European Review for Medical and Pharmacological Sciences 2024; 28: 2168-2178

Effects of *VDR* and *CYP24A1* gene polymorphisms on the outcome of supraglottic larynx cancer

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Abstract. – OBJECTIVE: Vitamin D has been demonstrated to play a protective role in carcinogenesis. Polymorphisms of the vitamin D receptor (*VDR*) genes and 24-α-hydroxylase (encoded by *CYP24A1*) may affect the outcome of some cancers. This study examines the effects of the *VDR* gene and *CYP24A1* single nucleotide polymorphisms on the outcome of supraglottic larynx cancer.

PATIENTS AND METHODS: Patients diagnosed with supraglottic larynx cancer between 2017 and 2022 were enrolled. Single nucleotide polymorphisms of the *VDR* gene (rs2228570, rs731236, rs7975232, rs11574113, rs11168267 and rs11168266) and *CYP24A1* gene (rs4809960, rs6022999, rs6068816, rs2259735 and rs2296241) were investigated. All patients were followed up for any evidence of local recurrence, regional recurrence, distant metastasis, and second primary tumor development. Cox regression analysis was performed to evaluate the prognostic value of single-nucleotide polymorphisms (SNPs). Kaplan-Meier method was used for survival analysis.

RESULTS: 87 patients were included. The mean follow-up time was 45.02±24.47 months. Cox regression analysis for locoregional recurrence revealed that the hazard ratio of rs731236 GG was 2.098 (95% CI, range: 1.047-4.202, p=0.037). Locoregional recurrence for rs731236 AA, AG, and GG were 38.6%, 23.1%, and 53.3%, respectively. In the presence of rs731236 GG polymorphism, disease-specific survival was significantly shorter (47.63±7.48 months, p=0.015), and disease-free survival (45.71±6.3 months) was significantly shorter (p=0.040). Rates of metastases and second primary tumors were not significantly different between SNPs.

CONCLUSIONS: This study has demonstrated the possible effects of *VDR* rs731236 SNP on the locoregional recurrence and prognosis of supraglottic larynx cancer.

Key Words:
CYP24A1, Polymorphism, Recurrence, Supraglottic larynx cancer, Survival, *VDR*.

Introduction

Laryngeal cancer remains a common type of cancer in the head and neck region despite a higher incidence of pharyngeal and oral cavity cancers in recent years[1]. Larynx cancer comprises 20% of the head and neck malignancies, and the vast majority of cases are squamous cell carcinoma[2]. Following introduction of organ preservation treatment strategies, quality of life has improved to some degree, however, the survival rates have not changed significantly[3]. More than 40% of patients have regional metastases, and about 7% have distant metastases at the time of diagnosis[4]. Larynx cancers also have a considerable rate of second primary tumor (SPT) development, which is attributed to field cancerization effect[4].

Larynx cancer should be investigated in separate subsites when performing survival analysis since cancer arising from three subsites of the larynx has unique properties[5]. Supraglottic cancers are the second most common larynx cancers and are characterized by a higher frequency of cervical metastases and advanced stage at presentation compared to glottic cancers, which result in a worse clinical behavior and a more ominous prognosis[6-8]. In recent times, there has been an exploration into the molecular characteristics of laryngeal cancers, potentially holding significance for diagnosis and prognosis.

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Diverse effects of vitamin D have been explored in addition to its traditionally known role on calcium and phosphate metabolism[9]. The active metabolite of vitamin D, $1.25(OH)_2$cholecalciferol or calcitriol, has been demonstrated to have considerable effects on carcinogenesis[10-12]. Following exposure to sunlight, vitamin D_3 is synthesized from 7-dehydrocholesterol and passes from two separate hydroxylation steps in the liver and kidney to form calcitriol. Calcitriol exerts its effects through interaction with the vitamin D receptor (*VDR*) nuclear receptor to regulate gene transcription involved in many cellular functions[12,13]. Calcitriol is then catabolized by the enzyme 24-α-hydroxylase (encoded by the *CYP24A1* gene). Although polymorphisms of the genes encoding *VDR* and the enzymes involved in metabolism were demonstrated to be associated with various cancers including breast, lung, prostate, kidney, and ovary, these polymorphisms were investigated in a relatively limited number of studies involving the head and neck region[14-17].

Patients with similar cancer features, including the stage and grade, may have different survival outcomes, which may suggest that some other factors may influence the prognosis. In this report, we aimed to investigate the prognostic role of *VDR* and *CYP24A1* gene polymorphisms in supraglottic larynx cancer. The survival rates, the incidence of local and regional failures, SPTs, and metastases were investigated.

Patients and Methods

In this retrospective study, patients diagnosed with supraglottic larynx cancer between 2017 and 2022 were included. Blood samples for whole genome sequencing were obtained. Patients with glottic and subglottic cancer were not included. Patients previously diagnosed with and treated for any cancer other than larynx cancer were excluded. Informed consent was taken from all patients. The study was approved by the Local Ethics Committee of Dokuz Eylul University (517-SBKAEK, 15th May 2020).

General demographic data of the patients were recorded, including age, gender, and smoking status. The stage of the disease was determined according to the American Joint Committee on Cancer Staging Manual, 8th edition, 2017. Margin positivity and adjuvant treatment, including radiotherapy, were noted. Those patients with positive surgical margins were treated with re-surgery to obtain tumor-free margins.

Follow-up examinations of the patients were performed every three months in the first three years and every six months thereafter. Examinations included clinical evaluation, computerized tomography, magnetic resonance imaging, and/or positron emission tomography (PET). All patients were followed up for any evidence of local recurrence (LR), regional recurrence (RR), distant metastasis, and SPT development. In case of any suspicious recurrence, histopathological confirmation and/or PET were used to delineate recurrence and distant metastasis. The follow-up time was determined from the date of the first treatment.

Genotyping for single-nucleotide polymorphisms (SNPs) for the *VDR* gene (rs2228570, rs731236, rs7975232, rs11574113, rs11168267, and rs11168266) and *CYP24A1* gene (rs4809960, rs6022999, rs6068816, rs2259735, and rs2296241) were performed. Primers were diluted and amplified, and the polymerase chain reaction (PCR) product was purified enzymatically (ExoSAP), which was then incubated and fragmented. Purification was performed using magnetic beads. A resuspension buffer was added to the DNA products, and the final solution was treated with magnetic beads. Following the complete drying of magnetic beads, a resuspension buffer was added. The supernatant was extracted, and the end-repair process was performed. After several purification steps, the final measurement was made with Qubit Fluorimetry (Thermo Scientific™, Waltham, MA, USA). An amplicon pool was produced following the union of PCR products. Gene sequencing was performed using Illumina Next Generation Sequencing (Illumina MiSeq, San Diego, CA, USA). FASTQ data is extracted from the device and evaluated with appropriate software, and variants are detected according to the reference genome.

Statistical Analysis

SPSS 28.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Mean, median, standard deviation, minimum, and maximum data were utilized for descriptive statistics. The distribution of variables was evaluated with the Kolmogorov-Smirnov test. Quantitative variables were analyzed with the Mann-Whitney U test. Qualitative variables were analyzed with the Chi-square test and Fischer extracts test if the Chi-square test was inappropriate. Kaplan-Meier

analysis and the Log Rank test were used for survival analysis. The Cox-regression test was utilized to evaluate variables' effects on mortality, recurrence, and metastasis. A *p*-value lower than 0.05 was considered statistically significant.

Results

87 patients with supraglottic larynx cancer were included. All patients but those with metastatic disease at the time of diagnosis were treated primarily by surgery. If the disease was stage III or IV, adjuvant chemoradiotherapy was applied. The type of surgery for local disease was supraglottic, supra-cricoid, or total laryngectomy, depending on the extent of the tumor. Bilateral neck dissection was performed for all patients except for those with metastatic disease at the time of diagnosis. The mean age of the patients was 60.69±10.89 years. Of the patients, 58 (66.7%) were men and 29 (33.3%) were women. The mean follow-up time was 45.02±24.47 (range: 7-94) months. 78 patients (90%) were active smokers before diagnosis. However, out of the total number of patients, 9 of them, which is 10% of the total, reported that they had never smoked before. A positive surgical margin for cancer was detected in 13 patients (15%). These 13 patients were re-operated, and resection was extended, either to supra-cricoid or total laryngectomy.

Total number of patients with LR was 22 (25.3%) and with RR was 21 (24.1%). The rate of combined locoregional recurrence (LRR) was 41.4% (36 patients). The mean time of LRR was 21.17±12.53 (range: 5-50) months. The number of patients with distant metastases was 19 (21.8%). However, 6 of them had metastatic disease at the time of diagnosis (stage IVC disease). The rate of development of metastasis during the disease was 14.9%. The rate of SPT development was 12.6% (11 patients). SPTs were oral cavity cancer in four patients, lung cancer in three patients, oropharynx cancer in two patients, pancreas cancer in one patient, and gastric cancer in one patient. Stage distribution and rates of recurrences, metastases, and SPT according to the stage are given in Table I.

The number of disease-specific deaths in this study was 40 (46%). The mean time of death was 28.25±16.36 (range: 7-78) months from the beginning of treatment. Additionally, 5 (5.7%) patients died unrelated to larynx cancer, and the causes included stroke (2 patients) and myocardial infarction (3 patients). Overall survival (OS), disease-specific survival (DSS), and disease-free survival (DFS) were 55.04±3.57 months, 60.71±3.86 months, and 53.07±4.04 months, respectively.

Table I. Rates of locoregional recurrence, metastasis and second primary tumors according to stage distribution.

	n	LRR	Metastasis	SPT
Stage I	11	2 (18.2%)	0 (0%)	1 (9%)
Stage II	11	2 (18.2%)	2 (18.2%)	1 (9%)
Stage III	26	9 (34.6)	4 (15.3%)	3 (11.5%)
Stage IV-A	29	14 (48.3)	5 (17.2%)	4 (13.7%)
Stage IV-B	4	4 (100%)	2 (50%)	1 (25%)
Stage IV-C	6	5 (83.3%)	6 (100%)	1 (16.6%)

LRR: locoregional recurrence, SPT: second primary tumor.

The distribution of *VDR* and *CYP24A1* gene polymorphisms and specific allele frequencies in this study and the general population (data extracted from the National Institutes of Health database) is given in Table II.

Age and gender distribution, smoking status, and T stage did not differ significantly among patients with and without LR ($p>0.05$). Patients with clinical neck metastasis at the time of diagnosis had a significantly higher rate of LR ($p=0.043$); similarly, patients with advanced disease (Stage III and IV) had a significantly higher rate of LR ($p=0.04$). The rate of RR was 31.8% in patients with LR, whereas it was 21.5% in patients without LR; however, the difference was not statistically significant ($p=0.33$). Rates of distant metastasis were 22.7% and 21.5% in patients with and without LR, respectively. The difference was not statistically significant ($p=0.907$). Rates of SPT development were not significantly different in patients with and without LR (13.6% *vs.* 12.3%; $p=0.871$). The rate of disease-specific death was 77.3% in patients with LR, whereas it was 35.4% in patients without LR ($p=0.01$). The rates of LR were not significantly different in *VDR* and *CYP24A1* gene polymorphisms ($p>0.05$) (Table III). Cox regression analysis did not reveal any statistically significant effect of SNPs in predicting LR-free survival ($p>0.05$) (Table IV).

Differences in age and gender distribution and smoking status between patients with and without RR were not statistically significant ($p>0.05$). Those patients with T3 disease at the time of diagnosis had a significantly higher rate of RR (28.8% *vs.* 38.1%, $p=0.012$). Patients with stage IV disease at the time of diagnosis had a

Table II. *VDR* and *CYP24A1* gene polymorphisms and allele frequencies.

SNP	Genotype	n (%)	Allele frequency (this study)	Allele frequency (population)	p
***VDR* rs2228570**	AA	19 (21.8%)	58%	33%	0.001*
	AG	33 (37.9%)			
	GG	35 (40.2%)			
***VDR* rs731236**	AA	44 (50.6%)	32%	32%	1.000
	AG	30 (34.5%)			
	GG	13 (14.9%)			
***VDR* rs7975232**	CC	21 (24.1%)	53%	45%	0.258
	CA	38 (43.7%)			
	AA	28 (32.2%)			
***VDR* rs11574113**	CC	59 (67.8%)	18%	11%	0.160
	CG	24 (27.6%)			
	GG	4 (4.6%)			
***VDR* rs11168267**	GG	57 (65.5%)	19%	9%	0.042*
	GA	26 (29.9%)			
	AA	4 (4.6%)			
***VDR* rs11168266**	CC	20 (23%)	55%	45%	0.157
	CT	36 (41.4%)			
	TT	31 (35.6%)			
***CYP24A1* rs4809960**	TT	50 (57.5%)	24%	20%	0.495
	TC	32 (36.8%)			
	CC	5 (5.7%)			
***CYP24A1* rs6022999**	AA	46 (52.9%)	28%	36%	0.225
	AG	33 (37.9%)			
	GG	8 (9.2%)			
***CYP24A1* rs6068816**	AA	66 (75.9%)	14%	10%	0.384
	AG	18 (20.7%)			
	GG	3 (3.4%)			
***CYP24A1* rs2259735**	TT	30 (34.5%)	45%	46%	0.887
	TC	34 (39.1%)			
	CC	23 (26.4%)			
***CYP24A1* rs2296241**	GG	26 (29.9%)	49%	46%	0.671
	GA	36 (41.4%)			
	AA	25 (28.7%)			

*Denotes statistical significance; SNP: single nucleotide polymorphism, VDR: vitamin D receptor.

significantly higher rate of RR (p=0.001). The metastasis and SPT development rates were not significantly different between patients with and without RR (p=0.391 and p=0.795, respectively). The rate of disease-specific death was 95.2% in patients with RR, whereas it was 30.3% in patients without RR; the difference was statistically significant (p=0.000). The rates of RR were not significantly different in *VDR* and *CYP24A1* gene polymorphisms (p>0.05) (Table III). Cox regression analysis revealed a significant effect of rs731236 in predicting RR-free survival (HR=1.892; p=0.01) (Table IV). RR rate was 40% for rs731236 GG, while it was 18.2% and 7.7% for rs731236 AA and AG, respectively.

Regarding total LRR, the recurrence rates were not significantly different between the investigated polymorphisms (p>0.05). Univariate Cox proportional hazards regression analysis revealed that the hazard ratio of rs731236 GG was 2.098 (95% CI, range: 1.047-4.202, p=0.037). LRR for rs731236 AA, AG, and GG were 38.6%, 23.1%, and 53.3%, respectively (Table III).

Age and gender distribution and smoking status were not significantly different between patients with and without metastasis (p>0.05). The rates of T and N stage of the disease at presentation were not significantly different in patients with and without metastasis (p>0.05). The rates of LR and RR were not significantly different in patients with and without metastasis (p=0.907 and p=0.391, respectively). The rate of disease-specific death was 94.7% in patients with metastasis, whereas it was 32.4% in patients without metastasis (p=0.000). The mean time of disease-specific death was 36.8±17.7 months in patients without metastasis and 24.7±17.3 months

Table III. Local and regional recurrences in *VDR* and *CYP24A1* polymorphisms.

SNP	Genotype	Total number	LR (n, %)	p	RR (n, %)	p	LRR (n, %)	p
***VDR* rs2228570**	AA	19	4 21.1%	0.631	4 21.1%	0.722	7 36.8%	0.764
	AG	33	6 18.2%		7 21.2%		10 30.3%	
	GG	35	12 34.3%		10 28.6%		19 54.3%	
***VDR* rs731236**	AA	44	10 22.7%	0.578	8 18.2%	0.189	17 38.6%	0.037*
	AG	30	2 15.4%		1 7.7%		3 23.1%	
	GG	13	10 33.3%		12 40%		16 53.3%	
***VDR* rs7975232**	CC	21	5 23.8%	0.858	4 19%	0.531	8 38.1%	0.363
	CA	38	12 31.6%		10 26.3%		18 47.4%	
	AA	28	5 17.9%		7 25.0%		10 35.7%	
***VDR* rs11574113**	CC	59	15 25.4%	0.966	14 23.7%	0.897	23 39%	0.381
	CG	24	7 29.2%		6 25%		12 50%	
	GG	4	0 0%		1 25%		1 1%	
***VDR* rs11168267**	GG	57	15 26.3%	0.761	14 24.6%	0.899	23 40.4%	0.405
	GA	26	7 26.9%		7 26.9%		13 50%	
	AA	4	0 0%		0 0%		0 0%	
***VDR* rs11168266**	CC	20	5 25%	0.973	3 15%	0.276	7 35%	0.322
	CT	36	11 30.6%		10 27.8%		17 47.2%	
	TT	31	6 19.4%		8 25.8%		12 38.7%	
***CYP24A1* rs4809960**	TT	50	13 26%	0.859	16 32%	0.066	24 48%	0.412
	TC	32	8 25%		5 15.6%		11 34.4%	
	CC	5	1 20%		0 0%		1 20%	
***CYP24A1* rs6022999**	AA	46	14 30.4%	0.242	14 30.4%	0.146	23 50%	0.404
	AG	33	8 24.2%		6 18.2%		12 36.4%	
	GG	8	0 0%		1 12.5%		1 20%	
***CYP24A1* rs6068816**	AA	66	16 24.2%	0.691	16 24.2%	0.968	27 40.9%	0.447
	AG	18	6 33.3%		5 27.8%		9 50%	
	GG	3	0 0%		0 0%		0 0%	
***CYP24A1* rs2259735**	TT	30	7 23.3%	0.761	7 23.3%	0.899	12 40%	0.923
	TC	34	10 29.4%		7 20.6%		15 44.1%	
	CC	23	5 21.7%		7 30.4%		9 39.1%	
***CYP24A1* rs2296241**	GG	26	7 26.9%	0.819	7 26.9%	0.692	11 42.3%	0.881
	GA	36	9 25%		9 25%		15 41.7%	
	AA	25	6 24%		5 20%		10 40%	

*Denotes statistical significance; SNP: single nucleotide polymorphism, LR: local recurrence, RR: regional recurrence, LRR: locoregional recurrence, VDR: vitamin D receptor.

Table IV. Cox regression analysis of single nucleotide polymorphisms for local and regional recurrences and metastasis.

SNP	Local recurrence			Regional recurrence			Metastasis		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
***VDR* rs2228570**	0.916	0.533-1.575	0.752	1.032	0.590-1.805	0.913	0.931	0.517-1.678	0.813
***VDR* rs731236**	1.430	0.900-2.273	0.130	1.892	1.165-3.072	0.010*	1.188	0.723-1.949	0.497
***VDR* rs7975232**	1.322	0.769-2.273	0.312	1.267	0.733-2.190	0.397	0.874	0.504-1.515	0.631
***VDR* rs11574113**	1.078	0.680-1.711	0.748	1.030	0.641-1.655	0.904	1.009	0.603-1.688	0.973
***VDR* rs11168267**	1.023	0.642-1.630	0.922	1.030	0.643-1.649	0.902	1.056	0.646-1.726	0.827
***VDR* rs11168266**	1.231	0.716-2.118	0.452	1.390	0.787-2.453	0.256	1.011	0.573-1.783	0.969
***CYP24A1* rs4809960**	1.013	0.649-1.580	0.955	0.694	0.411-1.174	0.173	1.310	0.822-2.088	0.257
***CYP24A1* rs6022999**	0.876	0.551-1.393	0.577	0.765	0.466-1.254	0.288	1.122	0.698-1.829	0.643
***CYP24A1* rs6068816**	1.179	0.729-1.908	0.501	1.075	0.642-1.799	0.784	1.102	0.652-1.864	0.716
***CYP24A1* rs2259735**	1.232	0.751-2.020	0.408	1.021	0.622-1.677	0.934	1.363	0.798-2.328	0.257
***CYP24A1* rs2296241**	0.957	0.580-1.579	0.864	0.980	0.588-1.634	0.939	1.226	0.710-2.118	0.465

*Denotes statistical significance; SNP: SINGLE nucleotide polymorphism, VDR: vitamin D receptor.

Table V. Metastases and second primary tumors in *VDR* and *CYP24A1* polymorphisms.

SNP	Genotype	Total number	Metastasis		*p*	SPT		*p*
			n	%		n	%	
***VDR* rs2228570**	AA	19	5	26.3%	0.593	2	10.5%	0.702
	AG	33	7	21.2%		3	9.1%	
	GG	35	7	20.0%		6	17.1%	
***VDR* rs731236**	AA	44	9	20.5%	0.752	5	11.4%	0.909
	AG	30	7	23.3%		4	13.3%	
	GG	13	3	23.1%		2	15.4%	
***VDR* rs7975232**	CC	21	6	28.6%	0.391	2	9.5%	0.783
	CA	38	7	18.4%		6	15.8%	
	AA	28	6	21.4%		3	10.7%	
***VDR* rs11574113**	CC	59	13	22%	0.949	7	11.9%	0.590
	CG	24	5	20.8%		3	12.5%	
	GG	4	1	25.0%		1	25%	
***VDR* rs11168267**	GG	57	12	21.1%	0.807	6	10.5%	0.506
	GA	26	6	23.1%		5	19.2%	
	AA	4	1	25%		0	0%	
***VDR* rs11168266**	CC	20	5	25%	0.697	2	10%	0.780
	CT	36	8	22.2%		4	11.1%	
	TT	31	6	19.4%		5	16.1%	
***CYP24A1* rs4809960**	TT	50	9	18%	0.314	8	16%	0.653
	TC	32	9	28.1%		3	9.4%	
	CC	5	1	20.0%		0	0%	
***CYP24A1* rs6022999**	AA	46	10	21.7%	0.981	6	13%	0.714
	AG	33	8	24.2%		5	15.2%	
	GG	8	1	12.5%		0	0%	
***CYP24A1* rs6068816**	AA	66	13	19.7%	0.391	10	15.2%	0.622
	AG	18	4	22.2%		1	5.6%	
	GG	3	2	66.7%		0	0%	
***CYP24A1* rs2259735**	TT	30	5	16.7%	0.397	6	20%	0.212
	TC	34	9	26.5%		2	5.9%	
	CC	23	5	21.7%		3	13%	
***CYP24A1* rs2296241**	GG	26	5	19.2%	0.701	3	11.5%	0.664
	GA	36	9	25.0%		6	16.7%	
	AA	25	5	20%		2	8%	

SNP: single nucleotide polymorphism, VDR: vitamin D receptor, SPT: second primary tumor.

in patients with metastasis; the difference was statistically significant (p=0.013). The rates of metastasis were not significantly different in *VDR* and *CYP24A1* gene polymorphisms (p>0.05) (Table V). Cox regression analysis did not reveal any statistical significance regarding SNPs in predicting metastasis-free survival (p>0.05) (Table IV).

The rates of SPT development were not significantly different in *VDR* and *CYP24A1* gene polymorphisms (Table V).

Mortality rates were not significantly different among polymorphism types (p>0.05) (Table VI). However, Cox proportional hazards regression analysis revealed that the hazard ratio of rs731236 GG polymorphism was 2.362 (95% CI, range: 1.218-4.582, p=0.011). Mortality rates for rs731236 AA, AG, and GG genotypes were 40.9%, 30.8%, and 60%, respectively.

DSS for all SNPs but rs731236 GG were not significantly different among the polymorphism types (p>0.05). In the presence of rs731236 GG polymorphism, DSS was significantly shorter (47.63±7.48 months, p=0.015) (Table VI).

DFS for all SNPs but rs731236 GG were not significantly different (p>0.05). In the presence of rs731236 GG, DFS (45.71±6.83 months) was significantly shorter (p=0.040) (Table VI).

Discussion

Vitamin D was reported to affect the risk of development and prognosis of cancer[18-20]. Vitamin D participates in the basic steps of carcinogenesis, including regulation of cell cycle, cell differentiation and apoptosis, autophagy, expression of

Table VI. Survival and mortality rates in *VDR* and *CYP24A1* polymorphisms.

SNP	Genotype	DSS (months)	*p*	DFS (months)	*p*	Mortality n, %	*p*
***VDR* rs2228570**	AA	58.24 ± 7.30	0.776	58.24 ± 6.59	0.169	8 (42.1%)	0.863
	AG	68.55 ± 6.63		62.16 ± 3.85		14 (42.4%)	
	GG	52.79 ± 4.64		45.76 ± 5.30		18 (51.4%)	
***VDR* rs731236**	AA	61.66 ± 3.87	0.015*	58.72 ± 4.51	0.040*	18 (40.9%)	0.011*
	AG	63.16 ± 8.39		60.76 ± 8.02		4 (30.8%)	
	GG	47.63 ± 7.48		45.71 ± 6.83		18 (60%)	
***VDR* rs7975232**	CC	57.47 ± 6.62	0.818	54.84 ± 5.78	0.551	11 (52.4%)	0.960
	CA	59.44 ± 5.93		55.86 ± 6.42		18 (47.4%)	
	AA	58.08 ± 5.68		56.82 ± 6.15		11 (39.3%)	
***VDR* rs11574113**	CC	62.83 ± 5.04	0.756	59.12 ± 4.61	0.601	29 (49.2%)	0.487
	CG	55.21 ± 5.14		44.98 ± 5,45		9 (37.5%)	
	GG	58.67 ± 15.79		49.00 ± 14.65		2 (50%)	
***VDR* rs11168267**	GG	61.39 ± 5.18	0.598	59.53 ± 4.83	0.194	27 (47.4%)	0.958
	GA	55.15 ± 5.45		46.64 ± 5.90		12 (46.2%)	
	AA	67.75 ± 8.88		61.00 ± 17.61		1 (25%)	
***VDR* rs11168266**	CC	58.94 ± 6.77	0.890	56.98 ± 6.08	0.597	9 (45%)	0.653
	CT	58.39 ± 5.93		56.66 ± 6.52		18 (50%)	
	TT	55.71 ± 5.52		54.55 ± 5.94		13 (41.9%)	
***CYP24A1* rs4809960**	TT	54.92 ± 4.17	0.529	51.18 ± 4.58	0.465	24 (48%)	0.869
	TC	57.89 ± 5.91		54.08 ± 5.41		15 (46.9%)	
	CC	80.00 ± 12.52		77.80 ± 14.49		1 (20%)	
***CYP24A1* rs6022999**	AA	53.31 ± 4.46	0.414	50.17 ± 4.86	0.155	24 (52.2%)	0.725
	AG	51.33 ± 4.43		51.07 ± 4.95		14 (42.4%)	
	GG	84.17 ± 8.98		77.23 ± 10.21		2 (25%)	
***CYP24A1* rs6068816**	AA	63.43 ± 4.36	0.569	61,25 ± 4.72	0.376	28 (42.4%)	0.360
	AG	49.93 ± 7.16		45.83 ± 7.64		10 (55.6%)	
	GG	43.67 ± 33.50		43.67 ± 15.79		2 (66.7%)	
***CYP24A1* rs2259735**	TT	60.85 ± 5.02	0.429	56.89 ± 5.71	0.742	12 (40%)	0.203
	TC	57.03 ± 6.95		55.28 ± 6.33		18 (52.9%)	
	CC	55.47 ± 6.47		55.44 ± 5.89		10 (43.5%)	
***CYP24A1* rs2296241**	GG	55.79 ± 5.83	0.949	53.17 ± 6.57	0.960	12 (46.2%)	0.825
	GA	54.85 ± 5.49		54.19 ± 5.23		17 (47.2%)	
	AA	62.61 ± 6.81		62.11 ± 7.62		11 (44%)	

*Denotes statistical significance; SNP: single nucleotide polymorphism, VDR: vitamin D receptor, DSS: disease specific survival, DFS: disease free survival.

adhesion molecules, angiogenesis, and inflammation[21,22]. Inducing a cell cycle arrest in head and neck cancer (HNC) lines through regulating the expression of *p21* and *p27* was also proposed[23]. Pu et al[24] reported that elevated vitamin D activity from dietary intake, genomic polymorphisms, and serum levels of 25(OH)D3 might protect against HNC and improve prognosis.

Vitamin D exerts its biological actions *via* its intracellular receptor, the *VDR*. Following ligand binding to *VDR*, it interacts with vitamin D response elements of the target genes and affects the expression level of these genes[25]. *VDR* gene is located on the long arm of chromosome 12 and has more than 200 SNPs[25]. rs2228570, rs731236, and rs7975232 were among the most widely studied polymorphisms associated with VDR in previous reports. rs2228570, located in the transcriptional initiation site of the *VDR* gene, results in the formation of a protein isoform leading to a change in post-transcriptional modification, and rs731236 polymorphism, located near the 3′ ends of the *VDR* gene, affects the level of the protein by regulating the stability of the *VDR* mRNA without changing the amino acid sequence of the encoded protein[17,26,27].

This analysis found no relation between rs2228570 and rs7975232 with supraglottic cancer outcomes. However, rs731236 was demonstrated to have some remarkable features. Although rates of LR and RR for rs731236 were not significantly different compared to other SNPs, Cox regression analysis resulted in a significant effect in predicting RR. RR rate was 40% in patients with rs731236 GG genotype (18.2% in AA and 7.7% in AG genotypes). From the perspective

of total LRR, although the recurrence rates were not significantly different between the SNPs, Cox regression analysis similarly resulted in a significant effect of rs731236 in predicting LRR. LRR rate was 53.3% in patients with rs731236 genotype (38.6% in AA and 23.1% in AG genotypes). The mortality rate was significantly higher in patients with rs731236 GG compared to rs731236 AA and AG genotypes. DFS and DSS were significantly shorter in patients with rs731236 GG compared to rs731236 AA and AG genotypes.

rs731236 was previously reported to be associated with a higher risk of progression and risk of death in non-small cell carcinoma of the lung[28]. No association was found between rs731236 and prostate cancer prognosis by Holt et al[29]. Yousaf et al[30] reported no significant association between rs731236 and prostate cancer, except for the heterozygote form, which might be associated with some degree of protective role. Mishra et al[31] revealed no association between rs731236 and the outcome of breast cancer. However, Perna et al[32] stated that rs731236 homozygote polymorphism tended to a breast cancer-specific mortality. Liu et al[33] demonstrated that homozygous variants of rs731236 and rs2228570 might reduce the risk of HNC. The study included cancers of the oral cavity, oropharynx, hypopharynx, and larynx. Hama et al[16] stated that rs731236 had no association with the prognosis of HNC. The authors studied some well-known polymorphisms of the *VDR* and concluded that only rs2228570 had correlated with poor prognosis. This well-designed study, however, included cancers of the oropharynx, hypopharynx, oral cavity, and nasal cavity, as well as larynx cancers.

An association between rs2238135 and an increased risk of oral cavity cancer (OCC) was reported by Małodobra-Mazur et al[34]. Bektas-Kayhan et al[35] reported a higher risk of OCC in the presence of rs731236 polymorphism, especially in female patients. Zeljic et al[36] stated that rs2228570 polymorphism had been associated with worse survival in OCC and could be considered an independent prognostic factor[36]. The authors found no association between rs731236 and OCC. Beysel et al[37] presented the effects of *VDR* single nucleotide polymorphisms (SNPs) in papillary thyroid cancer (PTC), and stated that rs731236, rs7975232, and rs1544410 were not associated with PTC risk in contrast to rs2228570, which was related to increased risk and poor prognosis for PTC. rs2228570 did not affect recurrence and prognosis in our analysis. Maciejewski et al[38] stated that no association was found between *VDR* SNPs and susceptibility to differentiated thyroid cancer. Cocolos et al[39] reported that rs731236 and rs2228570 SNPs were more frequent in patients with thyroid cancer. The authors also stated that local invasion, multifocality, and risk of cervical metastases were higher in rs2228570. However, no relation was reported between rs731236 and disease prognosis. Azad et al[15] investigated the effects of polymorphisms of the *VDR* and other genes involved in vitamin D metabolism. They presented no significant association between *VDR* SNPs and outcomes in HNC patients.

CYP24A1 gene is located on the long arm of chromosome 20, and increased expression of *CYP24A1* was reported in cancers of the breast, ovary, lung, and colon and associated with poorer prognosis[40-42]. Zhang et al[43] demonstrated that rs4809960 and rs6022999 SNPs of the *CYP24A1* gene were associated with cancer development in different ethnic populations. Our analysis found no association between *CYP24A1* polymorphisms with LRR, metastasis, and survival of supraglottic larynx cancer. The relationship between *CYP24A1* SNPs and HNC has been very rarely studied. Azad et al[15] investigated the effects of polymorphisms in vitamin D metabolism genes on the outcome of HNC and reported that *CYP24A1* rs2296241 polymorphism was associated with poor overall survival. Zeljic et al[36] stated that *CYP24A1* gene polymorphism might influence the susceptibility to OCC.

Conclusions

Survival rates of cancers with the same stage and similar treatment modalities may differ among individuals. This reality directs investigators to search for new prognostic factors that have the potential to affect survival[28]. SNPs can be considered as prognostic factors, and a variety of polymorphisms were investigated from the perspective of risk and recurrence/prognosis of many cancers. SNPs associated with vitamin D were rarely examined for HNCs compared to other more common cancers. In this report, we aimed to investigate the role of *VDR* and *CYP24A1* SNPs, specifically in supraglottic larynx cancers. Cancer arising from different compartments of the larynx has unique features that separate investigations in terms of etiology, symptomatology, and prognosis should be performed

for cancers arising from each compartment. We explored the possible effects of *VDR* rs731236 SNP on the LRR and prognosis of supraglottic larynx cancer, and no effect on the development of metastasis and SPT.

Conflict of Interest
The authors declare they have no conflict of interest to disclose.

Ethics Approval
All subjects provided written informed consent for inclusion before participating in the study. This study was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013), and the protocol was reviewed and approved by the Ethics Committee Dokuz Eylul University (517-SBKAEK, 15th May 2020).

Informed Consent
All subjects provided written informed consent for inclusion before they participated in the study.

Acknowledgments
The authors appreciate to every member of Dokuz Eylul University, Institute of Health Sciences, Department of Translational Oncology for their moral support.

Funding
This work was supported by the Dokuz Eylul University Scientific Research Fund (2021.KB.SAG.046).

Authors' Contributions
Deniz Tuna Edizer: Conception and design of the study, acquisition of data, or analysis and interpretation of data, drafting the article or making critical revisions related to the relevant intellectual content of the manuscript, supervision, validation and final approval of the version of the article.
Asim Leblebici: Acquisition of data, or analysis and interpretation of data, drafting the article, validation and final approval of the version of the article.
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Data Availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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