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Original Article

Association between *MDM2* rs769412 and rs937283 polymorphisms with alcohol drinking and laryngeal carcinoma risk

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Abstract: Target: To investigate the association between the interactions of murine double minute 2 (*MDM2*) polymorphisms (rs769412 and rs937283) with alcohol drinking and laryngeal carcinoma. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotypes status of *MDM2* rs769412 and rs937283 polymorphisms among 126 cases and 120 controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by the chi-squared test, which was adopted to analyze the association between *MDM2* rs769412 and rs937283 polymorphisms and the susceptibility to larynx carcinoma in the drinking population. Results: Genotypes distributions of *MDM2* rs769412 and rs937283 polymorphisms in the control group were in accordance with Hardy-Weinberg equilibrium (HWE). *MDM2* rs769412 GG genotype and G allele significantly increased laryngeal carcinoma risk (GG vs. AA: OR=3.17, 95% CI=1.25-8.04; G vs. A: OR=1.88, 95% CI=1.24-2.84). Furthermore, the mutant genotypes of *MDM2* rs937283and rs769412 were remarkablely associated with the increased risk for laryngeal carcinoma in drinking population (rs937283: OR=2.67, 95% CI=1.40-5.07; rs769412: OR=3.76, 95% CI=1.62-8.75). Conclusion: *MDM2* polymorphisms are correlated with the onset of laryngeal carcinoma. The relationship is strengthened by alcohol drinking.

Keywords: Laryngeal carcinoma, *MDM2,* polymorphisms, alcohol drinking

Introduction

Larynx carcinoma is one of the common malignant tumors on head and neck. Its incidence is only lower than nasopharyngeal carcinoma in head and neck tumors, which accounts for 1%~5% of body tumors. Laryngeal carcinoma generally occurs among people aged 50~70 years old, especially in males. In recent years, the incidence of laryngeal carcinoma is increasing yearly because of multiple carcinogenic factors [1-3]. Among the factors, genes play vital roles in the occurrence of laryngeal carcinoma [4-6].

Previous studies have indicated that murine double minute 2 (*MDM2,* also known as *HDMX* and *ACTFS*), located at chromosome 12q14.3-q15, is a newly proto-oncogene encoding a apoptosis inhibiting protein [7]. As a new member in the family of the inhibitor of apoptosis protein (IAP), *MDM2* can extend the survival time of cells and promote the cell proliferation and tumor growth by a feedback loop with *P53* [8]. Multiple researches in recent years show that *MDM2* takes part in the emergence and development of many tumors, especially digestive carcinomas [9-11] and is associated with the infiltration, metastasis and poor prognosis of malignancies [12, 13]. However, the relationship of *MDM2* rs937283A/G and rs769412A/G polymorphisms with laryngeal carcinoma risk was hardly reported.

To our knowledge, with the improvement of people's living, cigarette and alcohol are excessively consumed, which leads to some diseases indirectly. Therefore, the association of *MDM2* polymorphisms with the environmental factors and laryngeal carcinoma susceptibility was analyzed in 126 patients with larynx carcinoma and 120 healthy controls. The results may provide evidence for exploring the pathogenesis of laryngeal carcinoma.

Table 1. Primers sequences and amplification lengths of *MDM2* rs769412 and rs937283 polymorphisms

SNP	Forward/ Reverse	Primer sequence	Fragment length
rs769412 A/G	Forward	5' ACAGATGTTGGGCCCTTCGT 3'	279 bp
	Reverse	5' GCAATGTGATGGAAGGGGGG 3'	
rs937283 A/G	Forward	5' GAGCAAGAAGCCGAGCCCGA 3'	261 bp
	Reverse	5' CTCGGGCTCGGCTTCTTGCT 3'	

Table 2. Basic characteristics of cases and controls

Basic characteristics	Cases (n=126, %)	Controls (n=120, %)	*P* value
Sex			0.80
Male	78 (61.9)	72 (60.0)	
Female	48 (38.1)	48 (40.0)	
Age			0.07
≤50	29 (23.0)	41 (34.2)	
>50	97 (77.0)	79 (65.8)	
Smoking status			0.16
Smoking	70 (55.6)	55 (45.8)	
Non-smoking	56 (44.4)	65 (54.2)	
Drinking status			0.01
Drinking	85 (67.5)	62 (51.7)	
Non-smoking	41 (32.5)	58 (48.3)	

Table 3. Comparison of genotypes and alleles distributions in two groups

Genotypes/-Alleles	Cases- (n, %)	Controls- (n, %)	*P*	OR (95% CI)
rs937283 A/G				
AA	65 (51.6)	80 (66.7)	-	1.00
AG	43 (34.1)	33 (27.5)	0.12	1.60 (0.92-2.81)
GG	18 (14.3)	7 (5.8)	0.02	3.17 (1.25-8.04)
A	173 (68.7)	193 (80.4)	-	1.00
G	79 (31.3)	47 (19.6)	0	1.88 (1.24-2.84)
rs769412 A/G				
AA	92 (73.0)	99 (82.5)	-	1.00
AG	30 (23.8)	19 (15.8)	0.11	1.70 (0.90-3.23)
GG	4 (3.2)	2 (1.7)	0.46	2.15 (0.39-12.03)
A	214 (84.9)	217 (90.4)	-	1.00
G	38 (15.1)	23 (9.6)	0.08	1.68 (0.97-2.91)

Materials and methods

Research objects

126 patients with laryngeal carcinoma hospitalized in Affiliated Hospital of Weifang Medical College were enrolled as cases. All patients (82 males and 44 females) got neither radiotherapy nor chemotherapy, and they were confirmed by two pathologists. They were aged 46-78 with an average age of 60.3. At the same period, 120 healthy people frequency-matched by age and gender with cases carried out physical examination in the same hospital were enrolled as the control group. Among them, there were 64 males and 56 females aged 41-82 with a median age of 59.8. All participators were unrelated Chinese Han population and had no other malignancy histories. Written consents were obtained from all subjects and this project was supported by the Research Ethics Committee of the hospital.

Blood collection and DNA extraction

5 mL peripheral venous blood was collected from every participator and then undergone anticoagulant operation by ethylene diamine tetraacetic acid (EDTA). Genomic DNA was extracted using proteinase K digestion-saturation sodium chloride method from all samples and stored at -20°C refrigerator.

Genotyping

Genotypings of *MDM2* rs769412 and rs937283 polymorphisms were carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR primers were designed by Primer premier 5.0 software and primers sequences were shown in **Table 1**. PCR reaction system was a volume of 25 μL solution, including 100 ng genomic DNA, 1.0 μL forward primer and reverse primer, respectively, 12.5 μL Master Mix and 10.5 μL redistilled water. PCR reaction program was as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C

Table 4. The association of *MDM2* rs937283 and rs769412 polymorphisms with alcohol drinking with larynx carcinoma risk

SNPs	Variates	Cases	Controls	*P*	OR (95% CI)
rs937283					
+	+	30	48		1.00
+	-	35	32	1.31	1.75 (0.90-3.39)
-	+	11	10	0.32	1.76 (0.67-4.64)
-	-	50	30	0.00	2.67 (1.40-5.07)
rs769412					
+	+	35	47		1.00
+	-	57	52	0.24	1.47 (0.83-2.62)
-	+	6	11	0.79	0.73 (0.25-2.17)
-	-	28	10	0.00	3.76 (1.62-8.75)

Note: "+" and "-" represents wild and mutant genotypes in SNP column, respectively. In variate column, "+" and "-" represents no drinking and alcohol drinking, respectively.

for 30 s, annealing at 58°C for 45 s and extension at 72°C for 1 min, at last final extension at 72°C for 5 min. PCR amplification products were digested by HpaII and MboI, respectively. The enzyme-digested products were seperated by 2% agarose gel electrophoresis.

Statistical analysis

The genotypes distributions of *MDM2* rs769412 and rs937282 polymorphisms in control group were evaluated by Hardy-Weinberg equilibrium (HWE). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated by χ^2 test to evaluate the relationship strength between *MDM2* polymorphisms or *MDM2* with alcohol drinking and larynx carcinoma risk. SPSS18.0 software was applied for statistical analysis. Statistically significant level was $P<0.05$.

Results

Basic characteristics of objects

Genotypes distributions of *MDM2* rs769412 and rs937283 polymorphisms in the control group were satisfied with HWE, indicated that control samples were representative. There were no distinct differences of sex, age and smoking between two groups ($P>0.05$). However, significant difference was found in alcohol drinking ($P<0.05$) (**Table 2**).

Correlation analysis between MDM2 polymorphisms and the risk of larynx carcinoma

The results indicated that (**Table 3**) the frequency of *MDM2* rs937283 GG genotype in case group was obviously higher than in control group ($P<0.05$). The risk of laryngeal carcinoma was 3.17 times higher in people with GG genotype than AA genotype (OR=3.17, 95% CI=1.25-8.04). Also, the G allele was associated with increased risk of larynx carcinoma (OR=1.88, 95% CI=1.24-2.84). Rs769412 polymorphism had no significant relevance with the occurrence of larynx carcinoma. Meanwhile, further study about the association between *MDM2* polymorphisms with alcohol drinking and larynx carcinoma susceptibility was carried out. As shown in **Table 4**, the results showed that both of rs937283 and rs769412 polymorphisms remarkablely increased larynx carcinoma risk in people who drink all the time (rs937283: OR=2.67, 95% CI=1.40-5.07; rs769412: OR=3.76, 95% CI=1.62-8.75).

Discussion

Laryngeal carcinoma is one of serious diseases affecting human health and life. Because of different carcinogenic factors and accumulated exposure to various carcinogenic conditions, the incidence of larynx carcinoma has increased year by year during the past 10 years. Recent studies showed that the occurrence of laryngeal carcinoma was the combined effects of environmental and genetic factors [14, 15].

The relationship of genetic factors, especially genetic variant and laryngeal carcinoma attracts a lot of attention. A meta-analysis of Li et al. showed that *GSTT1* null genotype significantly increased laryngeal carcinoma risk [16]. A DNA repair gene *XRCC1* Arg399Gln polymorphism was proved to be associated with the increased risk of laryngeal carcinoma in Xinjiang by Ayiheng and Bogela [17]. While genetic factors are not the decisive elements. Mostly they work together with some environmental factors, such as smoking, alcohol drinking and air pollution to promote the tumors development. According to the results of Li et al., *ERCC1* rs11615 and *XPG/ERCC5* rs17655 polymorphisms are not the independent risk

factors for larynx cancer, but could increase larynx cancer risk in smokers and drinkers [18].

MDM2 can extend cell survival time, and promote cell proliferation and tumor growth. It can block cell cycle, induce cell apoptosis and contribute to DNA repair via *MDM2-p53-p21* pathway [19, 20]. Additionally, *MDM2* forms a feedback system with *p53* and a complex regulatory network with other signal transduction pathways to take part in the processes of cell growth inhibition, apoptosis and cell cycle regulation and is related to the occurrence and development of tumors as well as embryonic development and tissue differentiation. *MDM2* gene is highly expressed in various tumors [21, 22] and it appears to be a biomarker for highly malignant tumor and poor prognosis [23]. Since Bond et al. reported that *MDM2* 309T/G polymorphism might increase *MDM2* protein expression and inhibit the expression of *p53* in 2004 [24], the relevance of *MDM2* polymorphisms and various tumors has been studied widely [25-27].

Based on the above researches, our research chose *MDM2* rs769412 and rs937283 polymorphisms to discuss their relationships with the onset of larynx carcinoma. The GG genotype and G allele of *MDM2* rs937283 polymorphism were found in the study to have correlations with the risk of larynx carcinoma in Chinese Han population, while rs769412 had no association with the risk of larynx carcinoma. In present study, the interaction between *MDM2* polymorphisms and alcohol drinking was analyzed at the same time. The results showed that both of *MDM2* rs937283 and rs769412 were associated the significantly increased risk of larynx carcinoma among drinkers. This result is consistent with previous studies that environmental factors can affect phenotypes based on gene-environment interactions.

In conclusion, *MDM2* might be an important candidate proto-oncogene for larynx carcinoma. Like other tumors, laryngeal carcinoma is also a multiplegenes and multi-factor disease, so the mutation of a single locus is not enough to reveal the susceptibility to tumors completely. Our research is the first one to discuss the relationships between *MDM2* rs769412 and rs937283 polymorphisms and the susceptibility to laryngeal carcinoma. Also, our study demonstrated that *MDM2* polymorphism may work together with environmental factors to promote the occurrence of laryngeal carcinoma. However, the limited SNP locus, ethnic group and sample size may affect the reliability and veracity of results. Therefore, more experiments with multiple genes and larger sample size need to be operated in multi-region to deeply assist the study and explore the pathogenesis of laryngeal carcinoma.

Disclosure of conflict of interest

None.

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