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MTHFD1 c.1958G>A and TCN2 c.776G>C polymorphisms of folate metabolism genes and their implication for oral cavity cancer

Orginal Study

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Abstract

Background. Cancers of the head and neck can damage the brain, spinal cord, and nerves, as well as the sense organs responsible for contact with the outside world. Oncogenic transformation occurs following mutations that change the function of specific genes, such tumor suppressor genes or oncogenes, and their encoded protein products. Therefore, the aim of this study was to evaluate the relationship between the occurrence of *MTHFD1* c.1958G>A and *TCN2* c.776G>C gene polymorphisms and the risk of oral cavity cancer.
Materials and methods. The study population consisted of 439 patients and 200 healthy subjects. Genomic DNA was extracted from peripheral blood and from paraffin-embedded tissue. Analysis of the gene polymorphisms was performed using polymerase chain reaction–restriction fragment length polymorphism.
Results. Statistically significant differences were found in the distribution of genotypes of the rs2236225 and rs1801198 polymorphisms between patients and controls. Regarding *MTHFD1* c.1958G>A, the GA genotype ($p<0.0001$, OR = 2.91, 95% CI = 1.88-4.49-GA) was more common among patients than healthy subjects. Regarding *TCN2* c.776G>C, the frequency of CC genotype ($p< 0.0001$, OR = 0.17, 95% CI = 0.10-0.33-CC) was significantly less common among patients than healthy subjects. Tobacco smoking, alcohol use, and male gender ($p<0.0001$) were found to be predictors of the disease.
Conclusion. The results suggest that the *MTHFD1* c.1958G>A polymorphism may be associated with a greater risk of oral cavity cancer, whereas a protective effect has been found for the *TCN2* c.776G>C polymorphism.

Keywords

polymorphism • *MTHFD1* gene • *TCN2* gene • oral cavity cancer

Introduction

Head and neck cancer is the sixth most commonly diagnosed cancer, making it a serious and growing health problem [1]. The term *head and neck cancer* encompasses a very broad group of cancers caused by tumor growth in parts of the upper respiratory tract, such as the nasal cavity, paranasal sinuses, oral cavity, pharynx, and larynx [2]. The symptoms of head and neck cancer differ depending on the location of origin of the cancer. Common symptoms are hoarseness, ulceration, pain, and weight loss for cancers of the larynx, oral cavity, oropharynx, and hypopharynx, respectively [3]. The cancers are classified within the same group due to the similarity of the diagnostic and therapeutic tools used for their detection; they are classified according to the anatomically based TNM classification of malignant tumors [4]. More than 90% of head and neck cancers are squamous cell carcinoma (HNSCC) [1]. Other malignant neoplasms are diagnosed much less frequently, most often they are salivary gland tumors or even more rarely soft tissue tumors or lymphomas.

In 2018, tumors of the lip and oral cavity accounted for 2% of all malignant tumors registered worldwide (354,864 cases) and contributed to 177,384 deaths that year [4]. Epidemiological evidence indicates that the worldwide incidence of head and neck cancer increases with age, occurring most often in people over 50 years old, and they are more common in men than women. The risk of cancer is caused mainly by exposure to tobacco and alcohol abuse [1, 5]. Infection with human papilloma virus [6], environmental factors [7], dietary habits [8], and poor oral hygiene [9] also play a role in the pathogenesis of head and neck cancer. Intensive worldwide, multicenter research has been undertaken to discover new prognostic and treatment predictive biomarkers for these cancers [10, 11, 12, 13].

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Carcinogenesis is a complex and multistage process which involves the conversion of normal cells into tumor cells. Oncogenic transformation occurs following mutations that change the function of specific genes, such tumor suppressor genes or oncogenes, and their encoded protein products. Important changes in the transcriptional and translational regulation of genes also occur in the process of malignant transformation [14]. Recently, much attention has been given to epigenetic changes that influence the regulation of gene expression without affecting the sequence of nucleotides. These phenomena are reversible under the action of chemicals and act through two routes: the epigenetic modification of gene expression by DNA methylation and the remodeling of chromatin by the alteration of histone proteins [15]. DNA methylation is essential for normal cell growth and differentiation. That process occurs during X-chromosome inactivation, genomic imprinting, cell death, and cell cycle control. In mammalian cells, cytosine residues of CpG sites are methylated by methyltransferase (DNMT) at position C5 [16]. The methylation process plays an important role in genome stability. Disorders of methylation patterns may become useful biological markers associated with many forms of tumor. Therefore, despite being difficult to clearly define and with no well-defined mechanism of action, epigenetic phenomena are of great potential clinical importance [17].

DNA methylation is a biochemical process of adding a methyl group to the substrate. The following enzymes take part in that process: methylenetetrahydrofolate reductase (MTHFR) [18], methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1 (MTHFD1) [19], methionine synthase (MTR) [20], methionine synthase reductase (MTRR) [21], transcobalamin 2 (TCN2) [22], methyltransferase betaine – homocysteine (BHMT) [23], methyltransferase betaine – homocysteine 2 (BHMT2) [24] and synthase β – cystathionine (CBS) [25]. Numerous studies implicate abnormal methylation in cancer. Aberrant process may be related to the occurrence of a polymorphism in the gene encoding the corresponding protein.

MTHFD1 is an enzyme that performs three different activities (5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase, and 10-formylotetrahydrofolate synthetase). Each of these catalyzes the interconversion of tetrahydrofolate. That is a substrate for the biosynthesis of methionine, thymidylate, and purine nucleotides. The most analyzed polymorphism of the *MTHFD1* gene, c.1958G>A, is located within the domain responsible for 10-formyltetrahydrofolate synthetase activity. The polymorphism results in the substitution of glutamine (Gln) with arginine (Arg) at codon 653 [19].

TCN2 belongs to the vitamin B_{12} – binding protein family and allows cobalamin to be transported into the cell [26]. Vitamin B_{12} is a co-factor for the enzyme MTR, which catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate. That chemical as well as methionine are converted by MTHFR from 5,10-methylenetetrahydrofolate. Methionine may be then converted to S-adenosylmethionine, an important methyl donor for the methylation process, including DNA methylation. MTR links the methylation and synthesis of purine and pyrimidine pathways via generation of the methyl donor methionine and synthesis of tetrahydrofolate, respectively [27]. Both low MTR activity and low vitamin B_{12} level are determinants of DNA hypomethylation [28]. The most common *TCN2* polymorphism is the replacement of guanine to cytosine at position 776; this change will replace the amino acid arginine (Arg) with proline (Pro) at position 259 in the protein sequence of TCN2. This change in the TCN2 protein sequence affects the ability of the protein to bind to vitamin B_{12} and disrupts the binding of the transcobalamin-vitamin B_{12} receptor complex. The *TCN2* c.776G>C genotype has a significant influence on the cellular delivery system of vitamin B_{12} [29]. Healthy subjects with low vitamin B_{12} status manifest reduced levels of homocysteine [30].

Our previous study [31] showed that the *TCN2* gene polymorphism modulates the risk of oral cancer, but the number of patients and controls were limited. Recent studies have examined whether polymorphisms within the *MTHFD1* and *TCN2* genes correlate with increased risk of oral cavity cancer and may be associated with cancer risk, tobacco smoking, alcohol consumption and gender.

1. Materials and Methods

1.1 Patients

The study was conducted with the approval of the Local Ethics Committee of the Medical University of Lodz (RNN/142/09/KB). A total of 439 unrelated patients (201 women and 238 men) with histologically confirmed oral cavity squamous cell carcinoma (OCSCC) were examined for the *MTHFD1* c.1958G>A polymorphism and 220 patients (97 women and 123 men) for the *TCN2* c.776G>C polymorphism. The control group consisted of 200 (100 women and 100 men) unrelated patients with no confirmed malignancies, being treated for such diseases as nasal septum deviation, palatine uvula hypertrophy with sleep apnea, or epistaxis. Four hundred and fifteen (64.9%) participants were men (315 patients and 100 controls) and two hundred and twenty-four (35.1%) were women (124 patients and 100 controls). Alcohol was consumed by 68% of the experimental group, compared to 15% of the controls. Tobacco use also differed greatly between cases: 72% of the experimental group and 25% of the control group. The age of the patients in the study group ranged from 42 to 90 years (average age 63.7 years), and the control group was aged 26 to 84 years (average age 52.4 years). All subjects were cross-matched regarding age and sex. Samples of peripheral blood (5 ml) were taken in EDTA from the antecubital vein. A sample of postoperative tumor tissue was also taken from the mouth of all subjects in the experimental group and parts of the samples

were secured in paraffin blocks. All study materials were collected in the Department of Otolaryngology, Medical University of Lodz, Poland. Informed, written consent to participate in the study was obtained from all patients.

1.2 Polymorphism analysis

MTHFD1 c.1958G>A genotyping by PCR-RFLP

DNA was extracted from peripheral blood lymphocytes using Genomic Mini AX Blood Kits (A&A Biotechnology, Gdynia, Poland) and from tissues in the paraffin blocks using Genomic Mini AX Tissue (A&A Biotechnology, Gdynia, Poland). Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR was carried out in a volume of 10 µl. The reaction mixture consisted of 100 ng of genomic DNA, 0.5 µmol of each primer, and 3U Taq polymerase (A&A Biotechnology, Gdynia, Poland). The PCR cycling conditions consisted of an initial denaturation step of 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 s, 62°C for 30 s, 72°C for 30 s and then a final extension step at 72°C for 5 minutes (Bio-Rad, Hercules, California, USA). Following this, 10 µl of specifically positive PCR products was digested overnight with 1 µl of *Msp*I (New England Bio Labs, Ipswich, Massachusetts, USA) at 37°C, and the digested DNA fragments were resolved on a 3% agarose gel (Sigma-Aldrich, Saint Louis, Missouri, USA). Obtained restrictive fragments were visualized by staining with ethidium bromide (Sigma-Aldrich, Saint Louis, Missouri, USA) and imaged under a UV light source (Bio-Rad, Hercules, California, United States). Genotypes were designated as follows: 197, 70, 56, and 8 bp for G/G; 267, 197, 70, 56, 8 bp for G/A; and 267 bp for A/A. Figure 1 presents a representative gel obtained after digestion of PCR.

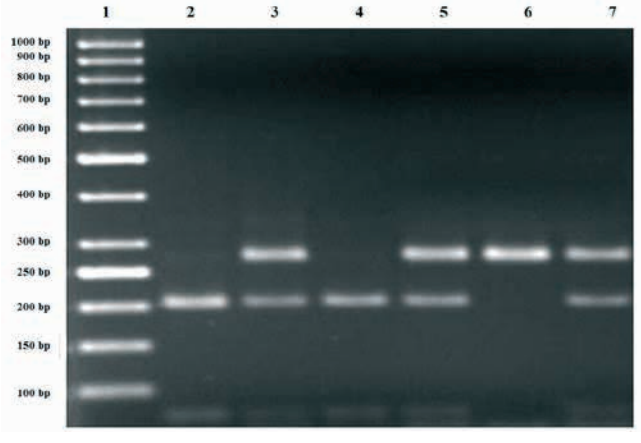


Fig. 1. Analysis of the genotype polymorphism MTHFD1 c.1958G>A after hydrolysis with restriction enzyme MspI; lane 1 - marker, lanes 3, 5, 7 - heterozygote GA, lanes 2, 4 - homozygous GG, lane 6 is homozygous AA

***1.3 TCN2* c.776G>C genotyping by PCR-RFLP**

DNA was extracted from peripheral blood lymphocytes using DNA Blood Mini Kits (A&A Biotechnology, Gdynia, Poland). Genotypes were determined by PCR-RFLP. PCR was carried out in a volume of 10 µl. The reaction mixture consisted of 100 ng of genomic DNA, 0.5 µmol of each primer, and 3 U Taq polymerase (A&A Biotechnology, Gdynia, Poland). The PCR was conducted under the following temperature conditions: pre-denaturation step at 94°C for 10 min, 34 cycles of denaturation at 56 °C for 45s oligonucleotide annealing at 55 °C for 30 s, and amplification at 72°C for 45 s, and final incubation at 72 °C for 10 min (Bio-Rad, Hercules, California, USA). Subsequently, the products were subjected to digestion by the *Scr*FI restriction enzyme (New England Bio Labs, Ipswich, Massachusetts, USA) at 37 °C. After 16 h of digestion, the samples were separated by electrophoresis (Bio-Rad, Hercules, California, USA) on a 2% agarose gel (Sigma-Aldrich, Saint Louis, Missouri, USA). Obtained restrictive fragments were visualized by staining with ethidium bromide (Sigma-Aldrich, Saint Louis, Missouri, USA) and imaged under a UV light source (Bio-Rad, Hercules, California, United States). Genotypes were designated as follows: 204 bp and 364 bp for C/C; 204, 364 and 568 bp for C/G; and 568 bp for G/G. Figure 2 presents a representative gel obtained after digestion of PCR products.

The primers, lengths of the PCR products, and restriction enzymes of *MTHFD1* and *TCN2*gene polymorphisms are summarized in Table 1. Detailed characterization has been shown in Table 2. The *MTHFD1* c.1958G>A and *TCN2* c.776G>C polymorphisms were investigated according to Cheng et al. [32] and Pietrzyk et al. [33] with some alterations.

2. Statistical analysis

Multiple logistic regression was used to determine the effect of the interaction between genetic polymorphisms and risk factors associated with head and neck cancer. The model includes gen-

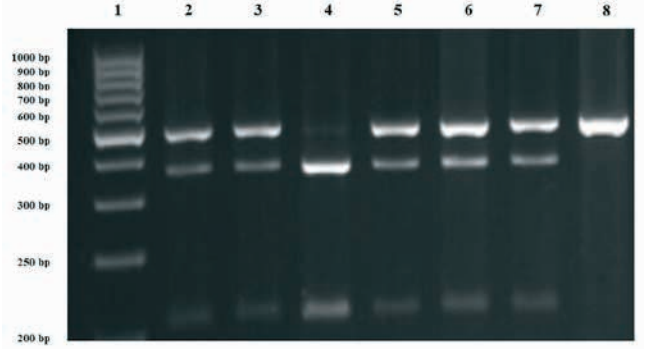


Fig. 2. Analysis of genotype polymorphism TCN2 c.776G>C after hydrolysis with restriction enzyme ScrFI; lane 1 - marker; lanes 2, 3, 5, 6, 7 - heterozygous CG, lane 4 - homozygous CC, lane 8 is homozygous GG

Table 1. The primers, length of PCR products, and restriction enzymes

Polymorphism	Primers	Product length	Restriction enzyme
MTHFD1 c.1958G>A	Sense 5'-CAC TCC AGT GTT TGT CCA TG-3' Antisense 5'-GCA TCT TGA GAG CCC TGA C-3'	331 bp	MspI (37°C)
TCN2 c.776G>C	Sense 5'-CAC TTC AAG ACA CAG CAG CCA-3' Antisense 5'-TCC CCT CAC TGG GAC GTC AAT-3'	568 bp	ScrFI (37°C)

MTHFD1 *- methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1;* ***TCN2*** *- transcobalamin 2*

Table 2. Detailed characterization of MTHFD1 and TCN2 gene polymorphisms

Gene	The encoded protein	Number of polymorphism	Location	Function of polymorphism[1]	Allele	MAF
MTHFD1	Methylene -tetrahydrofolate -dehydrogenase	rs2236225	14: 63978598	Arg653Gln	G/A	0,46
TCN2	Transcobalamin 2	rs1801198	22: 29341610	Arg259Pro	G/C	0,45

[1] *According to the Single Nucleotide Polymorphism Database (dbSNP), NCBI*
MAF - minor allele frequency; ***MTHFD1*** *- methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1;* ***TCN2*** *- transcobalamin 2*

der (reference: female), the consumption of tobacco products (reference: nonsmoking), and alcohol consumption (reference: nondrinkers). The frequencies of the analyzed polymorphisms among OCSCC patients and controls were evaluated using the Hardy-Weinberg (HW) equilibrium test. The distribution of the alleles was analyzed with the Yates chi-square test. Results are shown as odds ratio (OR) and 95% confidence intervals (95% CI). P-values were calculated as two-sided. Means were compared using a t-test or analysis of variance. Probabilities were considered significant at *p* values less than 0.05 (Statistica, Tulsa, Oklahoma, USA).

3. Results

The genotype and allele distributions of the *MTHFD1* c.1958G>A and *TCN2* c.776G>C gene polymorphisms are summarized in Table 3. The observed genotype frequencies of *MTHFD1* c.1958G>A and *TCN2* c.776G>C in the control group were in agreement with the Hardy-Weinberg equilibrium (HWE).

For the *MTHFD1* c.1958G>A polymorphism, GG, GA, and AA genotype frequencies were 12.5, 75.6, and 11.9%, respectively, for the subjects who had suffered from cancer and 26.5, 55 and 18.5%, respectively, for control patients. The 1958 G allele frequencies were 49% among the subjects who had suffered from cancer and 54% among the control patients, while the 1958 A allele frequencies were 51% among subjects who had suffered from cancer and 46% among controls.

For the *TCN2* c.776G>C polymorphism, GG, CG, and CC genotype frequencies were 34.5%, 48.7%, and 16.8% for the experimental group, and 17%, 35.5%, and 47.5%, respectively, for the control. In the gene *TCN2*, 776G allele frequencies were 59% among the subjects who had suffered from cancer and 35% among the controls, while 776C allele frequencies were 41% among subjects who had suffered from cancer and 65% among controls.

The results of comparison between groups showed that tobacco, alcohol use, and male gender ($p < 0.0001$) were predictors of the disease (Table 4).

The data of the interaction between genotype and tobacco smoking, alcohol consumption, and gender with respect to the risk for oral cavity cancer are shown in Table 5. The presence of the *MTHFD1* c.1958G>A variant genotype may increase the risk of oral cavity cancer for smokers ($p= 0.01$) and males ($p= 0.003$). On the other hand, the protective effect was observed for *TCN2* c.776G>C variant genotype for smokers ($p < 0.0001$), alcohol consumption ($p< 0.0001$), and males ($p = 0.003$).

4. Discussion

Malignant tumors have a significant impact on public health and constitute a significant economic burden on the population due to lost productivity and the costs associated with illness and treatment. It is expected that this problem will grow as the population ages [34].

Head and neck cancers are classified according to their location. Risk factors, diagnosis, and treatment may vary depending on the disease subtype. The head and neck region of the body is located close to other important life organs and systems. Cancers of the head and neck can damage the brain, spinal cord, and nerves, as well as the sense organs responsible for contact with the outside world. The tumors may also affect the endocrine or-

Table 3. Genotype and allele frequencies of MTHFD1 c.1958G>A and TCN2 c.776G>C polymorphisms in oral cavity cancer patients and controls

Genotypes	Controls (%) N=200	Patients (%) N=220/439	OR (95% CI)	P value
MTHFD1c.1958G>A				
GG	53 (26.5)	55 (12.5)	1.00	
GA	110 (55)	332 (75.6)	2.91 (1.88-4.49)	<0.0001
AA	37 (18.5)	52 (11.9)	1.35 (0.77-2.38)	0.29
G	216 (54)	442 (49)	1.00	
A	184 (46)	436 (51)	1.16 (0.91-1.47)	0.23
TCN2 c.776G>C				
GG	34 (17)	76 (34.5)	1.00	
CG	71 (35.5)	107 (48.7)	0.67 (0.41-1.12)	0.13
CC	95 (47.5)	37 (16.8)	0.17 (0.10-0.30)	<0.0001
G	139 (35)	259 (59)	1.00	
C	261 (65)	181 (41)	0.76 (0.58-1.01)	0.06

OR - odds ratio; N – number; P value - probability value; MTHFD1 - methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1; TCN2 - transcobalamin 2

Table 4. Relationships between the patient group and the control group regarding smoking, alcohol consumption, and gender

Variables value	Controls (%)	Patients (%) N=220/439	OR (95%CI)	P value
Tobacco				
(N=220)				
Non-smokers	150 (75.0)	31 (14.1)	Reference	
Smokers	50 (25.0)	189 (85.9)	18.29 (11.13-30.06)	<0.0001
(N=439)				
Non-smokers	150 (75.0)	124 (28.2)	Reference	
Smokers	50 (25.0)	315 (71.8)	7.62 (5.20-11.16)	<0.0001
Alcohol consumption				
(N=220)				
No	101 (50.5)	70 (31.8)	Reference	
Yes	99 (49.5)	150 (68.2)	2.19 (1.47-3.25)	<0.0001
(N=439)				
No	101 (50.5)	149 (33.9)	Reference	
Yes	99 (49.5)	290 (66.1)	1.98 (1.41-2.79)	<0.0001
Gender				
(N=220)				
Female	100 (50.0)	52 (23.6)	Reference	
Male	100 (50.0)	168 (76.4)	3.23 (2.13-4.90)	<0.0001
(N=439)				
Female	100 (50.0)	124 (28.2)	Reference	
Male	100 (50.0)	315 (71.8)	2.54 (1.80-3.59)	<0.0001

*OR - odds ratio; N- number; **P** value - probability value*

Table 5. Odds ratio of oral cavity cancer related to MTHFD1 and TCN2 genotypes according to tobacco use, alcohol consumption and gender in patients with head and neck cancer

Risk factor	OR (95%CI) - P value	
	MTHFD1c.1958G>A *	TCN2 c.776G>C **
Tobacco		
Non-smokers	2.10 (1.09-4.06) P=0.03	0.72 (0.29-1.77) P=0.63
Smokers	2.60 (1.29-5.23) P=0.01	0.15 (0.05-0.45) P<0.0001
Alcohol consumption		
No	1.23 (0.60-2.51) P=0.35	0.53 (0.27-1.01) P=0.08
Yes	1.01 (0.57-1.81) P=1	0.26 (0.13-0.52) P<0.0001
Gender		
Female	1.77 (0.93-3.39) P=0.1	0.48 (0.22-1.04) P=0.07
Male	2.28 (1.32-3.96) P=0.003	0.33 (0.17-0.61) P=0.003

OR - odds ratio; P value - probability value; MTHFD1 - methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1; TCN2 - transcobalamin 2
** MTHFD1c.1958G>A Reference: GG wild type genotype vs. variants GA+GG*
*** TCN2 c.776G>C Reference: GG wild type genotype vs. variants GC+CC*

gans, such as the thyroid or pituitary gland. Regardless of whether a tumor is malignant or benign, it often directly affects the performance of several structures simultaneously [2].

Non-healing ulcers, new or fast-growing growths, or warts on the skin, mouth, or nose can develop in response to anxiety and need to be investigated to determine whether they pose a threat to health or life. Particular attention should be paid to frequently occurring headaches, dizziness and changes in temperament and mood. In addition, frequent nosebleeds, ear or neck aches, persistent hoarseness, and voice alterations can be symptoms of cancer, as can problems with chewing and swallowing, choking, and the presence of ulcers in the mouth and blood in the saliva. Other symptoms may include numbness in the face or tongue, tooth loss, or a well-fitting prosthesis becoming mismatched [35].

Although genetic and environmental factors take part in the pathogenesis of head and neck cancer, the mechanisms underlying the process remain unclear. However, host genetic factors are the important determinants of the pathophysiology of the disease. Polymorphisms may be potentially used as a biomarker to evaluate cancer risk. Sequence variants in Toll-like receptor genes, which mediate the innate immune response defense against microorganisms, have been linked to head and neck cancer risk [36]. Moreover, strong evidence confirms that alterations in the genes associated with DNA methylation may contribute to carcinogenesis. Hypermethylation promotes gene silencing, and hypomethylation of a promoter can make it susceptible to induction by oncogenes [37].

The aim of the study was to determine the relationship between the occurrence of two polymorphisms of genes encoding selected enzymes associated with the methylation process, *viz. MTHFD1* (rs2236225) and *TCN2* (rs1801198), and the risk of developing cancer of the head and neck. This study included a group of 439 patients with histologically confirmed oral cavity cancer and a control group of 200 individuals without cancer.

Data indicate the presence of 30,000 CpG dinucleotides, also known as CpG islands, in the human genome; most of which are located in promotor regions. Under normal conditions, they remain unmethylated. Methylation of those structures leads to different diseases, including carcinogenesis [38]. Hazra et al. [39] found that aberrant methylation of the promoter CpG island may contribute to colorectal cancer development by silencing tumor suppressor genes. The inactivation of tumor suppressor genes by hypermethylation plays a key role in the process of malignant transformation [40].

MTHFR c. 677C>T gene polymorphism may be a risk factor for thyroid cancer. Furthermore, *MTR* c. 2756A>G is associated with tumor extent and aggressiveness [41]. Moreover, DNMT3B c. -283T>C gene polymorphism is linked to a higher risk of head and neck cancer [42]. The available literature data demonstrates the impact of the *MTHFD1* c.1958G>A gene polymorphism on the appearance of neural tube birth defects in children [43, 44] and loss in the second trimester of pregnancy [45]. Data indicate that SNPs in the *MTHFD* gene coexist with folate and homocysteine concentration disorders in families with congenital heart disease [46]. Kempisty et al. reported a higher prevalence of schizophrenia and bipolar disorder among patients with 1958AA and 1958AG genotypes [47], whereas Sutherland et al. found no evidence for an association of the *MTHFD1* c.1958G>A polymorphism with migraine susceptibility [48]. The *MTHFD1* gene

polymorphism is also regarded as the most common risk factor for cancer. Stevens et al. [49] noted an increased risk of developing malignant breast cancer in postmenopausal women who were carriers of the T and C alleles in the presence of at least one variant of the *MTHFD1* c.677C>T and *MTHFD1* c.1298A>C, respectively. Moreover, breast cancer patients who were carrying A allele for the *MTHFD1* c.1958G>A polymorphism showed a higher frequency of tumor CpG island hypermethylation [50]. Wang et al. reported a connection between the presence of several mutated variants of this gene and the increased risk of gastric cancer [51]. Another study found the *MTHFD1* c.1958G>A polymorphism to be related with a lower probability of event-free survival in children with lymphoblastic leukemia [52]. Despite expectations, *MTHFD1*c.1958G>A polymorphism has been found not to be a risk factor for prostate cancer [53]; however, *MTHFD1* c.1958G>A SNP was associated with the emergence of squamous cancer cells in the head and neck in alcohol abusers and smokers [54].

It has been found that the presence of the *TCN2* c.776CG+GG variant was related to a higher risk of occurrence of other disease entities such as colorectal adenoma [55] and Crohn disease [56]. The *TCN2* 776GG variant increased the susceptibility of individuals to peripheral nerve dysfunction [57]. Our earlier results indicate that the CG and GG genotypes and the G allele increase the likelihood of oral cancer [31]. Protein encoded by the *TCN2*c.776G>C G allele is associated with lower vitamin B_{12} transport from blood to tissues [30], and patients with GG genotype had significantly lower concentrations of holotranscobalamin [58].

In conclusion, the presence of the *MTHFD1* c.1958G>A polymorphism increases the risk of head and neck cancer associated with tobacco smoking and male sex, whereas *TCN2* c.776G>C variant has protective effects with tobacco smoking, alcohol consumption, and male sex.

Abbreviations

BHMT – methyltransferase betaine – homocysteine; **BHMT2** – methyltransferase betaine – homocysteine 2; **CBS** – synthase β – cystathionine; **DNMT** – DNA methyltransferase; **HNSCC** – head and neck squamous cell carcinoma; **HWE** – Hardy-Weinberg equilibrium test; **MTHFD1** – methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1; **MTHFR** – methylenetetrahydrofolate reductase; **MTR** – methionine synthase, **MTRR** – methionine synthase reductase, **OCSCC** – oral cavity squamous cell carcinoma; **OR** – odds ratio; **PCR-RFLP** – the polymerase chain reaction-restriction fragment length polymorphism; **SNP** – single nucleotide polymorphism; **TCN2** – transcobalamin 2.

Authors' Contribution

K.M.: research concept and design, final proofreading and approval of the version for publication, supervising the project; **A.N.Z.:** writing – original draft preparation, literature review; **A.M.S.:** data analysis and interpretation; **A.S.:** carrying out the experiments; **D.K.:** acquisition of data; **K.B.K.:** literature review; **H.Z.B.:** writing – review and editing.

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

Ethics Approval

Approval of the Bioethics Committee at the Medical University of Łódź was obtained, No. RNN/142/09/KB.

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