et al. (8) reported that the interaction between the 677TT genotype and 1298AC

**Table 4.** The distribution of MTHFR A1298C polymorphism between the study groups

| **MTHFR A12987C** | **Control group (n=107)** | **Patient group (n=107)** |
|---|---|---|
| Genotypes (n,%) | | |
| AA genotype | 44 (41.1) | 51 (47.7) |
| AC genotype | 45 (42.1) | 38 (35.5) |
| CC genotype | 18 (16.8) | 18 (16.8) |
| Alleles (n,%) | | |
| A allele | 133 (62.15) | 140 (65.42) |
| C allele | 81 (37.85) | 74 (34.58) |
| HWE p | 0.2721 | 0.026 |
| p | > 0.05 (Matches with HWE) | < 0.05 (Unmatches with HWE) |

Chi-square test was used to compare genotypes in the whole study group. For determining allelic frequencies, gene count method was used. n: number of individuals. p<0.05 denoted statistical significance

**Table 5.** The combined effect of MTHFR A1298C polymorphism and certain risk factors on the development of OSCC

| | **AA genotype** | **C allele (AC+CC genotype)** | **p** | **CC genotype** | **A allele (AA+AC genotype)** | **p** |
|---|---|---|---|---|---|---|
| Smoking | | | | | | |
| Absence | 42.1 | 57.9 | | 15.8 | 84.2 | |
| Presence | 50.8 | 49.2 | 0.396 | 16.9 | 83.1 | 0.881 |
| Cigarette consumption | | | | | | |
| < 20/ day | 41.8 | 58.2 | | 16.4 | 83.6 | |
| > 20/ day | 58.3 | 41.7 | 0.109 | 16.7 | 83.3 | 0.974 |
| First degree family history | | | | | | |
| Absence | 53.8 | 46.2 | | 18.5 | 81.5 | |
| Presence | 36.8 | 63.2 | 0.095 | 13.2 | 86.8 | 0.484 |
| Second degree family history | | | | | | |
| Absence | 60.7 | 39.3 | | 3.6 | 96.4 | |
| Presence | 14.3 | 85.7 | 0.041 | 42.9 | 57.1 | 0.019 |

Chi-square test was used to compare the related variants in OSCC patients group. p<0.05 denoted statistical significance

**Table 6.** The effects of MTHFR A1298C variants on BMI in the study groups

| | Patient Group | | | | | | Control Group | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA genotype | C allele (CC+AC genotype) | p | CC genotype | A allele (AA+AC genotype) | p | AA genotype | C allele (AC+CC genotype) | p | CC genotype | A allele (AA+AC genotype) | p |
| BMI (X±SD) | 24.18±5.00 | 24.83±4.40 | 0.571 | 23.81±4.95 | 24.70±4.63 | 0.539 | 26.35±3.06 | 26.50±3.01 | 0.873 | 28.44±3.11 | 26.26±2.97 | 0.232 |
| BMI (%) | | | | | | | | | | | | |
| ≥ 27.5 | 20.0 | 80.0 | | 50.0 | 50.0 | | 40.0 | 60.0 | | 8.00 | 92.0 | |
| < 27.5 | 16.7 | 83.3 | 0.758 | 33.3 | 66.7 | 0.223 | 57.1 | 42.9 | 0.308 | 7.1 | 92.9 | 1.000 |

BMI: body mass index; parametric results are shown as mean ± SD; % results were calculated by Chi-square test. p<0.05 denoted statistical significance

**Table 7.** The effects of MTHFR A1298C polymorphism on the clinical/demographic features of the patients group

| (%) | AA genotype | C allele (AC+CC genotype) | p | CC genotype | A allele (AA+AC genotype) | p |
|---|---|---|---|---|---|---|
| Tumor Grade | | | | | | |
| Early (Grade 1+2) | 48.9 | 51.1 | | 14.9 | 85.1 | |
| Late (Grade 3+4) | 44.4 | 55.6 | 0.652 | 18.5 | 81.5 | 0.627 |
| Differentiation Status | | | | | | |
| Moderately+Well | 48.2 | 51.8 | | 13.3 | 86.7 | |
| Poorly | 34.4 | 63.6 | 0.533 | 45.5 | 54.5 | 0.008 |
| Tumor diameter | | | | | | |
| < 4 cm | 54.0 | 46.0 | | 12.7 | 87.3 | |
| > 4 cm | 34.2 | 65.8 | 0.054 | 23.7 | 76.3 | 0.153 |
| Node metastasis | | | | | | |
| Absence | 47.0 | 53.0 | | 18.2 | 81.8 | |
| Presence | 45.7 | 54.3 | 0.904 | 14.3 | 85.7 | 0.618 |
| Node status (%) | | | | | | |
| < 3 cm (n0 + n1) | 47.0 | 53.0 | | 18.1 | 81.9 | |
| > 3 cm (n2 + n3) | 44.4 | 55.6 | 0.845 | 11.1 | 88.9 | 0.474 |

Chi-square test was used to compare the related variants in OSCC patients group. p<0.05 denoted statistical significance

**Table 8.** The effect of smoking and genotypes of MTHFR A1298C polymorphism on the clinical/demographic features of the patients group

| | Non- smokers | | | | | | Consumpted | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (%) | AA genotype | C allele (CC+AC genotype) | p | CC genotype | A allele (AA+AC genotype) | p | AA genotype | C allele (AC+CC genotype) | p | CC genotype | A allele (AA+AC genotype) | p |
| Tumor Grade | | | | | | | | | | | | |
| Early (Grade 1+2) | 52.6 | 47.4 | | 10.5 | 89.5 | | 46.4 | 53.6 | | 17.9 | 82.1 | |
| Late (Grade 3+4) | 33.3 | 66.7 | 0.236 | 16.7 | 83.3 | 0.585 | 50.0 | 50.0 | 0.777 | 19.4 | 80.6 | 0.872 |
| Differentiation Status | | | | | | | | | | | | |
| Moderately+Well | 45.5 | 54.5 | | 9.1 | 90.9 | | 50.0 | 50.0 | | 16.0 | 84.0 | |
| Poorly | - | 100.0 | 0.207 | 100.0 | - | 0.017 | 44.4 | 55.6 | 1.000 | 33.3 | 66.7 | 0.347 |
| Tumor diameter | | | | | | | | | | | | |
| < 4 cm | 54.5 | 45.5 | | 9.1 | 90.9 | | 53.7 | 46.3 | | 14.6 | 85.4 | |
| > 4 cm | 26.7 | 73.3 | 0.176 | 20.0 | 80.0 | 0.377 | 39.1 | 60.9 | 0.264 | 26.1 | 73.9 | 0.260 |
| Node metastasis | | | | | | | | | | | | |
| Absence | 44.4 | 55.6 | | 14.8 | 85.2 | | 48.7 | 51.3 | | 20.5 | 79.5 | |
| Presence | 40.0 | 60.0 | 1.000 | 10.0 | 90.0 | 1.000 | 48.0 | 52.0 | 0.955 | 16.0 | 84.0 | 0.751 |
| Node status (%) | | | | | | | | | | | | |
| < 3 cm (n0 + n1) | 45.5 | 54.5 | | 15.2 | 84.8 | | 48.0 | 52.0 | | 20.0 | 80.0 | |
| > 3 cm (n2 + n3) | 25.0 | 75.0 | 0.618 | - | 100 | 1.000 | 50.0 | 50.0 | 0.895 | 14.3 | 85.7 | 1.000 |

**Table 8.** The effect of smoking and genotypes of MTHFR A1298C polymorphism on the clinical/demographic features of the patients group (continued)

| | 20 years Smokers | | | | | | >20 years Smokers | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (%) | AA genotype | C allele (CC+AC genotype) | p | CC genotype | A allele (AA+AC genotype) | p | AA genotype | C allele (AC+CC genotype) | p | CC genotype | A allele (AA+AC genotype) | p |
| Tumor Grade | | | | | | | | | | | | |
| Early (Grade 1+2) | 46.7 | 53.3 | | 26.7 | 73.3 | | 64.3 | 35.7 | | 14.3 | 85.7 | |
| Late (Grade 3+4) | 43.3 | 56.7 | 0.832 | 23.3 | 76.7 | 1.000 | 52.4 | 47.6 | 0.486 | 19.0 | 81.0 | 1.000 |
| Differentiation Status | | | | | | | | | | | | |
| Moderately+Well | 45.5 | 54.5 | | 24.2 | 75.8 | | 61.5 | 38.5 | | 11.5 | 88.5 | |
| Poorly | 42.9 | 57.1 | 1.000 | 28.6 | 71.4 | 0.810 | 50.0 | 50.0 | 0.666 | 33.3 | 66.7 | 0.228 |
| Tumor diameter | | | | | | | | | | | | |
| < 4 cm | 52.2 | 47.8 | | 21.7 | 78.3 | | 68.4 | 31.6 | | 10.5 | 89.5 | |
| > 4 cm | 36.4 | 63.6 | 0.286 | 27.3 | 72.7 | 0.666 | 43.8 | 56.3 | 0.142 | 25.0 | 75.0 | 0.258 |
| Node metastasis | | | | | | | | | | | | |
| Absence | 48.0 | 52.0 | | 28.0 | 72.0 | | 60.9 | 39.1 | 17.4 | 82.6 | | |
| Presence | 40.0 | 60.0 | 0.592 | 20.0 | 80.0 | 0.729 | 50.0 | 50.0 | 0.537 | 16.7 | 83.3 | 1.000 |
| Node status (%) | | | | | | | | | | | | |
| < 3 cm (n0 + n1) | 47.1 | 52.9 | | 26.5 | 73.5 | | 60.7 | 39.3 | | 17.9 | 82.1 | |
| > 3 cm (n2 + n3) | 36.4 | 63.6 | 0.729 | 18.2 | 81.8 | 0.578 | 42.9 | 57.1 | 0.430 | 14.3 | 85.7 | 1.000 |

Chi-square test was used to compare the related variants in OSCC patients group. $p<0.05$ denoted statistical significance

or 1298CC genotypes and heavy smoking status increased the risk of nasophayngeal carcinoma. In the present study, classic risk factors such as male gender and first-degree family history were individually found to be associated with OSCC. Interestingly, a smoking status of either mild or heavy smoker did not seem to be a risk factor in our study groups, like the study by Taghavi et al. (21), however, this situation can be caused by lacking dietary behaviour for example, having a lack of vitamin or folate which was also a limitation of our study. Nevertheless, it was found that people who never smoked or mild smokers were a little more protected from OSCC development.

Skiloba et al. (12) reported a decreased risk of acute lymphocytic leukemia (ALL) in adults with 1298AC and 1298CC genotypes but no association with acute myeloid leukemia and suggested a link between folate inadequacy and the development of ALL. On the other hand, Blank et al. (9) investigated the prognostic significance of MTHFR gene polymorphisms in gastric cancer patients treated with neo-adjuvant chemotherapy in which it was formerly reported that the efficacy of these therapies, especially 5-fluorouracil treatment could be affected by folate metabolism and MTHFR function as well. However, they reported no effect of *MTHFR*A1298C and C677T polymorphisms on the prognostic impact of gastric cancer. A meta-analysis by Tan et al. (11) suggested an increased risk of esophageal cancer in Asian or Caucasion 1298CC genotype carriers. In addition, despite the findings of a rare distribution of 1298CC genotype in Chinese esophageal cancer patients and healthy controls, Song et al. (17), reported that possessing the 1298CC genotype increases esophageal cancer risk versus the AA1298 genotype. As for head and neck cancer (HNC) the results are still controversial. Galbiati et al. (5) reported an increased risk of HNC with 1298AC/CC genotypes in contrast with Neumann et al. (22) who showed a lower risk with the same genotypes. On the other hand, Suzuki et al. (23), Kruszyna et al. (24) and two meta-analyses by Boccia et al. (4) and Niu et al. (25) reported no association with the development of HNC. In line with all these previous studies, our results are somehow compatible with them. We also found no association between the distribution of MTHFR A1298C genotypes and the risk of OSCC development independently. However, and interestingly, it was found that while differentiation status was triggered by A1298 allele (AA+AC genotypes), >4 cm tumors were seen in 1298C allele (CC+AC genotypes) carriers. This result may be because of gene-nutrient interaction e.g. the level of folate intake. Unfortunately, our study lacks folate intake data which is one of the limitations in correlating such an association. Indeed, folate data may be a direct risk factor for evaluating the interaction of MTHFR variants and several cancers as a deficiency of folate causes several deleterious effects including an imbalance in DNA precursors and modified DNA, synthesis and repair which was re-improved by supplementation. On the other hand, it is well-known that the key enzyme in folate metabolism is MTHFR, the product of the MTHFR gene which has two common polymorphisms, C677T and A1298C, resulting in a looseness of enzyme activity, in different ratios. Independently from folate data it was seen that reduced enzyme activity by the variant MTHFR genotype did not affect the development of OSCC, however as seen in the effects on differentiation status and tumor size, MTHFR polymorphism may contribute to OSCC prognosis via DNA hypo-methylation through the variant MTHFR allele.

In conclusion, while our study did not individually demonstrate any association between MTHFR A1298C polymorphism and the risk of OSCC in the Turkish population, the prognosis of OSCC may be influenced by MTHFRA1298C variants. The limitation of our study is the size of study groups, and the lack of dietary behaviours. Therefore, the results obtained here should be supported by further studies.

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**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of İstanbul University School of Medicine.

**Informed Consent:** Written informed consent was obtained from the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

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