Arch Biol Sci. 2021;73(4):437-445 https://doi.org/10.2298/ABS210813037J

Cyclin D1 and p21 gene variants and oral squamous cell carcinoma risk and prognosis

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Received: August 13, 2021; **Revised**: September 7, 2021; **Accepted**: September 15, 2021; **Published online**: September 22, 2021

Abstract: Cyclin-dependent kinase inhibitor p21 (encoded by the *CDKN1A* gene) and cyclin D1 (encoded by the *CCND1* gene) are important regulators of cell cycle progression and could have important effects in the complex process of neoplastic transformation. The current study aimed to investigate variants in *CDKN1A* (rs1801270, rs1059234) and *CCND1* (rs9344) genes as potential risk and prognostic factors in oral squamous cell carcinoma (OSCC) patients. The study included 104 OSCC patients and 107 healthy individuals without a history of cancer. Genotypes were assessed by real-time PCR and TaqMan SNP genotyping. Significant differences in genotype distribution between OSCC cases and the control group were observed for the *CCND1* rs9344 variant (p=0.017). According to the odds ratio (OR), adjusted for age and sex, the rs9344 heterozygous GA and homozygous mutated AA genotypes were associated with an increased OSCC susceptibility (OR=2.295, p=0.007; OR=2.029, p=0.037, respectively). Variants rs1801270 and rs105923 in *CDKN1A* were not associated with OSCC risk. There were no differences in overall survival among OSCC patients stratified by genotypes of the analyzed variants in *CDKN1A* and *CCND1*. Variant rs9344 in the *CCND1* gene might be considered as a potential molecular risk factor for OSCC susceptibility but not for disease prognosis.

Keywords: p21; cyclin D1; gene polymorphism; oral squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common type of head and neck carcinoma, with an increasing number of cases every year worldwide [1]. OSCC is etiologically associated with risk factors, such as tobacco and alcohol use [2]. However, not all drinkers and smokers develop OSCC, which indicates the importance of an individual's genetic predisposition. A complex process of oral carcinogenesis encompasses various genetic changes. Oncogenes or tumor suppressor genes associated with cell cycle regulation are often altered in carcinogenesis [3]. The cyclins/cyclin-dependent kinase (CDKs) complexes and cyclin-dependent kinase inhibitors (CKIs) are key modulators of cell cycle progression. Phase-specific cyclins and CDKs are active at different stages of the cell cycle and are essential for cell cycle control. Cdk2 controls G1-S transition and S phase, while Cdk4 and Cdk6 are important for G1 progression [4]. Different mechanisms inhibit different cyclin-dependent kinases after DNA damage. Cdk2 is inhibited by p21, while the cooperation of p21 and other CDK inhibitors, such as p27, inhibits Cdk4, and Thr-14/Tyr-15 phosphorylation inhibits Cdk2 [4].

CDK inhibitor p21 (Waf1/Cip1/CDKN1A) is a member of the CIP/KIP family, along with p27 and p57, that regulates G1/S transition and modulates the cell cycle and apoptosis [5]. The p21 protein is encoded by a tumor-suppressor gene, *CDKN1A*, located on chromosome 6p21.2., and in response to DNA damage, p53 upregulates p21 expression [6]. To date, at least 40 *CDKN1A* genetic variants have been

described, of which seven have an allele frequency of more than 10% [7]. Among extensively studied variants are rs1801270 (C>A), a missense variant located in codon 31, and rs1059234 (T>C) in the 3'-untranslated region of the *CDKN1A* gene. Both variants may affect the functioning of p21 protein and consequently lead to cancerogenesis.

The *CCND1* protooncogene is located on chromosome 11q13 and encodes for the 45-kDa protein cyclin D1 (CycD1). CycD1 interacts with cyclin-dependent kinases 4 and 6 (CDK4/6) and is the main regulatory protein for G1-S transition. This complex catalyzes retinoblastoma (Rb) protein phosphorylation, promoting the release of E2F, which further favors cell cycle progression. The *CCND1* gene splice region variant rs9344 (G>A) is located in exon 4 at position 870 and leads to the synthesis of the truncated protein variant, cyclin D1b, which has a higher transforming activity [8].

To date, only a few studies have investigated variants in the *CDKN1A* and *CCND1* genes in OSCC, but the reported results are inconsistent. Copy number variations of the cycD1 gene were investigated in OSCC patients of the Serbian population but did not show association with OSCC progression [9]. To our knowledge, single nucleotide polymorphisms in *CDKN1A* and *CCND1* have not been studied in OSCC or other cancer patients in the Serbian population. The current study evaluated the association of gene variants rs1801270 and rs1059234 in *CDKN1A*, and rs9344 in *CCND1*, with risk for OSCC, clinicopathological features and patient prognosis.

MATERIALS AND METHODS

Ethics statement and study group

The institutional Ethics Committee of the Military Medical Academy, Belgrade, Serbia, approved the current study according to the Helsinki Declaration (1964) and subsequent amendments (Approval Number 18/2021). From all individuals included in the study informed consent was obtained, and the rights of confidentiality and privacy were protected. The study group consisted of 104 OSCC patients treated at the Clinic for Maxillofacial Surgery at the Military Medical Academy. For the control group, 107 healthy individuals without a previous cancer history were recruited. All participants in cases and control groups were Caucasian of the same population and ethnicity, matched by sex and age.

Biological samples, DNA isolation and gene variant analysis

From collected biological samples, tumor tissue from OSCC patients; peripheral blood from the patient and control groups and DNA was isolated by Qiagen QIAamp Mini Kit (Qiagen, Germany) and stored at -40°C until further use. Variants in *CDKN1A* and *CCND1* were chosen from the SNP database according to the minor allele frequency above 1. The selected gene variants within *CDKN1A* (rs1801270, rs1059234) and *CCND1* (rs9344) were genotyped using the commercial 40xTaqMan SNPs genotyping assay (C_14977_20, C_7514111_10, C_744725_1, respectively) and 2xUniversal TaqMan Master Mix (Applied Biosystems, USA), according to the manufacturer's instructions. Genotyping was performed by real-time PCR 7500 (Applied Biosystems, USA) under the following conditions: 95°C for 10 min, 40 cycles at 95°C for 15 s, at 60°C for 60 s. The obtained results of allelic discrimination were analyzed by SDS software (v.2.3).

Statistical analysis

The IBM SPSS software ver. 20.0 was used for the statistical analysis of collected data. Associations between categorical variables were analyzed by the nonparametric χ^2-test or Fisher's exact test. Studied gene variants were tested for Hardy-Weinberg (HW) equilibrium by the χ^2-test. The odds ratio (OR) with a 95% confidence interval (95% CI) was estimated by binary logistic regression. For adjusted OR calculation, adjustment for sex and age was done. Associations were considered significant when $P<0.05$. The nonparametric generalized multifactorial dimensionality reduction (GMDR) method was utilized for estimation of statistical epistatic interactions between genes and their variants [10-12]. Using GMDR software ver. 0.9, the best genes and variant interaction model was identified according to the parameters of cross validation consistency (CVC), testing balanced accuracy (TeBA),

training balanced accuracy (TrBA) and P<0.05. Overall survival (OS) was calculated from the date of diagnosis until death from any cause. Kaplan-Meier survival curves were compared using the log-rank test. Hazard ratio (HR) with 95% CI was calculated by the Cox proportional regression analysis.

RESULTS

Genotype and allele distribution of *CDKN1A* and *CCND1* gene variants

Table 1 presents the demographic characteristics, genotype and allele frequencies of the analyzed variants in *CDKN1A* and *CCND1* genes. The median age at diagnosis for OSCC patients in our cohort was 58, only 2 patients were younger than 40 years and the mean age was 64, which indicates that the data are not normally distributed. For this reason, it is commonly recommended to use the median values rather than the mean, as means are affected by extreme values. Thus, a median age of 58 was used as a cutoff point in our cohort and as a more appropriate value for data distribution. There were no differences related to sex, age, smoking status or alcohol intake between the studied groups. The genotype and allele frequency of the *CCND1* gene variant rs9344 was significantly different between OSCC patients and the control group (P=0.017, P=0.005, respectively). No differences in genotype distribution were noted for rs1801270 and rs1059234 in the *CDKN1A* gene. Gene variants were tested for HW equilibrium in the studied groups. There were no deviations from HWE (P>0.0.5), except in the case of the *CDKN1A* rs1801270 variant in the patient group (P<0.05) (Supplementary Table S1).

Table 1. Demographic features and gene variant prevalence in oral squamous cell carcinoma patients and controls.

Variables		OSCC		Controls		P*
		N	%	N	%	
Sex	Female	31	29.81	35	32.71	0.659
	Male	73	70.19	72	67.29	
Age#	< 58	54	51.92	51	47.66	0.583
	≥ 58	50	48.08	56	52.34	
Smoking	Never	28	26.92	43	40.19	0.058
	Ever	76	73.08	64	59.81	
Alcohol intake	No/Low	72	69.23	77	71.96	0.388
	High	32	30.77	30	28.04	
***CCND1* (rs9344)**	GG	20	19.23	38	35.51	**0.017**
	GA	54	51.92	50	46.73	
	AA	30	28.85	19	17.76	
	G	94	45.19	126	58.88	**0.005**
	A	114	54.81	88	41.12	
***CDKN1A* (rs1801270)**	CC	89	85.58	90	84.11	0.568
	CA	10	9.62	14	13.08	
	AA	5	4.81	3	2.80	
	C	188	90.38	194	90.65	0.925
	A	20	9.62	20	9.35	
***CDKN1A* (rs1059234)**	CC	84	80.77	90	84.11	0.804
	CT	18	17.31	15	14.02	
	TT	2	1.92	2	1.87	
	C	186	89.42	195	91.12	0.556
	T	22	10.58	19	8.88	

OSCC – oral squamous cell carcinoma patients; N – total number of patients / controls; #Age according to the median value of 58 years; *P<0.05 are presented in bold

Association between *CDKN1A* and *CCND1* gene variants and risk for OSCC occurrence

To estimate the association of the analyzed gene variants with the risk of OSCC susceptibility, binary logistic regression was performed. Since ge and sex are indicated as important confounding factors, they were incorporated into the calculation. Results obtained by the odds ratio analysis adjusted by sex and age indicated that *CCND1* rs9344 is associated with OSCC susceptibility (Table 2). Heterozygous GA, as well as the mutated AA genotype, were associated with an increased risk of OSCC when compared to the wild-type GG (OR=2.955, P=0.007; OR=2.029, P=0.037, respectively). A recessive genetic model that tests mutated AA vs combined heterozygous GA and wild-type GG showed association with increased OSCC risk (OR=2.285, P=0.010). No association was found for the dominant genetic model. Analyzed variants rs1801270 and rs1059234 in the *CDKN1A* gene were not associated with the risk of OSCC occurrence (Table 2).

CDKN1A and *CCND1* gene variant association with demographic and clinicopathological characteristics of OSCC patients

Table 3 shows the association between gene variants and the demographic and clinicopathological

Table 2. Association of analyzed *CCND1* and *CDKN1A* gene variants and oral squamous cell carcinoma risk.

Gene/gene variant	Model	Genotype	OSCC		Controls		Adjusted OR# [95% CI]	P*
			N=104	%	N=107	%		
***CCND1* rs9344**	**Genotype**	**GG**	20	19.23	38	35.51	1.000	*Reference*
		GA	54	51.92	50	46.73	2.955 [1.339-6.521]	**0.007**
		AA	30	28.85	19	17.76	2.029 [1.042-3.952]	**0.037**
	Dominant	**GG+GA vs. AA**	74	71.15	88	82.24	1.362 [0.968-3.582]	0.062
	Recessive	**GG vs. GA+AA**	84	80.77	69	64.49	2.285 [1.217-4.292]	**0.010**
***CDKN1A* rs1801270**	**Genotype**	**CC**	89	85.58	90	84.11	1.000	*Reference*
		CA	10	9.62	14	13.08	1.749 [0.403-7.589]	0.455
		AA	5	4.81	3	2.80	2.316 [0.445-12.044]	0.318
	Dominant	**CA+AA vs. CC**	15	14.42	17	15.89	1.073 [0.500-2.303]	0857
	Recessive	**AA vs. CA+CC**	99	95.19	104	97.20	0.551 [0.127-2.382]	0.425
***CDKN1A* rs1059234**	**Genotype**	**CC**	84	80.77	90	84.11	1.000	*Reference*
		CT	18	17.31	15	14.02	0.942 [0.129-6.872]	0.953
		TT	2	1.92	2	1.87	1.212 [0.151-9.713]	0.856
	Dominant	**CT + TT vs. CC**	20	19.23	17	15.89	0.794 [0.389-1.619]	0.526
	Recessive	**TT vs. CT+CC**	102	98.08	105	98.13	0.981 [0.135-7.126]	0.985

OSCC – oral squamous cell carcinoma; N – total number of patients / controls; # Adjusted for sex and age
OR [95% CI] – Odds Ratio [95% Confidence Interval]; *P<0.05 are presented in bold

characteristics of the OSCC patients. There was a statistical trend for association between the rs9344 variant in the *CCND1* gene and age according to the median (P=0.057). None of the variables were found to be associated with the analyzed variants in *CDKN1A* and *CCND1* genes (Table 3).

Epistatic interactions among analyzed gene variants in *CDKN1A* and *CCND1* genes

Variants rs1801270, rs1059234 and rs9344 in *CDKN1A* and *CCND1* were subjected to GMDR analysis. Since oral cancer is a disease with a complex etiology, an investigation into the potential existence of epistatic interactions among tested gene variants should not be neglected. None of the tested statistical models was significant (Fig. 1, Table 4.). However, as the best genetic model, the identified single tested variant was rs9344 in the *CCND1* gene.

Association of *CDKN1A* and *CCND1* gene variants with overall survival of OSCC patients

Kaplan-Meier analysis was used for the estimation of overall and disease-free survival in OSCC patients. Survival curves were compared by the log-rank test. No significant differences were observed in overall survival between OSCC patients with wild-type, heterozygous and mutated genotypes of the investigated variants rs1801270 (P=0.655), rs1059234 (P=0.816), and rs9344 (P=0.946) (Fig. 2A). No differences were noted in disease-free survival between OSCC patients with wild-type, heterozygous and mutated genotypes of rs1801270 (P=0.708), rs1059234 (P=0.092) and rs9344 (P=0.440) variants. There were no significant differences in survival among combined heterozygous and mutated genotypes vs the wild type of all analyzed variants in the *CDKN1A* and *CCND1* genes (Fig. 2B). No differences in overall survival were observed when the survival curves of mutated genotype vs the wild type and heterozygous variants were compared for all analyzed variants.

According to the results of Cox hazard ratio analysis, the independent prognostic factors in OSCC patients of alcohol intake, histological and nuclear grade, stage, tumor size, nodal status and recurrence were identified (Table 5).

DISCUSSION

Due to the rising incidence and poor prognosis of advanced OSCC, it is crucial to find novel biomarkers that reveal individual susceptibility to OSCC and to predict disease progression and prognosis. Since cell cycle control is commonly impaired in cancer cells,

Table 3. Association of *CCND1* and *CDKN1A* gene variants with demographic and clinicopathological features of OSCC patients.

Variables		N	*CCND1* rs9344			*CDKN1A* rs1801270			*CDKN1A* rs1059234		
			GG	GA	AA	CC	CA	AA	CC	CT	TT
Age (median)	<58	54	15	33	6	49	4	1	42	11	1
	≥58	50	15	21	14	40	6	4	42	7	1
	P*		0.057			0.228			0.692		
Sex	Male	73	21	38	14	62	8	3	58	14	1
	Female	31	9	16	6	27	2	2	26	4	1
	P*		0.999			0.698			0.626		
Smoking	Never	28	7	13	8	22	4	2	23	4	1
	Ever	76	23	41	12	67	6	3	61	14	1
	P*		0.340			0.467			0.686		
Alcohol intake	No/Low	72	22	34	16	61	7	4	57	13	2
	High	32	3	20	14	28	3	1	27	5	0
	P*		0.313			0.863			0.595		
Histological grade	G1/G2	83	22	44	17	71	8	4	65	17	1
	G3	21	8	10	3	18	2	1	19	1	1
	P*		0.804			0.583			0.159		
Nuclear grade	1/2	74	19	39	16	66	6	2	62	12	0
	3	30	11	15	4	23	4	3	22	6	2
	P*		0.774			0.344			0.139		
Stage	I/II	27	9	13	5	20	4	3	21	6	3
	III/IV	77	21	41	15	69	6	2	63	12	2
	P*		0.833			0.100			0.535		
Tumor size	T1/2	77	23	38	16	67	6	4	65	11	1
	T3/4	27	7	16	4	22	4	1	19	7	1
	P*		0.652			0.552			0.265		
Nodal status	N -	28	9	12	7	22	4	2	23	5	0
	N +	76	21	42	13	67	6	3	61	13	2
	P*		0.493			0.467			0.686		
Recurrences	No	43	15	21	7	36	5	2	35	8	0
	Yes	61	15	33	13	53	5	3	49	10	2
	P*		0.498			0.843			0.476		

N – total number of patients; #Age according to the median value of 58 years; *Significant values (P <0.05) are presented in bold' I/II – stage I and II combined; III/IV – stage III and IV combined; G1/G2 - well/moderate differentiated; G3 - poor differentiated

Table 4. Epistatic interactions between analyzed variants in *CCND1* (rs9344) and *CDKN1A* (rs1801270, rs1059234) genes obtained by generalized multifactorial dimensionality reduction analysis.

Model	TrBA	TeBA	CVC	Sign test (P)
rs9344	0.579	0.574	10/10	0.054
rs1801270-rs9344	0.600	0.564	7/10	0.054

TrBA – training balanced accuracy; TeBA – testing balanced accuracy; CVC – cross validation consistency

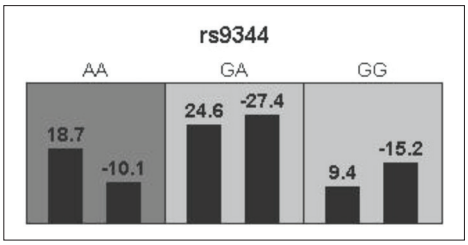

Fig. 1. *CCND1* rs9344 high-risk model for oral squamous cell carcinoma identified by generalized multifactorial dimensionality reduction analysis. The dark grey squares indicate high-risk genotypes, grey squares indicate low-risk genotypes. The figure was generated by GMDR software, v.0.9.

variants in the genes involved in regulations of cell cycle checkpoints are the most extensively studied. Gene variants are considered one of the most important factors that determine inter-individual susceptibility to cancer. Variants in the *CDKN1A* gene encoding for p21, and in the *CCND1* gene encoding for cyclin D1, are involved in apoptosis, playing roles in neoplastic transformation in various types of cancers, including head and neck carcinoma [13,14]. To the best of our knowledge, our study is the first to investigate *CDKN1A* and *CCND1* variants in OSCC patients in the Serbian population, and to provide first evidence for the association between the rs9344 variant in the *CCND1* gene and increased OSCC occurrence risk.

Our results indicate that variant rs9344 in the *CCND1* gene might be considered as a potential molecular risk factor for OSCC susceptibility, while *CDKN1A* variants rs1801270 and rs105923 were not associated with oral cancer risk. The control group was comprised of healthy individuals and was similar to the patient group in all aspects apart from cancer history. Both the cases and controls were age- and sex-matched and drawn from the same ethnic population. Case-controlled studies, which compare groups retrospectively to identify risk factors or disease biomarkers, provide valuable assessments of risk in smaller retrospective studies and data for studying rare diseases, such as oral carcinoma.

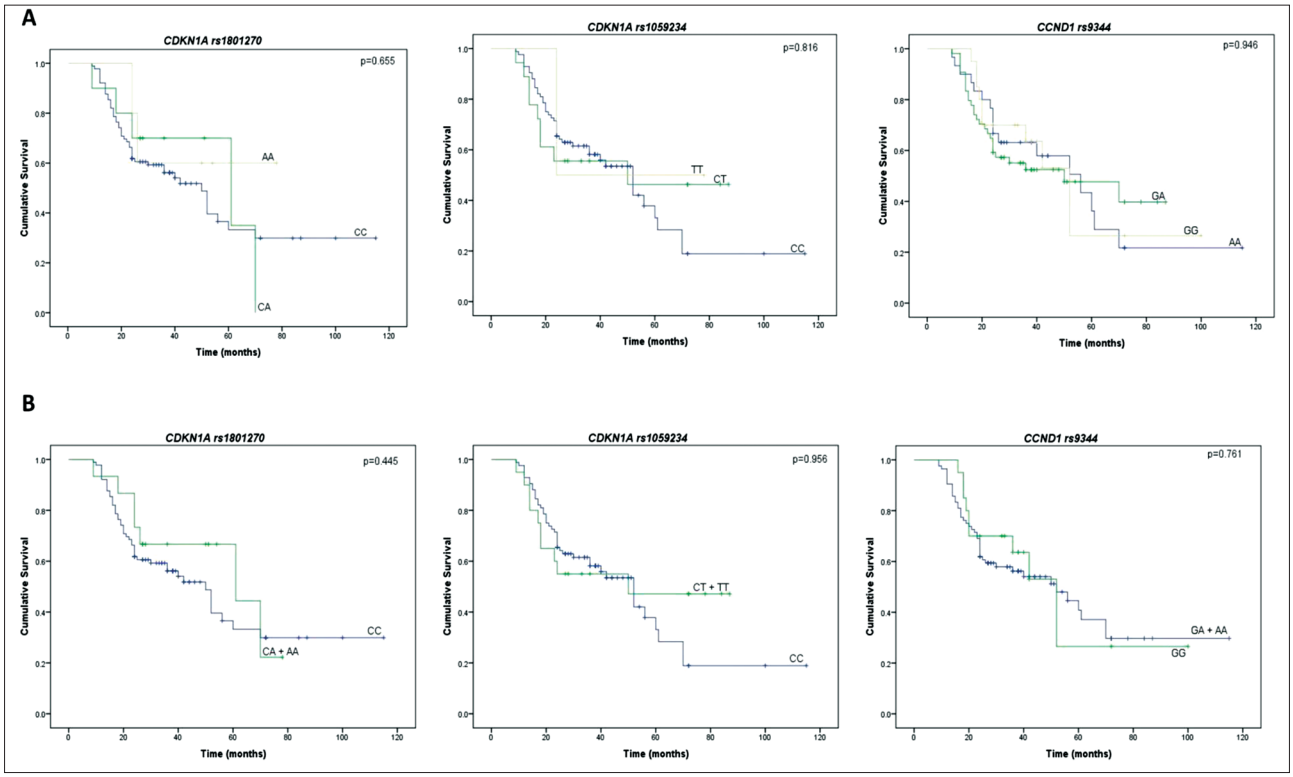

Fig. 2. Survival curves of oral squamous cell carcinoma patients according to (**A**) wild-type, heterozygous and mutated genotypes and (**B**) wild-type and combined heterozygous and mutated genotypes of *CDKN1A* rs1801270 and rs1059234 and *CCND1* rs9344 variants. P-values were calculated according to the log-rank test.

Table 5. Cox hazard risk ratio analysis of prognostic indicators in oral squamous cell carcinoma patients.

Variable	HR [95% CI]	P*
Age (median)	0.800 (0.464-1.381)	0.424
Smoking	1.589 (0.797-3.168)	0.188
Alcohol intake	2.571 (1.506-4.390)	**0.001**
Histological grade	1.370 (1.006-1.866)	**0.046**
Nuclear grade	1.465 (1.103-1.945)	**0.008**
Stage	4.980 (1.980-12.526)	**0.001**
Tumor size	2.470 (1.401-4.355)	**0.002**
Nodal status	3.674 (1.656-8.152)	**0.001**
Recurrence	6.008 (2.988-12.083)	**<0.0001**
***CCND1* rs9344**	0.971 (0.665-1.416)	0.876
***CDKN1A* rs1801270**	0.776 (0.440-1.368)	0.380
***CDKN1A* rs1059234**	0.831 (0.456-1.513)	0.544

HR [95% CI] – Hazard Ratio [95% Confidence Interval];
*P<0.05 are presented in bold

According to our results, *CCND1* rs9344 heterozygous GA and mutated AA genotypes might be risk factors for increased OSCC susceptibility. Patients with GA/AA genotypes had 2.95-/2.03-fold increased OSCC risk compared to the wild-type GG genotype. In the recessive genetic model, AA genotype was associated with a 2.3-fold increase in OSCC risk. This is in line with findings reported for the German population [15] and an earlier case-control study [16]. In addition, according to the results of meta-analysis conducted on the Indian population, rs9344 was identified as a risk factor for breast, colorectal and esophageal cancer [17]. Also, we identified the single tested variant rs9344 in the *CCND1* gene as the best genetic model according to GMDR analysis, which could partially confirm the statistical significance estimated for the same variant in our study group. The association could be attributed to the functional effect of the investigated variant. Namely, rs9344 is a splice site variant that results in an alternative splice site in mRNA, and consequently, translated cycD1 protein, also known as cycD1b, has an altered C-terminal domain and a more stable form [8,18]. Cells in which cycD1 is mutated could lose the G1 checkpoint and would not undergo cell-cycle arrest at the G1/S checkpoint transition. This is a potential explanation of how the *CCND1* rs9344 variant increases the risk of developing carcinoma in the

oral cavity. As a critical regulator of the cell cycle, the *CCND1* rs9344 variant that produces the cyclinD1b variant has an increased capacity to regulate CDK activity and serve as a potent oncogene [18,19]. Further research and functional studies are essential to evaluate the true oncogenic potential of the rs9344 variant in OSCC etiology. Apart from the rs9344 *CCND1* variant involved in oncogenic events, *CCND1* gene amplification, mutations and activation of different pathways result in cyclin D1 upregulation and overexpression in cells, promoting oncogenic action [20].

There are still inconsistent results in the literature regarding the association of rs9344 and OSCC occurrence. In different populations, opposite effects of rs9344 genotypes on OSCC risk have been reported. Thus, in the population of Taiwan, rs9344 was associated with decreased oral cancer risk [21], while in Brazilian and German populations no association was found [22,23]. Two independent meta-analyses showed an absence of association between rs9344 and oral cancer risk [23,24], while a positive association was noted in an Asian subgroup [24]. Such discrepancies might be due to a limited number of samples, differences in applied criteria for patients, population stratification, as well as ethnic differences in allele and genotype frequencies. Moreover, exposure to different environmental risk factors not included in the analysis could also yield inconsistent results.

Oral cancerogenesis is a complex process resulting from the interaction of genetic alterations and exposure to risk factors. Therefore, it is important to identify environmental factors which, together with the rs9344 risk allele, could potentially contribute to oral cancerogenesis. Even though the rs9344 genotype was not associated with smoking habits in our study group, the interaction between rs9344 and smoking in the development of oral cancer was previously demonstrated [21]. Known prognostic factors associated with OSCC, including lymph node involvement and tumor size and grade, were also found to predict poor survival of patients in our cohort; however, there were no differences in disease-free survival among OSCC patients stratified according to genotypes of the analyzed variants in *CDKN1A* and *CCND1*. Our results are consistent with the study that pointed to the absence of any association of the rs9344 variant with survival in German OSCC patients [23]. In contrast, previous studies have revealed the apparent importance of the *CCND1* rs9344 variant as a prognostic indicator in oral cancer, which was proposed as a biomarker for disease-free survival in oral cancer patients [26]. In advanced stages of head and neck carcinoma, rs9344 was associated with better survival of patients not treated with radiotherapy [27]. The observed discrepancies in survival reported in our and other studies could be attributed to the specificity of distinct types of head and neck carcinoma, but also to differences in sample size, as well as the duration of patient follow-up.

In addition to existing treatment options, which include surgical removal of the tumor, radiotherapy and chemotherapy, a novel therapeutic strategy of OSCC treatment includes the development of CDK inhibitors [28]. Therefore, information on the rs9344 genotype of patients might be of importance when planning the application of targeted therapy.

Our study did not reveal a statistically significant association between rs1801270 and rs1059234 variants and OSCC risk. A previous study has reported a nearly 1.5-fold increased risk associated with the combined heterozygous and mutated genotypes of the *CDKN1A* rs1059234 variant compared to the wild type among former and never-alcohol users, former and never-smokers in patients with oropharyngeal cancer patients [14]. In the same study, combined heterozygous and mutated genotypes of rs1801270 were associated with a 1.4-fold increase in risk when compared with the wild-type genotype among former alcohol users and smokers [14]. In addition, a meta-analysis showed that rs1059234 in the dominant genetic model (CT+TT vs CC) was associated with a 1.5-fold increased risk in head and neck carcinoma [29]. Heterozygous and mutated genotypes of *CDKN1A* rs1801270 and rs1059234 variants had an increased risk for the development of second primary malignancy in patients with head and neck carcinoma [30]. In contrast, the absence of association between *CDKN1A* gene variants and the risk of breast cancer [31], bladder cancer [32] and nasopharyngeal carcinoma [33], indicates cancer-specific association. Together, these results suggest that rs1801270 and rs1059234 *CDKN1A* variants contribute to a genetic susceptibility to cancer of the head and neck region [14]. The difference between this result and our

findings could be related to the smaller sample size of our study group, but also to genetic heterogeneity among different populations. In contrast, there was no significant association between rs1801270 genotypes and OSCC risk in a Brazilian population [22], which is in line with our findings. It is worth mentioning that in our OSCC case group, the rs1801270 variant was not in HW equilibrium, while a statistical trend was noted in the control group. Whereas HWE deviation in the case group could indicate potential association of a common allele with disease risk, HWE absence in the control group could be due to a variety of reasons [34]. Deviation from the HWE could influence estimation of the risk allele effect in genetic association studies, and it can also arise as a result of population stratification, confounding factors that are unaccounted for, etc. [34]; also, there is a possibility that other variants which are in strong linkage equilibrium with rs1801270 lead to HWE deviation.

CONCLUSIONS

The results of our study suggest that the rs9344 variant in the *CCND1* gene could be considered as a potential molecular biomarker of increased risk for oral squamous cell carcinoma. Due to its key role in cell-cycle regulation, further investigation should include more variants of the *CCND1* gene, gene-gene and gene-environmental interactions, combined with findings from transcriptomics and epigenetics analyses in order to fully elucidate the role of *CCND1* rs9344 in oral cancer etiology. Confirmation of our findings in future larger prospective studies might facilitate the early identification of individuals at risk for OSCC development and to potentially enable a personalized approach in treatment and targeted therapy of OSCC patients.

Funding: Medical Faculty of Military Medical Academy, University of Defense, Serbia [grant #MFVMA/02/20-22].

Author contributions: SJ and KZ contributed equally to this paper, and all authors made substantial contributions. GS conceived and designed the study. All authors participated in analysis of the results obtained from clinical data and interpretation of the data. SJ, KZ, GS wrote of the manuscript. All authors reviewed and edited the manuscript and approved the final version before submission.

Conflict of interest disclosure: The authors report no conflict of interest.

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Supplementary Material

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