CYP2E1 and ALDH2 Gene Polymorphisms in Squamous Cell Head and Neck Cancer in the Turkish Population

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ABSTRACT

Alcohol consumption and smoking habits are risk-enhancing factors for head and neck squamous cell carcinoma (HNSCC). The CYP450 2E1 (CYP2E1) and acetaldehyde dehydrogenase 2 (ALDH2) enzymes play an important role in the metabolism of these carcinogens. Various polymorphisms have been identified on genes that expresses these enzymes. The aim of this study was to investigate the associations between the ALDH2 and CYP2E1 polymorphisms and HNSCC susceptibility in the Turkish population. The genomic DNA was isolated from the peripheral blood leukocytes of 79 HNSCC patients and 98 healthy controls. ALDH2 genotyping was performed with the multiplex polymerase chain reaction (PCR) technique. CYP2E1-DraI, CYP2E1-RsaI and CYP2E1-PstI genetic polymorphisms were determined via the PCR restriction fragment length polymorphism (PCR-RFLP) technique. The results of our study demonstrated that CYP2E1-RsaI heterozygous (c1/c2) genotyped individuals have an increased risk for HNSCC susceptibility (OR= 7.79; 95% CI, 0.92-179.64; p= 0.04). No other relationship was found between the other studied polymorphisms and HNSCC. The results are consistent with previous studies, which examined the ALDH2 and CYP2E1 polymorphisms in Caucasian subjects. However, there are still conflicting results in Caucasian races for CYP2E1 RsaI/PstI polymorphisms. Consequently, the c1/c2 genotypes for RsaI polymorphisms may be a risk factor for HNSCC. Larger study populations and multi-centre studies are needed to more precisely demonstrate the susceptibility in the Turkish population.

Keywords: ALDH2, CYP2E1, Head and neck cancer, Polymorphism

ÖZET

Türk Populasyonunda CYP2E1 ve ALDH2 Polimorfizmleri ile Skuamoz Hücreli Baş ve Boyun Kanseri İlişkisi

Alkol ve tütün kullanma alışkanlıkları skuamoz hücreli baş ve boyun kanseri (SHBBK) için risk arttırıcı faktörlerdir. CYP450 2E1 (CYP2E1) ve asetaldehid dehidrojenaz 2 (ALDH2) enzimleri bu karsinojenlerin metabolizmasında önemli rol oynamaktadırlar. Bu enzimleri eksprese eden genlerde çeşitli polimorfizmler tanımlanmıştır. Çalışmamızın amacı Türk populasyonunda ALDH2 ve CYP2E1 gen polimorzfimleri ile SHBBK yatkınlığı arasındaki ilişkinin aydınlatılmasıdır. 79 SHBBK hastası ve 98 sağlıklı gönüllüden alınan periferal venöz kanların lökositlerinden genomik DNA'lar izole edildi. ALDH2 polimorfizmi multiplex PCR (polimeraz zincir reaksiyonu) yöntemi ile; CYP2E1-DraI, CYP2E1-RsaI ve CYP2E1-PstI genetik polimorfizmleri ise PCR-RFLP (kesim parçası uzunluğu polimorfizmi) yöntemi kullanılarak araştırıldı. Çalışmamızda CYP2E1-RsaI heterozigot (c1/c2) bireylerde anlamlı derecede yüksek SHBBK yatkınlığı bulunduğu belirlendi (OR= 7.79; 95% CI, 0.92–179.64; p= 0.04). İncelenen diğer polimorfizmler ile SHBBK yatkınlığı arasında ilişki saptanmadı. Çalışmanın sonuçları Türk populasyonunun da içinde yer aldığı Kafkas ırkı bireylerle ALDH2 ve CYP2E1 polimorfizmlerinin değerlendirildiği diğer çalışmalarla uyumludur. Diğer taraftan Kafkas populasyonlarında CYP2E1 RsaI/PstI polimorfimleri konusunda hala tartışmalı sonuçlar bulunmaktadır. Türk populasyonunda CYP2E1-RsaI heterozigot (c1/c2) genotipine sahip olmak SHBBK için artmış bir risk faktörü olabilir. Türk populasyonundaki yatkınlığın kesin olarak ortaya konulabilmesi için daha geniş katılımlı ve çok merkezli çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: ALDH2, CYP2E1, Baş ve boyun kanseri, Polimorfizm

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INTRODUCTION

Cancer is one of the leading causes of death and is a major public health problem. Amongst the many cancer types, head and neck cancer is the fourth most common malignancy. Annually, it is estimated that 650.000 new cases are diagnosed and 350.000 deaths occur from head and neck cancers worldwide. More than 90% of head and neck cancers are squamous cell tumours.[1-3]

The carcinogenesis of head and neck squamous cell cancer (HNSCC) is a complex process that results from the dynamic interaction of various factors; yet, 80% of cases have exposure history to well-known carcinogens, such as tobacco and alcohol.[2] Alcohol is also a major factor that increases the influence of tobacco and other carcinogens. The other important aetiological factors are increased age, diet, viruses (e.g., Ebstein-Barr virus and human papilloma virus), genetic predisposition, occupational exposure, air pollution, and radiation exposure.[4]

Alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1), and acetaldehyde dehydrogenase 2 (ALDH2) are the principle enzymes involved in alcohol metabolism. Alcohol is predominantly metabolised by ADH, whilst its metabolism via other pathways ranges from 10-30%. ADH and CYP2E1 convert alcohol to acetaldehyde, which is a highly toxic and carcinogenic substance. Subsequently, ALDH2 metabolises acetaldehyde to acetate. Acetate is a less active compound and is broken down into H_2O and CO_2 for easy elimination.[5,6]

Several gene polymorphisms have been identified for ADH, ALDH2, and CYP2E1. In the 1510 position of ALDH2, guanine and adenine nucleotide replacement causes a single nucleotide polymorphism (Glu487Lys). Whilst the 487Glu allele (ALDH2*1) shows full enzyme activity, the 487Lys allele (ALDH2*2), does not show enzymatic activity. As a result, in polymorphic individuals, acetaldehyde accumulates in the tissues and shows toxic effects. At the same time, the carcinogenicity of acetaldehyde has been shown in several experimental animal studies. Exposure to elevated acetaldehyde concentrations is an increased risk factor for HNSCC.[7]

CYP2E1 is a phase I enzyme and located most abundantly in the liver. Moreover, the presence of the enzyme has been shown in several other tissues, including the lungs, bronchial tissue, the kidneys, the small intestine, the colon, nasal mucosa, placenta and the lymphocytes.[8] CYP2E1 plays an important role in the biotransformation of industrial chemicals, tobacco smoke, and alcohol. In the 5' flanking region of wild-type alleles, the 1019 position of CYP2E1 contains the cytosine nucleotide. However, in the CYP2E1*5B RsaI (rs2031920) polymorphism, thymine takes place in the same position, instead of cytosine, in the mutant allele.

In the 5' flanking region of wild-type alleles, the 1259 position of CYP2E1 contains the guanine nucleotide. On the other hand, in CYP2E1*5B PstI (rs3813867) polymorphic individuals, cytosine, instead of guanine, is found in the same position. It is reported that, whilst the homozygous wild-type c1/c1 genotyped individuals has normal enzyme activity, heterozygous c1/c2 and homozygous mutant c2/c2 genotyped CYP2E1 RsaI/PstI polymorphic individuals show decreased enzyme activities. In cases when high amounts of CYP2E1 substrate are consumed by polymorphic individuals, these substances are inevitably slowly metabolised. Consequently, DNA damage may occur due to the accumulated toxic compounds in the tissues.[9,10]

CYP2E1*6 DraI (rs6413432) polymorphic individuals have the adenine nucleotide, instead of thymine, in the intron 6 region 7678 position. The DraI polymorphism results in three different genotypes, known as DD for the wild-type, DC for heterozygous individuals and CC for the homozygous mutant type. It has been reported that CC genotyped homozygous mutant individuals have increased enzyme activity. As a result, polymorphic CYP2E1 metabolises the substrate to mutagens and procarcinogens more quickly, leading to an increased HNSCC risk due to the more active intermediates, which occur in higher amounts in mutant genotypes during normal phase I reactions compared with the wild-types.[11,12]

The CYP2E1 and ALDH2 genes show genetic polymorphisms that vary markedly in frequency

amongst different ethnic and racial groups. These gene polymorphisms have been associated with increased genetic susceptibility to HNSCC.[10-12] The aim of this study was to investigate the associations between the ALDH2 and CYP2E1 polymorphisms and HNSCC susceptibility in the Turkish population.

MATERIALS AND METHODS

This case-control study was carried out according to the principles of the Declaration of Helsinki and was approved by the Non-Invasive Clinical Research Ethics Committee of the Pamukkale University. Cases were consisted patients with HNSCC diagnosed at University hospital's Ear, Nose and Throat Clinic. Subjects older than 18 years of age who had pathologically confirmed squamous cell carcinoma of the oral cavity, tongue, lip, maxilla and larynx, without a history of previous malignancy or a diagnosis of a genetic disease or syndrome, were considered eligible for the study.

Subjects

Seventy nine patients (previously diagnosed, alive at the time of initial contact, 75 males and 4 females; age range between 28-81 years old) were successfully interviewed and included as eligible cases. The localizations of primary tumor sites were as follows: larynx (73), oral cavity and tongue (3), lip (2) and maxilla (1).

The control group was consisted of ninety eight Turkish healthy volunteer individuals (94 males and 4 females; age range between 29-78 years old). They have similar epidemiological characteristics with the case group. None of them had a history of any types of cancer and alcohol or tobacco related diseases. Neither control group nor case group members had a history of kidney or liver diseases.

Case and control groups were individually informed about the study with the face-to-face interviews in the hospital clinic and written informed consent were taken from all participants. The experienced interviewer's questions related to lifetime alcohol and tobacco consumption, medical history, familial history of cancer, demographics and diet. Data were recorded to the information sheets.

Genomic DNA Isolation

2 millilitres (ml) of venous blood samples were taken from the subjects. The samples were placed into tubes containing 1ml of 2% Ethylene diamine tetra acetic acid (EDTA) and stored at –20°C until DNA isolation. The ALDH2 (rs671), CYP2E1-DraI (rs6413432), CYP2E1-RsaI (rs2031920), and CYP2E1-PstI (rs3813867) gene polymorphisms were analysed using the polymerase chain reaction (PCR) and gel electrophoresis methods.

1ml of venous blood was taken from each sample, and the leukocyte DNA was isolated using a method based on DNA extraction with phenol-chloroform and ethanol precipitation, as described by Lewin and Stewart-Haynes.[13] The purity of the DNA samples was analysed with the gel electrophoresis and spectrophotometric methods. A Cleaver® horizontal gel electrophoresis tray connected to a Wealtec® Elite 300 Plus model power supply container was used for the gel electrophoresis processes. In the study, all gel images were obtained with a computer-assisted Intas® gel imaging system under UV light. The absorbance values were measured with an Eppendorf® spectrophotometer to determine the concentration and purity of the DNA samples.

PCR Analysis

The deoxiribonücleotide triphosphate (dNTP), thermus aquaticus (Taq) DNA polymerase, primers, and restriction endonucleases that were used in the study were purchased from New England Biolabs GmbH (Frankfurt, Germany). A Peqlab® Primus 25 model thermocycler was used for the PCR processes. Each PCR product was mixed with 1μl loading buffer and loaded into the wells of the gel. Each electrophoresis process throughout the study was conducted for 30 minutes at 90 volts. General protocols followed in the replication programmes are presented in Table 1. The amounts of components used in the PCR programmes are presented in Table 2.

Table 1. General protocols followed in PCR programmes*

	CYP2E1 DraI	CYP2E1 RsaI/PstI	ALDH2
Denaturation	94°C - 3 min	94°C -1 min	95°C -3 min
Annealing	61°C - 1 min	55°C -1 min	55°C -0,5 min
Extension	72°C - 1 min	72°C -1 min	72°C -1 min
Final Extension	72°C - 1 min	72°C -1 min	63°C -1 min
Cycle	35 cycle	35 cycle	35 cycle

10 μL of all CYP2E1 amplified genomic fragments were subjected to reaction with their specific restriction endonucleases (5U) at 37°C for 24 hours. Digestion products (10 μl) were electrophoresed and then analysed by gel imaging instrument.

RsaI restriction endonuclease cuts the amplified genomic fragment from the recognition site in wild-type alleles. The length of the fragment was 412 base pair (bp), and those of the digested products were 351 bp and 61 bp. The subjects that had a 412 bp fragment length after the reaction with RsaI were considered mutant alleles.

PstI restriction endonuclease cuts the 400 bp amplified genomic fragment from the recognition site in mutant alleles. As a result, two separate parts occur as 300 bp and 100 bp, respectively. The subjects that had a 400 bp fragment length after the reaction with RsaI were considered wild-type alleles.

The DraI restriction endonuclease enzyme has two recognition sites in wild-type alleles. The length of the amplified genomic fragment was 1000 bp, and those of the digested products were 100 bp, 300 bp, and 600 bp. On the other hand, mutant alleles have only one recognition site for DraI. Therefore, DraI separates the genomic fragment into two parts at 900 bp and 100 bp in mutant alleles.

ALDH2 (rs671) genotyping was performed with a modified PCR technique called multiplex PCR using the method of Tamakoshi et al.[14] This technique allows for the detection of the presence of polymorphisms without using restriction enzymes. Two forward and two reverse primers (F1: 5'-TGCTATGAT GTGTTTGGAGCC-3', F2: 5'-GGGCTGCAGGCATACACTA-3', R1:5'-CCCACACTCACAGTTTTCACTTC-3', R2:5'-GGCTCCGAGCCACCA-3') were used. These primer pairs allowed for the replication of adjacent regions.

Statistical Analysis

The results were analyzed with SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Chi-square (χ^2) and Fisher's exact tests were used for the statistical comparison of the allele frequencies between cases

Table 2. Amount of the PCR mixture components in PCR programmes

Compound	CYP2E1-PstI	CYP2E1-RsaI	CYP2E1-DraI	ALDH2
Template DNA	~4 ng/ µl	~4 ng/ µl	~4 ng/ µl	~8 ng/ µl
PCR buffer	1x	1x	1x	1x
MgCl2	1.5 mM	1.5 mM	1.5 mM	2 mM
dNTP	200 µM	200 µM	200 µM	200 µM
Forward primer(s)*	20 pmol	20 pmol	20 pmol	40 pmol
Reverse primer(s)*	20 pmol	20 pmol	20 pmol	40 pmol
Taq DNA Polimerase	5 U	5 U	5 U	5 U
Sterile water	Add to 50 µl			

*Two forward and two reverse primers were used for detecting ALDH2 polymorphism by multiplex PCR. ng: nanogram, µl: microliter, mM: millimolar, µM: micromolar, pmol: picomole, U: unit

Table 3. Genotypes and alleles distribution between case and control groups and comparative values in CYP2E1-RsaI (rs2031920) polymorphism

Genotypes	Case Group (n= 79)			Control Group (n= 98)					
	n	%	95% CI	n	%	95% CI	OR	95% CI	p
c1/c1	71	89.9	85.2 -94.5	95	96.9	94.4-99.3	0.28	0.05- 1.21	0.06
c1/c2	6	7.6	3.4-11.7	1	1.0	0.0- 2.3	7.97	0.92- 179.64	0.04
c2/c2	2	2.5	0.0- 4.9	2	2.1	0.0- 4.1	1.24	0.12- 12.72	1.00
Alleles									
c1	148	93.7	91.0- 96.3	191	97.5	95.9- 99.0	0.38	0.11- 1.26	0.11
c2	10	6.3	3.6- 8.9	5	2.5	0.9- 4.0	2.58	0.79- 8.88	0.11

and control groups. Hardy-Weinberg equation was used from the allele frequencies to calculate the expected frequencies of the genotypes. The expected and observed frequencies were compared with the χ^2 test. Odds ratios (OR) were calculated using logistic regression model and 95% confidence interval (95% CI) were used to estimate the risk for HNSCC. In order to evaluate the differences in terms of ages, tobacco and alcohol comsumptions between the case and control groups, Student's t-test was used. $p< 0.05$ was accepted as statistically significant.

RESULTS

The genotype and allele distributions between the case and control groups in the CYP2E1-RsaI (rs2031920) polymorphism are presented in Table 3. According to the results, heterozygous (c1/c2) individuals in the case group were found to be statistically significantly higher than the control group in the CYP2E1-RsaI (rs2031920) polymorphism ($p= 0.04$).

The genotypes and allele frequencies of the CYP2E1-PstI (rs3813867) and CYP2E1-DraI (rs6413432) polymorphisms showed no statistical difference between the two groups. The genotype and allele distributions of the case and control groups in the CYP2E1-PstI (rs3813867) and CYP2E1-DraI (rs6413432) polymorphisms are presented in Table 4 and Table 5, respectively.

In this study, all subjects in the case and control groups (100%) were determined as being homozygous wild genotypes for the ALDH2 (rs671) polymorphism. Neither heterozygous nor homozygous mutant genotypes were found in either the case or control group.

There was no statistical difference between the case and control groups in terms of age, gender, diet, alcohol consumption, or smoking ($p> 0.05$). However, alcohol consumption was found to be

Table 4. Genotype and allele distribution between case and control groups in CYP2E1- PstI (rs3813867) polymorphism

Genotypes	Case Group (n= 79)			Control Group (n= 98)					
	n	%	95% CI	n	%	95% CI	OR	95% CI	p
c1/c1	72	91.1	86.6-95.5	85	86.7	81.9-91.4	1.57	0.54-4.63	0.47
c1/c2	6	7.6	3.4-11.7	10	10.2	5.9-14.4	0.72	0.22-2.13	0.60
c2/c2	1	1.3	0-3.0	3	3.1	0.6-5.5	0.37	0.01-4.17	0.62
Alleles									
c1	150	94.9	92.4-97.3	180	91.8	89.0-94.5	1.66	0.65-4.38	0.29
c2	8	5.1	2.6-7.5	16	8.2	5.4-10.9	0.60	0.22-1.53	0.29

Table 5. Genotypes and alleles distribution between case-control groups and comparative values in T-7678A (DraI) polymorphism

Genotypes	Case Group (n= 79)			Control Group (n= 98)					
	n	%	95% CI	n	%	95% CI	OR	95% CI	p
DD	70	88.6	83.6- 93.5	89	90.8	86.7-94.8	0.78	0.26-2.30	0.62
DC	8	10.1	5.4- 14.7	9	9.2	5.1-13.2	1.11	0.36-3.35	1.00
CC	1	1.3	0-3.0	-	-	-	-	-	-
Alleles									
D	148	93.7	91.0-96.3	187	95.4	93.3-97.4	0.71	0.25-1.95	0.48
C	10	6.3	3.6-8.9	9	4.6	2.5-6.6	1.40	0.51-3.87	0.48

higher in the case group compared to the control group (cases: 21.1 drinks/week, controls: 15.0 drinks/week). There was no statistical difference between the groups according to allele and genotype frequency when we divided the case group into subgroups in terms of alcohol consumption. The data regarding the characteristics of the case and control groups are presented in Table 6.

DISCUSSION

Our study is the first case-control study that evaluated both the allele and genotype distributions of the CYP2E1 and ALDH2 polymorphisms for HNSCC risk in the Turkish population. The results of the study demonstrated that CYP2E1-RsaI heterozygous (c1/c2) genotyped individuals have an increased risk of HNSCC susceptibility (OR= 7.79; 95% CI, 0.92–179.64; p= 0.04). No other relationship was found between the other studied polymorphisms and the risk of HNSCC. Although various important data were obtained in the study, it was conducted in only one region in Turkey, with a small number of case and control subjects. Therefore, the results of the study cannot be generalised for the entire Turkish population. These were the limitations of our study.

It has been reported in several case-control studies conducted in Caucasian populations regarding CYP2E1-RsaI (rs2031920) polymorphisms that no significant difference was found between allele and genotype frequencies and susceptibility to HNSCC.[15-22] However, only one study reported a correlation between the frequency of c2 mutant alleles and HNSCC risk in Caucasian populations.[23] On the other hand, the CYP2E1-RsaI (rs2031920) polymorphism has been determined as a risk factor for HNSCC in Asian populations.[9,24] Additionally, a meta-analysis study reported no significant risk in Caucasian populations, but an important risk of HNSCC is present in c2 homozygous individuals in Asian populations.[11] These different results may be attributed to ethnic differences, since the distributions of the less common c2 allele were different between various races, with a prevalence of ~25-50% and 5-10% amongst Asians and

Table 6. The characteristics of case and control groups

Characteristic	Case group (n= 79)	Control group (n= 98)
Gender (male / female)	75/4	94/4
Age (years)	61.1 (10.1*)	58.40 (11,4*)
Smoking (pack / year)	40.17 (39.8*)	56.8 (31.4*)
Alcohol consumption (drink**/ week)	21.1 (19.2*)	15.0 (4.0*)
Dietary history	Do not show any properties	Do not show properties

* Standard deviation values. ** Clinical; the amount of alcohol drunk is expressed with the 'drink' unit. A 'drink' is the amount of alcohol that equivalent to 13 grams pure ethanol (about 16 ml)

Caucasians, respectively.[25] The c2 variant allele frequency did reach a statistically significant level amongst Asians, whilst in the Caucasian populations, the lack of significant association might be explained by the substantially lower statistical power caused by the lower prevalence of the CYP2E1 c2 allele (5-10%, compared to 25-50% for Asians).[11]

The results of this study are consistent with the case-control studies conducted within Caucasian populations regarding the CYP2E1-PstI (rs3813867) polymorphism.[15,16,19,26] These studies indicated no significant difference in HNSCC susceptibility in terms of allele and genotype frequencies for the CYP2E1-PstI polymorphism in Caucasian populations. This polymorphism is common in East Asian people, but it is rarely seen in Caucasians.[11,26]

The results of our study are also consistent with similar studies conducted in Caucasian populations regarding the CYP2E1-DraI (rs6413432) polymorphism.[16,17,21] These studies indicated no significant difference in HNSCC susceptibility in terms of allele and genotype frequencies for the CYP2E1-DraI polymorphism. On the other hand, some studies have reported that the mutant (C) allele is related to increased HNSCC risk.[15,23] Moreover, some meta-analyses have reported that the CYP2E1-DraI polymorphism is associated with increased HNSCC risk in Asian populations but not in Caucasian populations.[11,27] Nevertheless, more studies are needed to verify the effects of the CYP2E1-DraI polymorphism on the risk of head and neck cancer in Asian populations.[11]

The risk-enhancing mechanisms of the CYP2E1 PstI/RsaI and DraI polymorphisms relative to HNSCC are still unclear. It has been reported that the mutant (c2) allele is associated with decreased enzyme activity in the CYP2E1 PstI/RsaI polymorphisms.[10,28] Likewise, strong evidence exists regarding increased HNSCC risk from accumulated toxic substances in tissues due to the decreased enzyme activity in CYP2E1 PstI/RsaI polymorphic individuals.[9,29] The CC mutant allele is associated with increased HNSCC risk in CYP2E1 DraI polymorphic individuals due to the more active intermediates that occur in higher amounts during normal phase I reactions. Whilst the relationship between CYP2E1 polymorphisms and HNSCC risk is well demonstrated in Asian populations, more studies are needed of Caucasian subjects.[11,12]

In this study, the ALDH2 (rs671) polymorphism was not identified in either the case or control group. Thus, no association was found between HNSCC risk and the ALDH2 polymorphism in our study population. The results of our study are consistent with similar studies conducted in Caucasian populations regarding the ALDH2 polymorphism. The ALDH2*2 allele, which is associated with susceptibility to HNSCC, is common in Far East countries, such as in China and Japan.[28,30,31] This mutant allele is, however, rare amongst Caucasians, such as our study population, and has not been associated with HNSCC.

In conclusion, this study demonstrated that heterozygous (c1/c2) genotypes for the CYP2E1-RsaI polymorphism may be a risk factor for HNSCC. On the other hand, no relationship between the CYP2E1-DraI, CYP2E1-PstI, or ALDH2 polymorphisms and susceptibility to HNSCC was detected. These results are consistent with previous studies of the ALDH2 and CYP2E1 polymorphisms in Caucasian populations. However, there are still conflicting results in Caucasian populations for the CYP2E1 RsaI/PstI polymorphisms. Larger case groups and multi-centre studies are needed to more precisely demonstrate the susceptibility to HNSCC in the Turkish population.

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REFERENCES

1. Farris C, Petitte DM. Head, neck, and oral cancer update. Home Healthc Nurse 31: 322-328, 2013.
2. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 26: 123-141, 2014.
3. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.

4. Pezzuto F, Buonaguro L, Caponigro F, et al. Update on Head and Neck Cancer: Current Knowledge on Epidemiology, Risk Factors, Molecular Features and Novel Therapies. Oncology 89: 125-136, 2015.
5. Salway, JG. Metabolism at a glance. 3rd ed. Oxford, UK: Blackwell Publishing; 2004: 34-35.
6. Cederbaum AI. Alcohol metabolism. Clin Liver Dis 16: 667-685, 2012.
7. Seitz HK, Mueller S. Alcohol and cancer: an overview with special emphasis on the role of acetaldehyde and cytochrome P450 2E1. Adv Exp Med Biol 815: 59-70, 2015.
8. Neafsey P, Ginsberg G, Hattis D, et al. Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. J Toxicol Environ Health B Crit Rev 12: 362-388, 2009.
9. Zhuo X, Song J, Liao J, et al. Does CYP2E1 RsaI/PstI polymorphism confer head and neck carcinoma susceptibility?: A meta-analysis based on 43 studies. Medicine (Baltimore) 95: e5156, 2016.
10. Cadoni G, Boccia S, Petrelli L, et al. A review of genetic epidemiology of head and neck cancer related to polymorphisms in metabolic genes, cell cycle control and alcohol metabolism. Acta Otorhinolaryngol Ital 32: 1-11, 2012.
11. Tang K, Li Y, Zhang Z, et al. The PstI/RsaI and DraI polymorphisms of CYP2E1 and head and neck cancer risk: a meta-analysis based on 21 case-control studies. BMC Cancer 10: 575, 2010.
12. Cury NM, Russo A, Galbiatti AL, et al. Polymorphisms of the CYP1A1 and CYP2E1 genes in head and neck squamous cell carcinoma risk. Mol Biol Rep 39: 1055-1063, 2012.
13. Lewin HA, Stewart-Haynes JA. A simple method for DNA extraction from leukocytes for use in PCR. Biotechniques 13: 522-524, 1992.
14. Tamakoshi A, Hamajima N, Kawase H, et al. Duplex polymerase chain reaction with confronting two-pair primers (PCR-CTPP) for genotyping alcohol dehydrogenase beta subunit (ADH2) and aldehyde dehydrogenase 2 (ALDH2). Alcohol Alcohol 38: 407-410, 2003.
15. Lucas D, Ménez C, Floch F, et al. Cytochromes P4502E1 and P4501A1 genotypes and susceptibility to cirrhosis or upper aerodigestive tract cancer in alcoholic caucasians. Alcohol Clin Exp Res 20: 1033-1037,1996.
16. Matthias C, Bockmühl U, Jahnke V, et al. Polymorphism in cytochrome P450 CYP2D6, CYP1A1, CYP2E1 and glutathione S-transferase, GSTM1, GSTM3, GSTT1 and susceptibility to tobacco-related cancers: studies in upper aerodigestive tract cancers. Pharmacogenetics 8: 91-100, 1998.
17. Neuhaus T, Ko YD, Lorenzen K, et al. Association of cytochrome P450 2E1 polymorphisms and head and neck squamous cell cancer. Toxicol Lett 151: 273-282, 2004.
18. Gajecka M, Rydzanicz M, Jaskula-Sztul R, et al. CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma. Mutat Res 574: 112-123, 2005.
19. Li G, Liu Z, Sturgis EM, et al. CYP2E1 G1532C, NQO1 Pro187Ser, and CYP1B1 Val432Leu polymorphisms are not associated with risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol Biomarkers Prev 14: 1034-1036, 2005.
20. Buch SC, Nazar-Stewart V, Weissfeld JL, et al. Case-control study of oral and oropharyngeal cancer in whites and genetic variation in eight metabolic enzymes. Head Neck 30: 1139-1147, 2008.
21. Boccia S, Cadoni G, Sayed-Tabatabaei FA, et al. CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4, and NAT2 polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer. J Cancer Res Clin Oncol 134: 93-100, 2008.
22. Brocic M, Supic G, Zeljic K, et al. Genetic polymorphisms of ADH1C and CYP2E1 and risk of oral squamous cell carcinoma. Otolaryngol Head Neck Surg 145: 586-593, 2011.
23. Bouchardy C, Hirvonen A, Coutelle C, et al. Role of alcohol dehydrogenase 3 and cytochrome P-4502E1 genotypes in susceptibility to cancers of the upper aerodigestive tract. Int J Cancer 87: 734-740, 2000.
24. Lu D, Yu X, Du Y. Meta-analyses of the effect of cytochrome P450 2E1 gene polymorphism on the risk of head and neck cancer. Mol Biol Rep 38: 2409-2416, 2011.
25. Garte S, Gaspari L, Alexandrie AK, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 10: 1239-1248, 2001.
26. González MV, Alvarez V, Pello MF, et al. Genetic polymorphism of N-acetyltransferase-2, glutathione S-transferase-M1, and cytochromes P450IIE1 and P450IID6 in the susceptibility to head and neck cancer. J Clin Pathol 51: 294-298, 1998.
27. Hirschhorn JN, Lohmueller K, Byrne E, et al. Comprehensive review of genetic association studies. Genet Med 4: 45-61, 2002.
28. Hiyama T, Yoshihara M, Tanaka S, et al. Genetic polymorphisms and head and neck cancer risk (Review). Int J Oncol 32: 945-973, 2008.

29. Guo Y, Zhou S, Liu F, et al. CYP2E1 RsaI/PstI polymorphisms contributed to oral cancer susceptibility: a meta-analysis. Int J Clin Exp Pathol 8: 14685-14692, 2015.

30. Eriksson CJ. Genetic-epidemiological evidence for the role of acetaldehyde in cancers related to alcohol drinking. Adv Exp Med Biol 815: 41-58, 2015.

31. Tsai ST, Wong TY, Ou CY, et. al. The interplay between alcohol consumption, oral hygiene, ALDH2 and ADH1B in the risk of head and neck cancer. Int J Cancer 135: 2424-2436, 2014.

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