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Association of snps of aif-1 gene with susceptibility to oral cancer in chinese population

Liqiong ZHANG[1], Shaolin DENG[1]*

Abstract

To investigate the association between allograft inflammatory factor-1 (AIF-1) gene polymorphism and susceptibility to oral cancer in Chinese population. A case-control study was conducted to collect 400 cases of newly diagnosed oral cancer patients diagnosed by pathology from January 2015 to June 2019 and 400 cases of physical examination in the same period. Single nucleotide polymorphisms (SNPs) of the AIF-1 gene were detected using TaqMan probe technology. Unconditional logistic regression analysis was used to calculate the odd ratios (OR) and 95% confidence interval (CI) of the association between AIF-1 gene polymorphism and oral cancer susceptibility. GG genotypes reduced the risk of oral cancer in co-dominant models (*OR* = 0.499, 95% *CI*: 0.328-0.760, *P* = 0.001) and recessive models (*OR* = 0.496, 95% *CI*: 0.342-0.729, *P* < 0.001). There was no significant difference between the case group and the control group in the dominant model genotype (*OR* = 0.836, 95% *CI*: 0.623~1.121, *P* = 0.836). Conclusion AIF-1 gene rs2857595 gene SNPs can reduce the risk of oral cancer.

Keywords: AIF-1 gene; oral cancer; case-control study.

Practical Application: In the future, the AFS-1 gene rs2857595 locus GG genotype should be considered in the development of precise prevention strategies and measures for oral cancer.

1 Introduction

Head and neck cancer can occur in the paranasal sinuses, sinuses, nasopharynx, oropharynx, mouth, pharynx, and throat. Head and neck cancer affects more than 500,000 people worldwide each year, and the incidence of oral cancer is increasing in the young population (Anderson et al., 2019; Bachok et al., 2018; Balaguer et al., 2020; Funahara et al., 2017). The occurrence of head and neck cancer has a serious impact on the quality of life of patients, such as communication, breathing and difficulty swallowing (Matar & Haddad, 2011; Lin et al., 2012). The treatment of head and neck cancer often causes problems such as pain, diet and communication, which seriously affects the mental health of patients (Devins et al., 2013; Sreekumar, 2019).

Studies (Alkhubaizi et al., 2018; Kadashetti et al., 2017; Wei et al., 2014) have shown that smoking, drinking and other factors are the main risk factors for oral cancer, and the proportion of patients with oral cancer in non-smokers and non-drinkers is about 15% to 20% (Laco et al., 2011). It is suggested that genetic factors may play a non-negligible role in it. The results of a genome-wide association study (GWAS) by Wei et al. (2014) suggest that single nucleotide polymorphisms (SNPs) of the rs2857595 locus of the AIF-1 gene are associated with the susceptibility of laryngeal squamous cell carcinoma (LSCC) in Chinese population. However, the subjects were all male and did not include females.

Obesity is a risk factor for many tumors (Renehan et al., 2008; Barnes et al., 2016; Zhao et al., 2017), it have found that obesity can reduce the risk of head and neck cancer (such as oral cancer) (Maasland et al., 2015; Radoï et al., 2013). AIF-1 gene is associated with body mass index (BMI) (Rouskas et al., 2012; Thorleifsson et al., 2009). Therefore, effective head and neck cancer markers have been found to play a key role in understanding tumor causes, patient outcomes, and in-depth research. This study used a case-control study to explore the association between AIF-1 gene SNPs and susceptibility to oral cancer, and to provide a scientific basis for the development of accurate prevention strategies and measures for oral cancer.

2 Materials and methods

2.1 Methods

A case-control study was conducted to collect 400 cases (case groups) of newly diagnosed oral cancer patients diagnosed by pathology from January 2015 to June 2019 and 400 (control group) of the hospital for the same period. The investigators from the case group and the control group conducted an epidemiological questionnaire survey to collect the general demographic characteristics of the subjects and the exposure history of smoking and drinking. At the same time, 5 mL of peripheral blood of each subject was collected for DNA extraction and genotyping. This study was approved by the our hospital. All participants participated in the written informed consent form.

2.2 DNA extraction

DNA extraction mainly concludes as follows: (1) The whole blood sample was transferred to a 10 mL centrifuge tube, and

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[1]*Department of Stomatology, The Central Hospital of Wuhan Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China*
*Corresponding author: dengshaolin@aliyun.com

then the red blood cell lysate was added, and the mixture was inverted and mixed for about 200 minutes, and then allowed to stand for 30 minutes. (2) Centrifuge at 4000 r × 6 min, discard the supernatant, resuspend the pellet by adding 1 mL of phosphate buffered saline (PBS), mix by pipetting and transfer to a 1.5 mL EP tube. (3) Centrifuge again at 5000 r × 6 min, discard the supernatant, add 0.1 mL PBS and 0.7 mL DNAzol, mix by pipetting. (4) Add 0.5 mL of 100% ethanol, mix by inversion, centrifuge at 12000 r × 5 min, and discard the supernatant. (5) Add 1 mL of 75% ethanol, shake well, centrifuge at 12000 r × 2 min, and discard the supernatant. (6) When drying naturally to the micro-dry, add 400 μL of double distilled water. (7) After taking 1 μL of the measured DNA content, four tubes were dispensed and stored in a refrigerator at -20 °C.

2.3 Genotyping

Genotyping assays were performed using TaqMan probe technology. The probe and primer sequences are shown in Table 1. The specific experimental procedures are as follows: (1) Pre-denaturation at 95 °C for 10 min. (2) Following denaturation at 95 °C for 5 s, then annealing at 60 °C for 30 s, this step is carried out for 40 cycles. (3) Finally placed in an environment of 4 °C. The polymerase chain reaction (PCR) total reaction system is 5 μL: 1 μL DNA template, 2 × TaqMan Universal Master Mix 2. 5 μL, upstream and downstream primers each 0. 225 μL, P1/P2 each 0. 095 Ml, ddH2O 0. 86 μL. All assays were performed in 96-well plates with 8 replicates and 8 blanks in each plate and 5% of the samples were randomly selected for repeated determination. The success rate of all the sites was over 99.0%, and the agreement rate was 100%.

2.3 Statistical analysis

The chi-square analysis was used for the count data and the t-test was used for the measurement data. Unconditional logistic regression model was used to analyze the association of ANP-1 gene rs2857595 SNPs with oral cancer and the odd ratios (OR) and 95% CI were calculated. All statistical analyses were performed using the Stata 23.0 software package with a test level of $\alpha = 0.05$. A two-sided test $P < 0.05$ considered the difference between the case group and the control group to be statistically significant.

3 Results

3.1 General characteristics of the research objects

A total of 400 Chinese patients with oral cancer were enrolled in the study, including 365 patients with squamous cell carcinoma, 35 patients with adenocarcinoma and other pathological types, and an average age of (55.74 ± 12.19) years. There were 400 patients in the control group with an average age of (54.06 ± 13.03) years. In the case group, 216 cases were 56 years old and above, accounting for 54.0%; in the control group, 177 cases were 56 years old and above, accounting for 44.3%. There were 259 males in the case group, accounting for 64.8%; 262 males in the control group, accounting for 65.5%. The case group was full-time 281 cases, accounting for 70.3%; the control group was full-time 316 cases, accounting for 79.0%. The marital status of the case group was 345 cases of marriage, accounting for 86.3%; the marital status of the control group was 352 cases of marriage, accounting for 88.0%. The education level of the case group was 238 cases of primary school and below, accounting for 59.5%; the control group had 68 cases of primary school and below, accounting for 17.0%. The case group lived in 203 cities, accounting for 50.8%; the control group lived in 130 cities, accounting for 32.5%. There were 253 cases of smoking in the case group, accounting for 63.3%; 139 cases of smoking in the control group, accounting for 34.8%. In the case group, 168 cases were alcoholic, accounting for 42.0%; in the control group, 95 cases were drinking, accounting for 23.8%. In the case group, 100 cases of BMI were 24 or above, accounting for 25.0%; in the control group, 194 cases of BMI were 24 or above, accounting for 48.5%. Univariate analysis showed that compared with the control group, the differences in age, occupation, education level, place of residence, smoking, drinking, BMI and other factors were statistically significant ($P<0.05$). The results of the analysis are shown in Table 2.

3.2 Association of rs2857595 with susceptibility to oral cancer

The analysis showed that the distribution of genotype frequencies of rs2857595 was statistically significant between the case group and the control group (chi-square value = 13.24, $P = 0.001$). Moreover, this locus conforms to the law of genetic balance of hardy-weinberg (H-W) (chi-square value = 2.051, $P = 0.152$). Logistic regression analysis suggested that GG genotypes can reduce the risk of oral cancer in the codominant model ($OR = 0.499$, 95% CI: 0.328-0.760, $P = 0.001$) and the recessive model ($OR = 0.496$, 95% CI: 0.342-0.729, $P < 0.001$), and the results are shown in Table 3. There was no significant difference between the case group and the control group in the dominant model genotype ($OR = 0.836$, 95% CI: 0.623~1.121, $P = 0.836$).

4 Discussion

Smoking is considered to be an important risk factor for oral cancer (Alkhubaizi et al., 2018; Rogers et al., 2019;

Table 1. Probe and primer sequences in PCR.

Number	Primer/probe	Base order(5'……3')
rs2857595-P1	Probe	FAM-AGAAGTCACCCAATCT-MGB
rs2857595-P2	Probe	HEX-AGTCACTCAATCTC-MGB
rs2857595-F	Primer	GGGAGCCATTCATTAGTTGAAAAATAT
rs2857595-R	Primer	AAAGTCCACAATCCAGCACAGG

Table 2. Comparison of general characteristics between the two groups of respondents.

Variables	Case group		Control group		*χ2 or t*	*P (Probability)*
	No.	*col %*	*No.*	*col %*		
Age						
23~55	184	46.0	223	55.8	7.607	0.006
56~	216	54.0	177	44.3		
Gender						
Female	141	35.3	138	34.5	0.05	0.824
Male	259	64.8	262	65.5		
Occupation						
Part time	119	29.8	84	21.0	8,086	0.004
Full time	281	70.3	316	79.0		
Marital status						
Unmarried	55	13.8	48	12.0	0.546	0.46
Married	345	86.3	352	88.0		
Education						
Elementary school and below	238	59.5	68	17.0	153.807	<0.001
Junior school	106	26.5	202	50.5		
High school and above	56	14.0	130	32.5		
Place of residence						
Rural	197	49.3	279	69.8	27.414	<0.001
City	203	50.8	130	32.5		
Smoking						
None	147	36.8	262	65.5	65.006	<0.001
Yes	253	63.3	139	34.8		
Drinking						
None	232	58.0	305	76.3	30.186	<0.001
Yes	168	42.0	95	23.8		
BMI						
<18.5	58	14.5	17	4.3	58.985	<0.001
18.5-24	242	60.5	189	47.3		
≥24	100	25.0	194	48.5		

Table 3. The unconditional Logistic regression of rs2857595 on oral cancer.

Type	Case group		Control group		*Odds Ratio (95% CI)*	*P (Probability)*
	No.	*col %*	*No.*	*col %*		
Co-dominant genetic model						
AA(Genotype AA)	143	35.8	127	31.8	1.0	
AG(Genotype AG)	207	51.8	184	46.0	0.999 (0.732-1.363)	0.996
GG(Genotype GG)	50	12.5	89	22.3	0.499 (0.328-0.760)	0.001
Dominant genetic model						
AA	143	35.8	127	31.8	1.0	
AG+GG	257	64.3	273	68.3	0.836 (0.623-1.121)	0.836
Recessive genetic model						
AA+AG	350	87.5	311	77.8	1.0	
GG	50	12.5	89	22.3	0.496 (0.342-0.729)	<0.001

Yoon et al., 2019). Polycyclic aromatic hydrocarbons, heterocyclic amines and nitrosamines in cigarettes are well-defined chemical carcinogens. At present, most chemical carcinogens are known to require biotransformation activation or detoxification in vivo. The metabolic enzymes involved in this process are divided into two categories, namely, phase I metabolic enzymes and phase II metabolic enzymes. Phase I metabolic enzymes are metabolically activated enzymes, and many procarcinogens are metabolically activated to become the ultimate carcinogen. The phase II metabolic enzyme is a metabolic detoxifying enzyme that converts carcinogens or their toxic metabolites into substances that are harmless to the human body and excreted from the body. Whether a carcinogen causes cancer of a target cell depends to a large extent on the activity of the two enzymes and their equilibrium relationship.

Most of the genes of metabolic enzymes are polymorphic, and the polymorphism of some genes can cause changes in enzyme activity, leading to differences in individual tumor susceptibility. The development of oral cancer is a multi-step process in which many causes and host susceptibility interact. Most environmental carcinogens require carcinogenesis after metabolic activation in the body. Many of the former carcinogens in tobacco also need to be activated and detoxified by in vivo phase I and phase II metabolic enzymes. CYP1A1 is one of the important phase I metabolic enzymes, which can catalyze the phase I oxidation of foreign compounds such as acetaldehyde into the body, and convert the inactive procarcinogen activation into an electrophilic compound (Divya et al., 2018; Shahid et al., 2019). The electrophilic compound can attack biological macromolecules in cells and form adducts with DNA or proteins. Eventually it causes changes in oncogenes and tumor suppressor genes, leading to cancer.

Oral cancer is a general term for malignant tumors that occur in the mouth. In recent years, the incidence of oral cancer has been increasing (Hou et al., 2015). Previous studies have found that AIF-1 gene rs2857595 locus polymorphism can reduce the risk of laryngeal squamous cell carcinoma (Wei et al., 2014), which is basically consistent with the results of this study. AIF-1 is a gene located in the genomic region of human leukocyte antigen class III on chromosome 6. Although the mechanism of rs2857595 gene polymorphism and susceptibility to oral cancer has not been reported, there is a linkage disequilibrium between rs2857595 locus and multiple inflammatory genes (Wei et al., 2014). In addition, a recent animal study found that AIF-1 protein as a biomarker for intestinal mucosal barrier repair can promote intestinal mucosal damage healing by reducing the intestinal mucosal inflammatory response and increasing the differentiation of intestinal mucosal cells (Roman et al., 2017). Although this study only explored the relationship between AIF-1 and intestinal mucosal injury repair, the results suggest that the relationship between AIF-1 gene and oral cancer may be related to the healing of oral mucosal injury, but the specific mechanism remains to be further studied.

Obesity is a risk factor for many malignant tumors, which could reduce the risk of head and neck cancer (Maasland et al., 2015; Radoï et al., 2013). Many carcinogens (such as polycyclic aromatic hydrocarbons) are lipophilic and can accumulate in adipose tissue, so the body fat content may affect the distribution of carcinogens in the human body, which may lead to tumors (Gaudet et al., 2010). 8-hydroxydeoxyguanosine (8-OHdG) is a marker of DNA oxidative damage (Mizoue et al., 2006). Previous studies have found a negative correlation between BMI levels and 8-OHdG levels in the body. Low body weight may increase the oxidative damage of DNA by smoking and other factors (Nigam et al., 2019; Tang et al., 2018a, b; Chang et al., 2016), thereby promoting tumorigenesis.

Because of the case-control study used in this study, there may be some selection bias and recall bias. However, the investigators in this study have undergone unified training, focusing on quality control, and the selected participants are new cases, which minimizes selection bias and recall bias. Therefore, there is still a need to further expand the sample size of the multicenter study and conduct a prospective cohort study to validate the results of this study.

5 Conclusion

In summary, the AFS-1 gene rs2857595 locus GG genotype can reduce the risk of oral cancer. In the future, the AFS-1 gene rs2857595 locus GG genotype should be considered in the development of precise prevention strategies and measures for oral cancer.

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