

# Association study between miR-149 gene polymorphism and nasopharyngeal carcinoma

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**Abstract.** Association studies between single-nucleotide polymorphism (SNP) rs2292832 on miR-149 gene and cancer risk have been previously analyzed in several types of cancer. The aim of this study was to evaluate the association between miR-149 polymorphism and risk of nasopharyngeal carcinoma (NPC). miR-149 gene polymorphism was genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) in 158 patients with NPC and 242 healthy individuals. Associations with cancer risk and clinicopathological characteristics were analyzed by  $\chi^2$  test. No significant difference was observed for miR-149 gene polymorphism in NPC patients and healthy controls in either genotype (P=0.427 for CC vs. CT vs. TT, P=0.247 for CT vs. TT and P=0.323 for CC vs. TT, respectively) or allelic analysis (P=0.216). No significant difference was noted between the genotypes and the clinicopathological parameters examined with the exception of clinical stage. A significantly higher CC distribution in clinical stage I-II compared with III-IV was observed under the dominant model (CC vs. CT vs. TT, P=0.026) and the co-dominant model (CC vs. TT, P=0.030). The results of this study suggested that the CC genotype of miR-149 contributes to the progression and development, rather than the initiation of NPC.

## Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy with an unusual ethnic and geographic disparity. It has been estimated that 92% of new cases occur in economically developing countries (1). High incidence is observed in Malaysia, Indonesia, Singapore, a number of provinces in South-Eastern China, including Guangdong and Hong Kong, and in other parts of Southern Asia (1,2). Aetiological factors for NPC carcinogenesis include genetic susceptibility, early-age exposure to chemical carcinogens (particularly Cantonese salted fish), and latent Epstein-Barr virus (EBV) infection (3). A few genes were reported to contribute to the risk of NPC by genetic linkage and association studies (4-6). However, the precise genetic alterations during NPC carcinogenesis remain to be clarified.

MicroRNAs (miRNAs) are a large family of gene regulators that have been shown to repress the expression of important cancer-related genes and might be useful in the diagnosis and treatment of cancer (7). The association between microRNA polymorphism and cancer risk or tumor clinicopathological characteristics has been reported in a number of studies. The SNP rs11614913 in hsa-miR-196a2 gene was associated with patient survival in non-small cell lung cancer (8). A significant association between miR-196a2 polymorphism and risk of digestive system cancers, particularly of colorectal and hepatocellular cancer, was identified (9). A significant association between single-nucleotide polymorphism (SNP) rs2910164 in miR-146a gene and an increased risk of gastric (10) and breast (11) cancer was also identified.

Association studies between SNP rs2292832 on miR-149 gene and cancer risk were analyzed in several types of cancer, including lung cancer (8,12,13), breast cancer (14), squamous cell carcinoma of the head and neck (15), gastric cancer and colorectal cancer (16,17). miR-149 CT/CC genotype carriers had increased susceptibilities to colorectal cancer among those that engaged in smoke inhalation, whereas a significantly protective effect of miR-149 CT/CC on the incidence of gastric cancer exists among tea drinkers (16). However, to the best of our knowledge, no previous study has reported on the association

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Table I. Characteristics of patients and controls.

Characteristics	Patients	Controls	P-value
Mean age (mean ± SD, years)	46.6±11.1	47.2±10.5	0.588
Age (years)			
<45	77	112	0.631
≥45	81	130	
Gender			
Male	111	157	0.264
Female	47	85	
T stage <sup>a</sup>			
T1-T2	51	-	
T3-T4	102	-	
N stage <sup>a</sup>			
N0	9	-	
N1-N2-N3	144	-	
Metastasis <sup>a</sup>			
No	140	-	
Yes	10	-	
Clinical stage <sup>a</sup>			
I-II	10	-	
III-IV	145	-	

<sup>a</sup>The sum of certain characteristics does not equal the total number of patients due to unavailable data. SD, standard deviation.

between miR-149 polymorphism and NPC risk. To elucidate the NPC molecular mechanism, the association between SNP rs2292832 on miR-149 gene and risk of NPC was evaluated in this study.

## Materials and methods

**Study population.** This case-control study included 158 patients with nasopharyngeal carcinoma (NPC) and 242 healthy individuals undergoing routine medical examination without any medical illness in the Hunan Tumor Hospital between April and October, 2011. The patients were diagnosed via histopathological evidence. The subjects were unrelated ethnic Chinese adults, who were resident in Changsha City (Changsha, China) or the surrounding regions. Informed consent was obtained from all participants for the use of their blood samples in the present study. This study was approved by the institutional review board of Hunan Tumor Hospital.

**Genotyping assay.** The SNP rs2292832 on miR-149 gene, which was reported to be associated with tumor risk for genotyping was selected (8,16). Genomic DNA was extracted from the whole blood of all participants, then genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The PCR mixture consisted of 5 pmol of each primer, 1X GoTaq Master Mix (Promega Corporation, Madison, WI, USA) and 2.0 μl extracted DNA at a total volume of 15 μl. The PCR procedure included an initial melting step at 95°C for 5 min, then 30 cycles at 95°C for 30 sec, at 60°C for 30 sec, at

72°C for 1 min and a final extension step at 72°C for 10 min. The primers for PCR amplification were as follows (8): forward, 5'-TGT CTT CAC TCC CGT GCT TGT CC-3' and reverse, 5'-TGA GGC CCG AAA CAC CCG TA-3'. The PCR product of 254 base pairs (bp) (the CC genotype) was digested by *PvuII* (Promega Corporation) into fragments of 194 and 60 bp for TT and into fragments of 254, 194 and 60 bp for CT. PCR products were randomly selected for DNA sequencing validation.

**Statistical analysis.** The Hardy-Weinberg equilibrium was utilized to compare the observed with expected genotype frequencies in the control groups. Genotype and allele frequencies were compared between patients and controls using the  $\chi^2$  test. Odds ratios and 95% confidence intervals for the risk of NPC were calculated to estimate the relative risk using the multivariate logistic regression analysis adjusted by age and gender. The  $\chi^2$  test or Fisher's exact test was performed in a subsequent analysis of the association between the polymorphisms and clinical characteristics where appropriate. Statistical analyses were performed with SPSS 16.0 software.  $P < 0.05$  was considered to indicate a statistically significant difference and all statistical analyses were two-sided.

## Results

**Characteristics of patients with NPC and healthy controls.** A total of 400 samples were included in this case-control study, including 158 patients with NPC and 242 healthy controls (Table I). Age and gender did not show a statistically different

Table II. Genotype and allele distribution of rs2292832 in patients and controls.

Polymorphism	Patient	Control	$\chi^2$	P-value	OR	95% CI
rs2292832 T>C						
Genotype						
CC	22 (13.9)	39 (16.1)	1.702	0.427		
CT	67 (42.4)	113 (46.7)				
TT	69 (43.7)	90 (37.2)				
CT vs. TT			1.34	0.247	0.761 <sup>a</sup>	0.491-1.179
CC vs. TT			0.977	0.323	1.023 <sup>a</sup>	0.754-1.387
Allele						
C	111 (35.1)	191 (39.5)	1.53	0.216	0.831	0.619-1.115
T	205 (64.9)	293 (60.5)				

<sup>a</sup>Data were calculated by unconditional logistic regression with adjustment for age and gender. OR, odd ratio; CI, confidence interval.

Table III. Clinicopathological characteristics and the genotypes of rs2292832 in nasopharyngeal carcinoma patients.

Parameters	rs2292832			P-value	P-value (CC vs. TT)
	CC	CT	TT		
Age (years)					
<45	8 (10.7)	28 (37.3)	39 (52.0)	0.121	0.099
≥45	14 (16.9)	39 (47.0)	30 (36.1)		
Gender					
Male	16 (14.0)	50 (43.9)	48 (42.1)	0.803	0.777
Female	6 (13.6)	17 (38.6)	21 (47.7)		
T stage					
T1-T2	7 (13.7)	25 (49.0)	19 (37.3)	0.386	0.757
T3-T4	15 (14.9)	38 (37.6)	48 (47.5)		
N stage					
N0	3 (33.3)	4 (44.4)	2 (22.2)	0.182	0.094
N1-N2-N3	19 (13.3)	59 (41.3)	65 (45.5)		
Metastasis					
No	21 (15.1)	57 (41.0)	61 (43.9)	0.884	1.000
Yes	1 (10.0)	4 (40.0)	5 (50.0)		
Clinical stage					
I-II	4 (44.4)	3 (33.3)	2 (22.2)	0.026	0.030
III-IV	18 (12.4)	61 (42.1)	66 (45.5)		

distribution in patients and controls. Most patients were in the late stage disease as shown in clinical stage (Table I).

*Distribution of genotype and allele frequencies and their association with risk of NPC.* Genotype distribution of the SNP rs2292832 in the control group was in accordance with the Hardy-Weinberg ( $P>0.05$ ), suggesting the genetic equilibrium of the population enrolled (Table II). The frequencies of genotype CC, CT and TT of rs2292832 were 13.9, 42.4 and 43.7%, respectively, in the patient group, and 16.1, 46.7 and 37.2%, respectively, in the control group. The allele frequen-

cies for C and T were 35.1 and 64.9%, respectively, in the patient group, and 39.5 and 60.5%, respectively, in the control group. The SNP rs2292832 on miR-149 showed no significant difference between NPC patients and healthy controls in either genotype ( $P=0.427$  for CC vs. CT vs. TT,  $P=0.247$  for CT vs. TT and  $P=0.323$  for CC vs. TT, respectively, Table II) or allelic analysis ( $P=0.216$ , Table II) for the comparison models.

*Association between genotypes and clinicopathological characteristics of NPC.* We analyzed the clinicopathological characteristics to investigate whether there was any association

between the genotype distribution and NPC progression. The clinicopathological parameters for comparison including primary tumor extension, regional lymph node status, metastatic status and clinical stages were dichotomized as follows: The primary tumor extension, T1-T2 vs. T3-T4; the regional lymph node status N0 vs. N1-N2-N3; the metastatic status, negative vs. positive and the clinical stages I-II vs. III-IV. No significant difference was found between the genotypes and clinicopathological parameters examined, with the exception of clinical stage. A significant higher CC distribution in clinical stage I-II vs. III-IV was observed under the dominant model (CC vs. CT vs. TT,  $P=0.026$ , Table III) and the co-dominant model (CC vs. TT,  $P=0.030$ , Table III).

## Discussion

miR-149 was reported to be downregulated in various types of cancer, including squamous cell carcinoma of tongue (18), clear cell renal cell carcinoma (19), prostate carcinoma (20) and astrocytomas (21). A pattern of six miRNAs containing miR-149 received an overall correction rate of 80% in the diagnosis of prostate carcinoma (20). miR-149 expression levels were negatively correlated with the WHO grade in astrocytomas (21). Overexpression of miR-149 inhibited glioblastoma cell proliferation and migration (21). miRNA-149 inhibited the proliferation and cell cycle progression in human gastric cancer (22). These results suggested miR-149 was a tumor suppressor. However, in NPC, an upregulated miR-149 level was reported in tumor tissue (23) and NPC cell lines (24). miR-149 promoted the proliferation, invasion and mobility of NPC cell lines (24). Thus, miR-149 appeared to function as a tumor promoter of NPC.

The single nucleotide polymorphism of miR-149 gene was first reported by Hu *et al* (8). These authors analyzed the relationship between the polymorphism and non-small cell lung cancer survival, however no significant association was identified. Association studies between miR-149 polymorphism and cancer risk were reported for several types of cancer. Significant associations were observed in colorectal and gastric cancer (16). In this study, we genotyped the SNP rs2292832 on miR-149 gene in 158 patients with NPC and 242 healthy controls. No associations were observed between NPC patients and healthy controls in either the genotype or allelic analysis for the comparison models. The result is similar to the studies conducted on lung cancer (8,12,13), breast cancer (14), squamous cell carcinoma of the head and neck (15), and colorectal cancer in a Korean population (17), where no association between miR-149 polymorphism with cancer risk was observed. In the study of squamous cell carcinoma of the head and neck by Liu *et al* (15), a combined risk genotype of four SNPs on miR-146a, miR-149, miR-196a2 and miR-499 was found to be significantly associated with cancer risk, although miR-149 polymorphism alone did not prove to be significant.

The association between genotypes and clinicopathological parameters of NPC was also analyzed. A significant higher CC distribution in clinical stage I-II compared with III-IV was observed for the dominant model (CC vs. CT vs. TT) and the co-dominant model (CC vs. TT). In the study of lung cancer, CC genotype of miR-149 demonstrated a significant

maximal expression in tumor tissue (13). A maximal, but not significant expression of CC genotype was observed in gastrointestinal cancer and other types of intestinal mucosal tissue samples (16). In that study on gastrointestinal cancer miR-149 CT/CC genotype carriers were found to have increased susceptibilities to colorectal cancer among those that engaged in smoke inhalation; whereas a significantly protective effect of miR-149 CT/CC on gastric cancer incidence exists among tea drinkers (16). The results suggested that the CC genotype was able to regulate the expression of miR-149 and therefore affect cancer risk or cancer progression.

In this study, no significant association was observed between miR-149 polymorphism and NPC risk. However, CC distribution was significantly higher in clinical stage I-II compared with III-IV in the clinicopathological parameter analysis. The results suggested that the CC genotype contributes to the progression and development, rather than the initiation of NPC.

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