

# Lack of Association of CYP2C9 genetic polymorphism with oral squamous cell carcinoma

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## Research article

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## Abstract

**Background:** There is increasing evidence for the role of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HCAs) in carcinogenesis, including oral squamous cell carcinoma (OSCC). Several of these mutagenic substances are cytochrome (CYP)2C9 enzyme substrates.

**Methods:** In this study, we examined the association of CYP2C9\*2 and \*3 genetic polymorphisms in 58 OSCC patients and 174 healthy, age, and sex-matched controls. Genotyping was done with allele-specific polymerase chain reaction followed by agarose gel electrophoreses, while selected samples were sequenced for confirmation of genotyping.

**Results:** The wild type genotype (CYP2C9\*1\*1) was observed at 83%, \*1\*3 at 8%, \*1\*2 at 5%, \*2\*2 at 2% and \*2\*3 at 2% in combined case and control groups. On further analysis, however, our results did not reveal an association of these variants with OSCC samples (Odds ratio: 0.608, 95% Confidence Interval: 0.289 - 1.281, p-value: 0.190). While larger studies are needed to confirm or refute these results, they show a lack of association of CYP2C9\*2 and \*3 polymorphisms with OSCC in this population.

**Conclusions:** This study demonstrates that the genetic polymorphisms in CYP2C9 genes (CYP2C9\*2 and \*3) are not causing the risk and are not associated with OSCC. Also, CYP2C9 has no role in the pathogenesis of OSCC in this population of patients.

**Trial registration:** Not applicable.

## Background

Head and neck squamous cell cancers (HNSCC) account for about 5% of all cancers worldwide. Among the HNSCC, approximately 90% are squamous cell carcinomas of the oral cavity, pharynx, and larynx (1). The main risk factors include tobacco and alcohol use (2). The risk is further enhanced by polymorphisms in genes involved in the metabolism of alcohol and tobacco (3). These factors strongly influence a patient's vulnerability to cancer. Specifically, polymorphisms in cytochrome P450 (CYP) genes such as *CYP2E1* and *CYP1A1* are associated with HNSCC (4).

Individuals at higher risk of tumor development may be identified by polymorphisms in the genes of drug-metabolizing enzymes. *CYP1A1\*2A* genotype as associated with an enhanced risk of HNSCC in a cohort of Brazilian patients (5). *GSTM1* and *GSTT1* genotypes exhibited modest association with the risk of HNSCC in a meta-analysis comprised of 8536 controls and 6969 cases (4). A combined effect of *GSTM1* homozygous deletion and the *CYP1A1\*1A/\*2A* genotype on the risk of developing cancer was also known (6). In a similar study done in the Polish population, the risk of developing HNSCC was associated with *CYP1A1\*2A* genotype and *GSTM1* homozygous deletion (7). These studies indicate that polymorphisms in *GST* and *CYP* genes confer high risk for developing cancer, especially HNSCC.

CYP2C9 is one of the most important drug-metabolizing enzymes in humans and carries out the metabolism of ifosfamide, cyclophosphamide, tamoxifen, warfarin, and etoposide (8). *CYP2C9\*2* and *CYP2C9\*3* genotypes result in a ‘poor-metabolizer’ phenotype and consequently slow down the metabolism of drugs and other substrates metabolized by CYP2C9 (9). In a yeast expression system, *CYP2C9\*2* and *CYP2C9\*3* were associated with a significant increase in cyclophosphamide clearance (10). Apart from metabolizing various drugs, many mutagens and carcinogens are detoxified by CYP2C9 (11,12). The frequencies of *CYP2C9\*2* and *CYP2C9\*3* have been reported for multiple populations and display significant variations between Caucasian and Oriental populations (13). A study has previously published a higher frequency of *CYP2C9\*2* polymorphism in lung cancer patients (14). The risk of distal colorectal adenoma is also reported to be increased with polymorphisms in *CYP2C9* (15). However, the association of *CYP2C9* genetic polymorphisms with HNSCC patients has not been established yet. The present study was started to find out if these polymorphisms in *CYP2C9* gene are associated with the risk of OSCC in a Pakistani cohort.

## Methods

This study was conducted between January 2015-December 2017 at the Dow University hospital, Karachi. The cases group contained 58 OSCC patients, diagnosed and confirmed by a panel of experts. For control arm, 174 age and sex-matched, unrelated healthy individuals were recruited. Informed written consent was obtained from each participating individual. This research was approved by the Ethics Committee of Shifa International Hospital, Islamabad, and as per the GCP regulations. DNA was isolated from the cancer tissues, embedded in paraffin and fixed in formalin, using QiaAmp FFPE tissue kit (QIAGEN, Germany). The extracted genomic DNA was stored at -20°C until PCR and sequencing experimentation.

### Genotyping

Polymorphisms in the *CYP2C9* gene were determined using allele-specific PCR. The thermal profile used was as follows; initial denaturation for 10 minutes at 95°C, then 37 cycles of denaturation, annealing and extension at 95°C, 58°C, and 72°C, respectively, each for 45 seconds, followed by a final extension for 7 minutes at 72°C. Two sets of primers were used for the determination of each genotype. The outer set of primers served as internal control, while the inner set of primers were designed to determine the polymorphism. PCR reactions were carried out in PCR tubes containing 25 µL reaction volume, including 3 µL of DNA template, sets of outer and inner primers, and PCR master mix (Thermo Scientific, USA). The resulting PCR products were identified by resolving them on 5% agarose gel stained with ethidium bromide and visualized under UV light. Results were confirmed by sequencing about half of the samples and can be submitted upon request.

### Statistical Analysis

Observed genotypes were compiled and analyzed to find out allelic and genotype frequencies in case and control groups. Data were recorded and analyzed using SPSS version 23.0. The association between disease status and genotype was investigated using the chi-square and Fisher exact test. A logistic regression model was run to determine the odds ratio and 95% confidence interval for estimating the risk of OSCC. P-value of less than 0.05 was considered to be statistically significant.

## Results

This study included 58 patients with OSCC and 174 healthy controls. The mean age of the patients was 42.64 years ( $SD \pm 12$  years) in cases while 40.25 years ( $SD \pm 11.64$ ) in controls. Males were 82% and female 18% in cases while in the control group, 75.9% were male, and 24.1% were female. Of the 58 cases, 24 (41.37%) were localized to buccal cavity, 17 (29.3%) to the tongue, 11 (18.96%) to lips, and 6 (10.34%) to the palate (Figure 1).

In cases, \*1\*1 genotype was found at a frequency of 77.6%, \*1\*2 at 5.2%, \*1\*3 at 7.5%, \*2\*2 at 5.2% and \*2\*3 at 1.7% while in control group, \*1\*1 genotype was found at a frequency of 85.1%, \*1\*2 at 3.4%, \*1\*3 at 12.1%, \*2\*2 at 0.6% and \*2\*3 at 1.7% (Figure 2). Overall, wild type genotype was observed at 83%, \*1\*3 at 8%, \*1\*2 at 5.5%, \*2\*2 and \*2\*3 at 2% each in combined case and control groups (Figure 3).

In patients with OSCC localized to buccal cavity, 16.7% had genotypes \*1\*3, 4.2% had \*2\*3 while remaining (79.2%) had \*1\*1 genotype. Of the 17 cases affecting the tongue, \*1\*3 and \*2\*2 genotypes were 11.8% each while the remaining 76.5% were \*1\*1 genotype (Figure 4). In OSCC patients in which lesions were localized to lips, genotypes \*1\*2, \*1\*3, and \*2\*2 were found in 9.1% cases each. In 6 cases in which lesions were confined to the palate, 5 had wild type genotype while only one had \*1\*2 genotype.

To determine whether polymorphisms in the *CYP2C9* gene were able to predict the presence of OSCC, genotyping data were compared (Table 1). The distribution of variant alleles did not show a higher incidence in HNSCC than in control subjects. A statistically insignificant difference was detected between these groups for *CYP2C9\*1\*1* and *CYP2C9\*1\*2, \*1\*3, \*2\*3, \*2\*2* combined ( $P = 0.190$ ). Similarly, no statistically significant association was found for these genotype groups when samples were compared from various anatomic sites (Buccal,  $p = 0.459$ , Tongue,  $p = 0.358$ , Lip,  $p = 0.285$ , Palate = 0.907) with controls (Table 1).

**Table 1.** Association among genotype groups from various anatomic sites.

Location	Categories	Controls/Cases	OR	95% CI	P Value
OSCC	*1*1	148/45			
(Overall)	*1*2+ *1*3+ *2*2+ *2*3	26/13	0.608	0.289 - 1.281	0.190
Buccal	*1*1	148/19	1	ref	
	*1*2+*1*3+ *2*2+*2*3	26/5	0.668	0.229 - 1.946	0.459
Tongue	*1*1	148/13	1	ref	
	*1*2+*1*3+ *2*2+*2*3	26/4	0.571	0.173 - 1.887	0.358
Lip	*1*1	148/8	1	ref	
	*1*2+*1*3+ *2*2+*2*3	26/3	0.468	0.117 - 1.887	0.285
Palate	*1*1	148/5	1	ref	
	*1*2+*1*3+ *2*2+*2*3	26/1	0.878	0.099 - 7.826	0.907

Odds ratio (OR), which quantifies the strength of the association between two events is given for cases and controls along with confidence intervals (CI) (a range of their probabilities).

## Discussion

The risk of developing HNSCC is enhanced by association with selected polymorphisms in the genes of certain CYP enzymes, which, apart from metabolizing drugs, are also responsible for the metabolism of several important environmental carcinogens (16–18). It is suggested that the interaction of these polymorphic CYP enzymes interact with these carcinogens to enhance susceptibility to cancer (19). Therefore, in the present study, selected polymorphisms in *CYP2C9* gene were investigated to find out if these are associated with HNSCC.

As mentioned earlier, variation in interindividual sensitivity towards carcinogens is a significant risk factor for developing various cancers. However, in the present study, *CYP2C9* polymorphisms did not reveal any significant association with HNSCC patients as compared with the control group. A similar lack of association was observed in another study with 210 HNSCC patients, in which various genetic polymorphisms were studied in important metabolic enzymes, including NAT2, EPHX1, GSTM1, GSTT1, CYP1A1, and CYP2E1 (19). However, another study reported a significant association of genetic polymorphisms in *CYP1A2* and *CYP2E1* genes in HNSCC patients (20).

The tumors arising at four major sites of HNSCC: buccal cavity, lip, tongue, and palate behave differently because of their different properties (21). However, in terms of genetic polymorphisms, minor differences were observed among cancers from these major anatomic sites. Our study revealed that the frequency of *CYP2C9* genotypes observed in samples obtained from all the anatomic areas was not statistically

different (Table 1). These results indicate that polymorphisms in *CYP2C9* gene do not play a significant role in oral tumor development in the Pakistani population.

In the majority of squamous cell cancers, *CYP1A1*, *CYP2A6*, *CYP2E1*, and *CYP3A* genotypes are not frequently expressed in the early stage. Still, in non-small cell lung cancer, they do appear in early-stage and differentiated adenocarcinoma (22). It is speculated that independent of tumor initiation, CYP levels affect various signaling transduction pathways, which alter cell cycle and cause apoptosis or aberrant cell growth and, therefore, might be correlated with carcinogenesis and tumor progression (23,24). However, our study could not find any association of *CYP2C9* genetic polymorphisms with OSCC. There are a large number of studies that have reported a similar lack of association of various CYP genetic variants with OSCC, as reviewed by Vukovic and colleagues (25) and Qiu and colleagues in excellent meta-analyses (26). However, this was the first study attempting to find out the association of *CYP2C9* genetic polymorphisms with OSCC.

## Conclusions

These findings suggest that the genetic polymorphisms in *CYP2C9* genes (*CYP2C9\*2* and *\*3*) are not predictors of risk and are not associated with OSCC. This indicates a lack of a role for *CYP2C9* in the pathogenesis of OSCC in this population of patients.

## List Of Abbreviations

PAHs: polycyclic aromatic hydrocarbons

HCA: heterocyclic aromatic amines

OSCC: oral squamous cell carcinoma

HNSCC: head and neck squamous cell cancers

(CYP) genes: cytochrome P450

ARMS-PCR: Allele Refractory Mutation System- Polymerase Chain Reaction

## Declarations

Ethics approval and consent to participate: This research protocol was approved by the Ethics Committee of Shifa International Hospital, Islamabad and is per the GCP regulations.

Consent to participate: Written informed consent was obtained from each participating volunteer and they are available from the corresponding author on request.

Consent for publication: Not applicable

Availability of data and material: Not applicable

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: SA and SK conceptualized the study. SUN and ZZ, HTA, MM and AI searched the literature, collected the data and helped in manuscript preparation. SA, and KJ helped prepare the manuscript. SA, SK, and KJ refined the manuscript for publication. All authors read and approved the final manuscript for publication.

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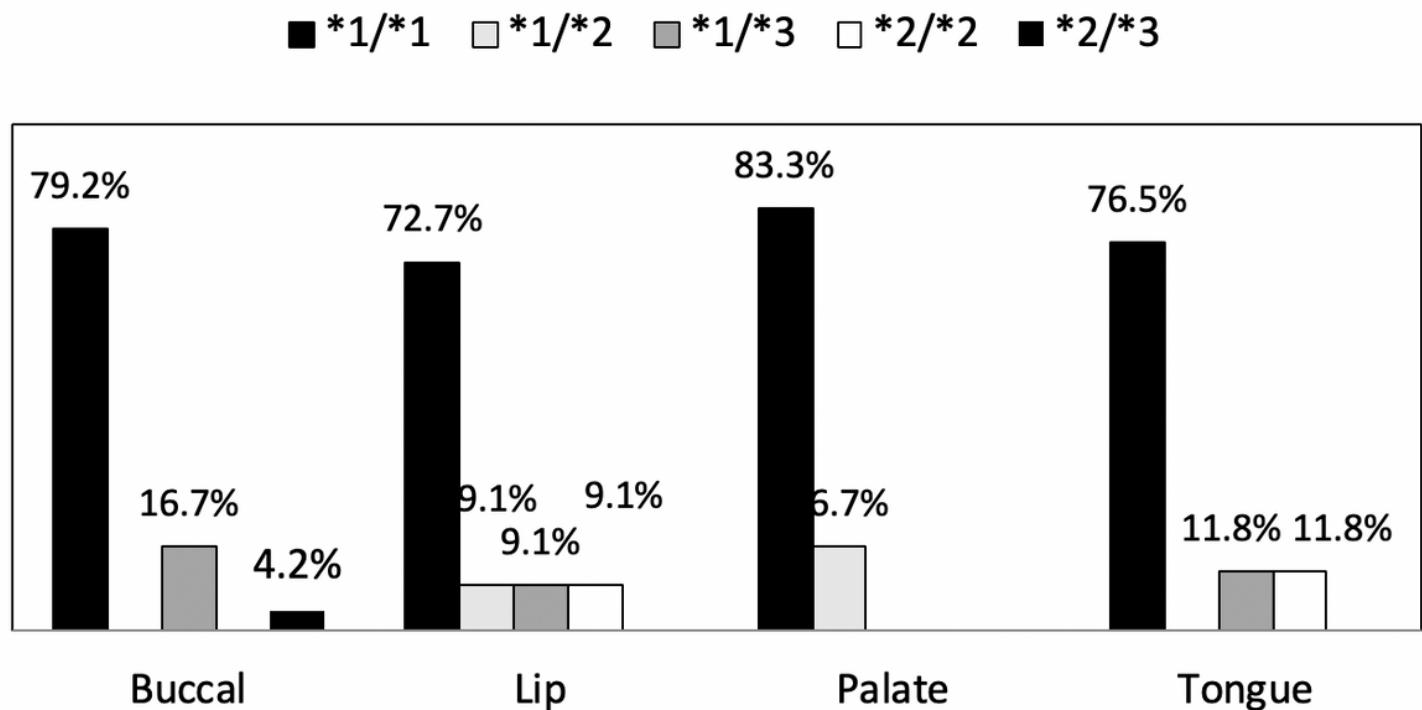
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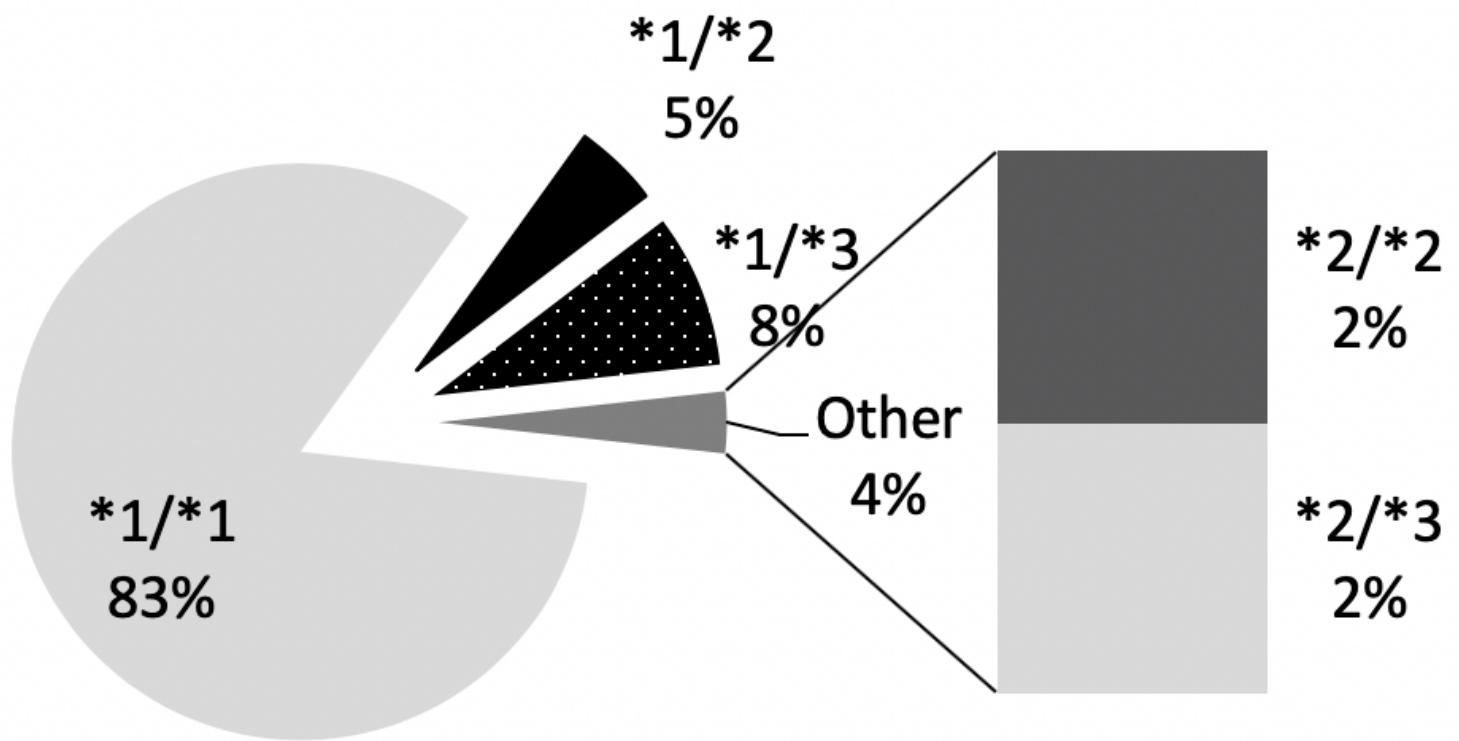
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## Figures



**Figure 1**

Locations of lesions in the oral cavity in OSCC patients.

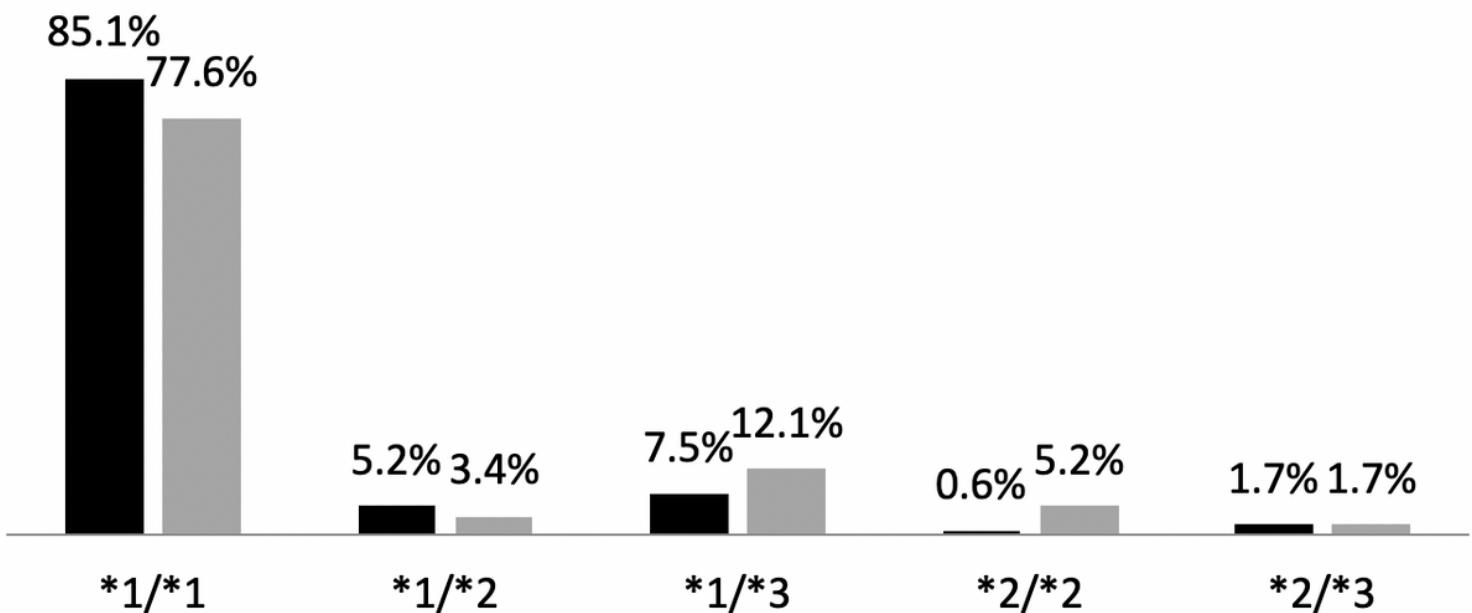


**Figure 2**

Relative distribution of different CYP2C9 genotypes in samples obtained from cases and controls.

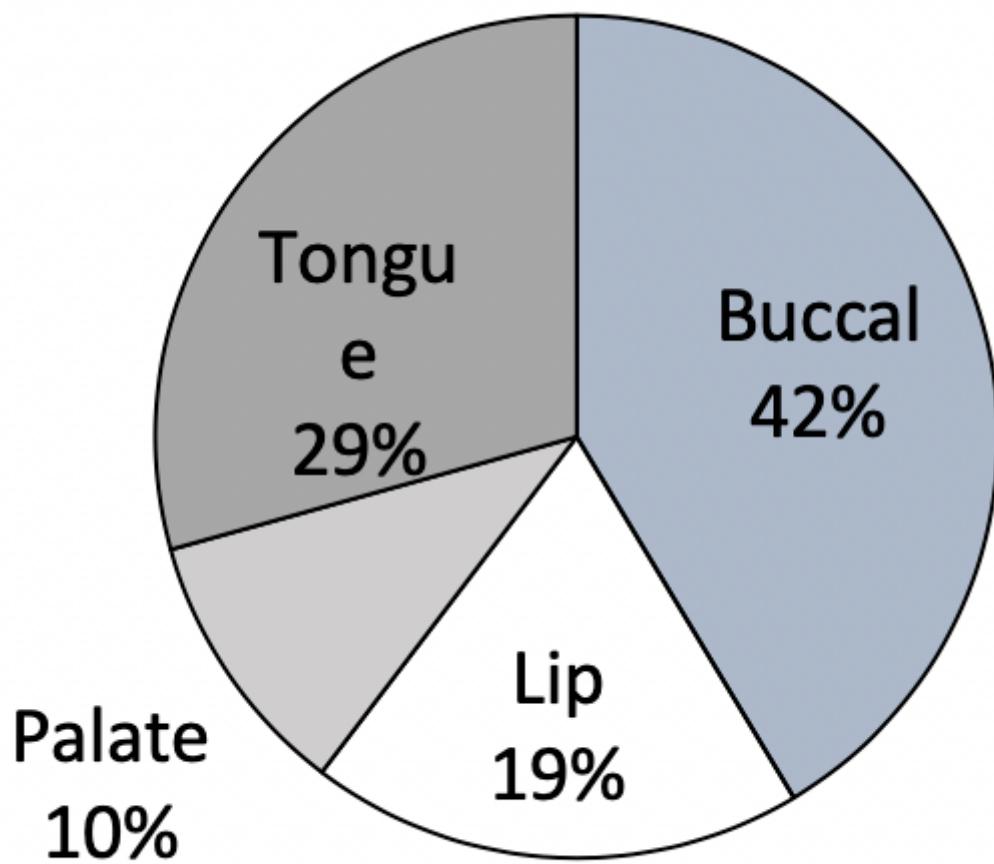
■ Controls ■ Cases

p-value = 0.138



**Figure 3**

Overall distribution of CYP2C9 genotypes in cases and controls combined.



**Figure 4**

Relative distribution of different CYP2C9 genotypes in OSCC samples obtained from various anatomic sites in the oral cavity