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*Original Article*

# Correlation between OPN gene polymorphism and susceptibility to nasopharyngeal carcinoma

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**Abstract:** We studied the correlation between osteopontin (OPN) gene polymorphism and susceptibility to nasopharyngeal carcinoma (NPC) among Han population in Jiangsu Province. The case-control study design was used. From February 1st 2011 to February 20th 2014, 197 patients of NPC treated at our hospital were included. For the control group, 197 healthy volunteers receiving physical examination at the same hospital from January 1st 2013 to August 1st 2013 were randomly selected. Single base extension (SBE)-PCR and DNA sequencing were performed to detect single nucleotide polymorphisms (SNPs) at rs11728697 and rs4754 locuss in OPN gene for all cases. The haplotype frequency of the OPN gene was analyzed. At rs11728697 locus, the carriers of CT genotype had a higher risk of NPC than those of CC genotype (OR=1.56, 95% CI: 1.01~2.76, $X^2$=4.592, P=0.042). However, TT genotype was not indicative of a higher risk of NPC (OR=0.83, 95% CI: 0.46~1.58, $X^2$=0.043, P=0.876). Allele frequency at this locus was not significantly different between the two groups. At rs4754 locus, the two groups also showed no significant difference in allele and genotype frequencies or haplotype frequency of the OPN gene (P>0.05). In summary, among Zhuang population in Guangxi Province, CT genotype at rs11728697 locus in the OPN gene can increase the susceptibility to NPC, while SNP at rs4754 locus was not relevant.

**Keywords:** Nasopharyngeal carcinoma, osteopontin, polymorphism, haplotype, susceptibility

## Introduction

Nasopharyngeal carcinoma (NPC) is an epithelium-derived malignancy of the head and neck [1, 2]. NPC can be induced by combined action of environmental and genetic factors, and its incidence is rising in China [1, 2]. Susceptibility genes of NPC have been intensively studied in recent years [3-5]. Osteopontin (OPN) is an important cell adhesion molecule, which has close associations with tumor occurrence. Given its role in cell adhesion, migration and survival, tumor angiogenesis and metastasis, OPN gene can be used as the diagnostic marker [6-8]. Although the pathogenesis of NPC is not fully known, the role of cytokines in NPC attracts increasing attention [9, 10]. OPN is a phosphorylated glycoprotein expressed in various cells, which acts as the cell adhesion molecule. OPN upregulation has been noted in the plasma from cancer patients. OPN can inhibit anti-tumor immunity and apoptotic pathways of tumor cells [6-10]. OPN gene is mapped to the long arm of human chromosome 4, consisting of 7 exons and 6 introns. According to the literature, OPN is highly expressed in the plasma from NPC patients, which correlates positively with the malignancy degree and metastasis and usually predicts poor prognosis [11, 12]. It is uncertain whether OPN gene polymorphism correlates with NPC. This study discussed the correlation between SNP of the OPN gene and susceptibility to NPC among Chinese population in China.

## Subjects and methods

### *Subjects*

The case group consisted of 197 patients with NPC treated at Department of Otolaryn-

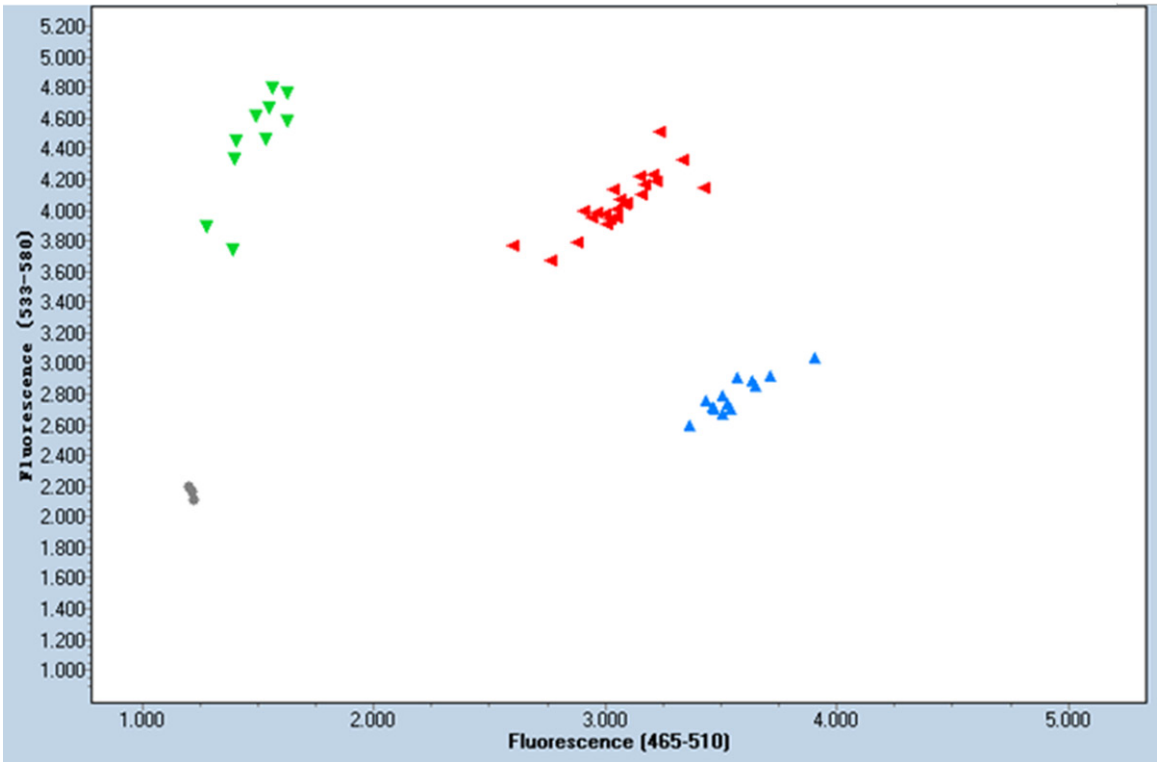


**Figure 1.** Genotyping of rs1172869 locus in the OPN gene (Green: CC genotype; Red: CT genotype; Blue: TT genotype).

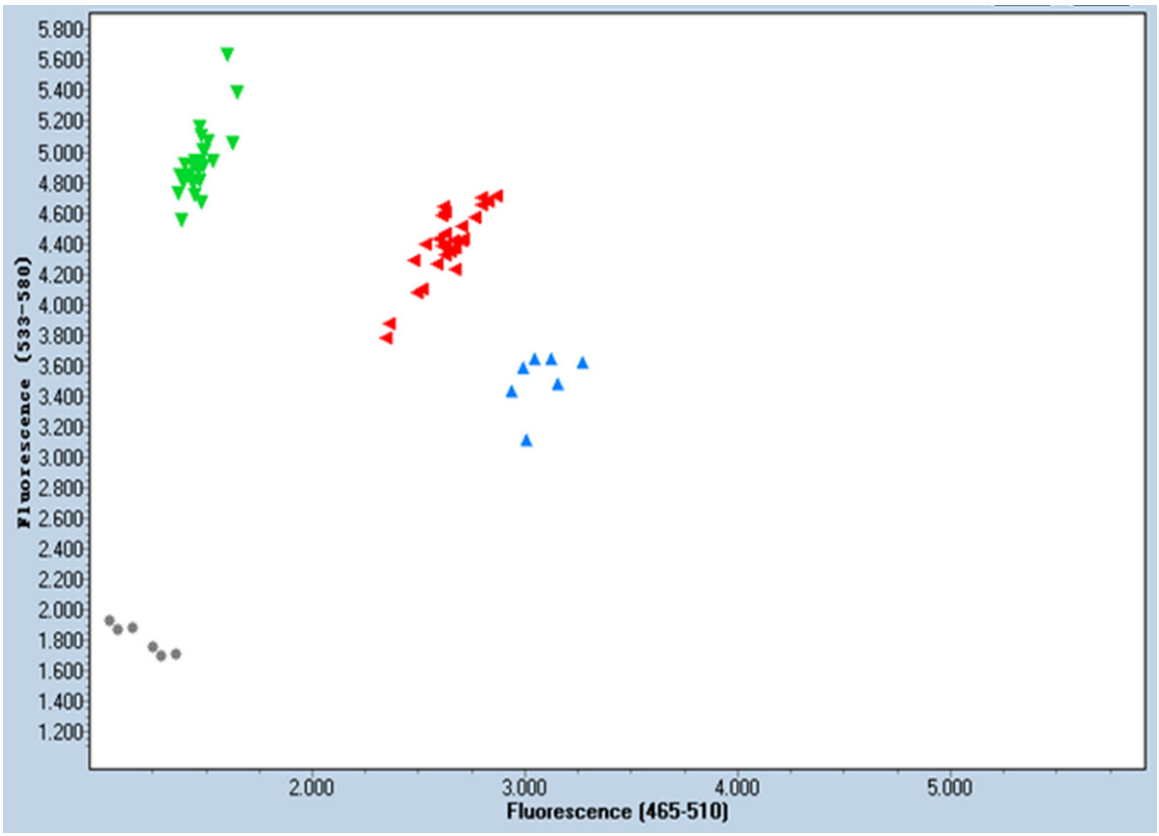


**Figure 2.** Genotyping of rs4754 locus in the OPN gene (Green: CC genotype; Red: CT genotype; Blue: TT genotype).

**Table 1.** SNPs at rs11728697 and rs4754 loci in the OPN gene and susceptibility to NPC [n (%)]

| SNPs | Control group (n=197) | NPC group (n=197) | OR (95% CI)[a] | $X^{2a}$ | P |
|---|---|---|---|---|---|
| Rs11728697 | | | | | |
| CC | 86 (43.7) | 62 (31.5) | 1.00 | | |
| CT | 77 (39.1) | 111 (56.3) | 1.56 (1.01~2.76) | 4.592 | 0.042 |
| TT | 34 (17.2) | 24 (12.2) | 0.83 (0.46~1.58) | 0.043 | 0.876 |
| C | 249 (63.2) | 235 (59.6) | 1.00 | | |
| T | 145 (36.8) | 159 (40.4) | 1.18 (0.61~1.38) | 0.745 | 0.513 |
| Rs4754 | | | | | |
| CC | 114 (57.9) | 115 (58.4) | 1.00 | | |
| CT | 74 (37.6) | 66 (33.5) | 0.75 (0.52~1.46) | 0.351 | 0.482 |
| TT | 9 (4.5) | 16 (8.1) | 1.72 (0.57~4.24) | 1.839 | 0.235 |
| C | 302 (76.6) | 296 (75.2) | 1.00 | | |
| T | 92 (23.4) | 98 (24.8) | 1.12 (0.61~1.53) | 0.158 | 0.572 |

Note: [a]corrected for the gender factor.

**Table 2.** Haplotype distributions of rs11728697 and rs4754 locuss in the OPN gene in the two groups [n (%)]

| Haplotypes | Control group (2n=394) | NPC group (2n=394) | OR (95% CI) | $X^2$ | P |
|---|---|---|---|---|---|
| CC | 163 (41.1) | 157 (39.5) | 1.00 | | |
| CT | 72 (18.1) | 83 (20.9) | 1.46 (0.82~2.13) | 2.645 | 0.142 |
| TC | 124 (31.2) | 142 (35.8) | 1.48 (0.91~1.84) | 3.638 | 0.125 |
| TT | 38 (9.6) | 15 (3.8) | 0.89 (0.42~1.86) | 0.231 | 0.649 |

gology, Kunshan Hospital of Traditional Chinese Medicine from February 2011 to February 2015 (129 males and 68 females, aged 24-92 years, with a mean of 50 ± 14 years and a median of 32 years).

Inclusion criteria for NPC patients: (1) people living in Jiangsu Province for 3 generations; (2) confirmed as NPC; (3) having received no radiotherapy or chemotherapy. The control group consisted of 197 healthy volunteers receiving physical examination at the same hospital from January 2013 to 2015 (112 males and 85 females, aged 25-84 years, with a mean of 52 ± 15 years and a median of 40 years). Inclusion criteria for controls: (1) People living in Jiangsu Province for 3 generations; (2) No current or past history of malignancies. The two groups differed insignificantly in age distribution or smoking status (P>0.05), but the difference was of statistical significance in terms of gender distribution (P<0.05). So the gender factor was considered in logistic regression. The experiment was approved by the ethics committee of Kunshan Hospital of Traditional Chinese Medicine and the informed consent was obtained.

*Genomic DNA extraction*

From each subject, 2 ml of venous blood was drawn. Genomic DNA extraction was performed using sodium iodide method and the DNA samples were preserved at -20°C.

*Genotyping methods*

We selected two SNPs (rs11728697 and rs4754) in the present study. The genotyping was performed according to the instruction of TaqMan Kit for genotyping as described previously [13].

*Statistical process*

All statistical analyses were carried out using SPSS 17.0 and SHEsis software. Sample means were compared using t-test and Hardy-Weinberg equilibrium was tested to see whether the samples were representative of the population. $X^2$ test was adopted to compare the differences in genotype and allele frequencies between the two groups. Dichotomous unconditional Logistic regression was performed to assess the correlation between SNPs and vulnerability to NPC. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated and corrected for the gender factor. SHEsis software was used for haplotype analysis with a=0.05.

## Result

*SNPs of the OPN gene*

As shown in **Figures 1** and **2**, the different genotype of rs1172869 and rs4754 were identified from three color dots.

*Testing of Hardy-Weinberg equilibrium*

The genotype and allele frequencies at rs1172869 and rs4754 locuss are shown in

**Table 1**. The genotype distributions at these two loci (P=0.406, P=0.722) obeyed Hardy-Weinberg equilibrium in the control group, which indicated good representativeness of the samples.

*Correlation between SNPs of the OPN gene and susceptibility to NPC*

As shown in **Table 1**, at rs1172869 locus, CC genotype is taken as the common genotype with OR=1.00. CT genotype contributed significantly to the susceptibility (OR=1.56, 95% CI 1.01~2.76, $X^2$=4.592, P=0.042), while TT genotype was irrelevant. As compared with C allele, T allele did not increase the susceptibility to NPC. At rs4754 locus, CC genotype is also taken as the common genotype with OR=1.00.

*Correlation between haplotype frequency of the OPN gene and susceptibility to NPC*

Haplotype frequency distributions at the two loci were analyzed using SHEsis in the two groups. As shown in **Table 2**, four haplotypes were identified, namely, CC, CT, TC and TT. The frequencies of these four haplotypes did not differ significantly between the two groups (P>0.05).

## Discussion

The present study identified SNPs of the OPN gene, which may affect gene transcription and expression and promote tumor occurrence. It is inferred that OPN gene SNPs correlates with susceptibility to NPC.

Lee et al. [14] detected OPN gene polymorphisms in 146 gastric cancer patients and 128 healthy controls. The results showed that TC or CC genotype at SNP-443 locus along with T/T or T/G genotype at SNP-616 locus increased the susceptibility to gastric cancer. Chiu et al. [15] reported that the frequency of insGG/insGG genotype at SNP-156 locus in the OPN gene was significantly higher among patients with oral squamous cell carcinoma than among the healthy population in Taiwan. We found that CC, CT and TT genotypes or C and T alleles at rs4754 locus did not increase the susceptibility to NPC. At Rs1172869 locus, CC and TT genotypes or C and T alleles did not increase the susceptibility to NPC either. However, CT genotype correlated with a higher risk of NPS, with OR=1.56, 95% CI 1.01~2.76. The haplotype analysis indicated that the frequencies of the four haplotypes at the two loci did not differ significantly between the two groups.

Susceptibility genes play an important role in the occurrence of NPC. According to our results, CT genotype at rs1172869 locus in the OPN gene may increase the susceptibility to NPC. However, SNPs at the rs4754 locus were irrelevant. To further elucidate the influence of OPN gene SNPs on NPC on the molecular level, more experiments are needed.

## Disclosure of conflict of interest

None.

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