The contribution of *interleukin-8* genotypes and expression to nasopharyngeal cancer susceptibility in Taiwan

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**Abstract**

The incidence rate of nasopharyngeal cancer (nasopharyngeal carcinoma [NPC]) is much higher in Southeast Asia than in western countries. *Interleukin-8* (IL-8), a chemokine produced by macrophages, epithelial cells, airway smooth muscle cells, and endothelial cells, is an important immuno-mediator in the development and progression of many types of cancer. Genetic variations in IL-8 have been associated with the risks of NPC and other cancers. In the current study, we evaluated the role of IL-8 in NPC at the levels of DNA, RNA, and protein in a Taiwanese population. First, in a case-control study, 176 NPC patients and 352 cancer-free controls were genotyped, and the associations of IL-8 T – 251A, C + 781T, C + 1633T, and A + 2767T polymorphisms with NPC risk were evaluated. Second, the NPC tissue samples were assessed for their IL-8 mRNA and protein expression by real-time quantitative reverse transcription polymerase chain reaction (PCR) and Western blotting, respectively. Regarding the IL-8 promoter T – 251A, the TA and AA genotypes were associated with significantly decreased risks of NPC compared with the wild-type TT genotype (adjusted odds ratio = 0.61 and 0.52, 95% confidence interval = 0.47–0.93 and 0.37–0.91, \(P = 0.0415\) and 0.0289, respectively). The mRNA and protein expression levels for NPC tissues revealed no significant associations among the 20 NPC samples with different genotypes. These findings suggest that IL-8 may play an important role in the carcinogenesis of NPC in Taiwan.

**Abbreviations:** ASR = age-standardized incidence rate, CI = confidence interval, EBV = Epstein–Barr virus, ECL = enhanced chemiluminescence, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, IL-8 = interleukin-8, NPC = nasopharyngeal carcinoma, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, RIPA = radio immunoprecipitation assay, SDS-PAGE = sodium dodecyl sulphate polyacrylamide gel electrophoresis, SNP = single nucleotide polymorphism.

**Keywords:** genotype, IL-8, nasopharyngeal cancer, polymorphism, Taiwan.

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1. Introduction

Nasopharyngeal carcinoma (NPC) is a relatively rare cancer in Western and most countries (age-standardized incidence rate [ASR] of <1/100,000), but its incidence rates are much higher in southern China (ASR 30–50/100,000), Southeast Asia (ASR 9–12/100,000), and Taiwan (ASR 8.2–8.4/100,000).\(^{[1–3]}\) This geographical pattern of NPC incidence suggests an interaction of complicated environmental and genetic factors. The epidemiologic factors that have been associated with increased risks of NPC included Epstein–Barr virus (EBV) infection,\(^{[4]}\) tobacco smoking,\(^{[5–8]}\) occupational exposure,\(^{[9]}\) and unhealthy dietary habits.\(^{[10]}\) Previous association studies have indicated that genetic susceptibility also plays an important role in the etiology of NPC.\(^{[11–15]}\)

*Interleukin-8* (IL-8) is produced by a wide variety of normal cells, including macrophages, epithelial cells, airway smooth muscle cells, endothelial cells, as well as tumor cells. It plays a critical role in the initiation and amplification of acute inflammatory reactions. IL-8 is a major mediator of inflammation, acting as a chemoattractant for neutrophils, basophils, and T cells.\(^{[16]}\) IL-8 has been reported to overexpress in various human malignancies,\(^{[17–19]}\) and in saliva of patients with oral cancer.\(^{[20]}\) Additionally, elevated levels of IL-8 has been reported to correspond to an increased disease severity such as the metastatic potential of melanoma,\(^{[21]}\) breast,\(^{[22]}\) ovarian,\(^{[23]}\) renal,\(^{[24]}\) prostate,\(^{[25]}\) pancreatic,\(^{[26]}\) gastric,\(^{[27,28]}\) and colorectal cancers.\(^{[29,30]}\) Furthermore, IL-8 overexpression can cause disease progression of bladder cancer\(^{[31]}\) and prostate cancer.\(^{[32]}\)
In the center of solid tumors under hypoxic microenvironments, IL-8 expression may help cancer cells to proliferate, survive, and escape programmed cell deaths.[26] To sum up, IL-8 is closed involved in cancer development and progression.

IL-8 gene locates in 4q12-q13 of human genome, consisting of 4 exons.[33] The IL-8 single nucleotide polymorphisms (SNPs) at promoter region A – 251T (rs4073) and C + 781T (rs2227306) have been reported to affect IL-8 expression.[34–36] Previously studies have investigated the associations of IL-8 SNPs with the risks of many cancers including NPC.[37–41] However, the role of IL-8 polymorphisms in NPC ethology in Taiwanese population have not been reported. Thus, in the present study, we performed a case-control study to evaluate the impacts of IL-8 SNPs on the susceptibility of NPC in Taiwan.

2. Materials and methods

2.1. Study population

One hundred and seventy-six patients diagnosed with NPC were recruited at the general surgery outpatient clinics of the study hospital in Taichung, Taiwan, between 2003 and 2009. All patients participated voluntarily, completed a self-administered questionnaire, and provided peripheral blood samples. The questionnaire included questions on history and frequency of alcohol consumption, betel quid chewing, and smoking habits, and “ever” was defined as more than twice a week for at least 1 year. Self-reported alcohol consumption, betel quid chewing, and smoking habits were evaluated and classified as categorical variables.

For each case patient, 2 age- and gender-matched healthy controls, who had no NPC or other types of cancer, were selected from those attending the hospital for a health examination (age matching was done within less than 5 years of the case patient’s first diagnosis). These volunteers attended the hospital for regular health assessments by multidisciplinary team approach with registered health practitioners during the years 2002 to 2012; most of the volunteers underwent health examinations every 5 to 6 months. Finally, 352 participants were included for analysis in the present study. The overall agreement rate in this study was more than 85% in collection. The study was approved by the institutional review board of the medical university hospital (DMR101-IRB1-306).

2.2. Genotyping protocols

Genomic DNA from the peripheral blood leucocytes of each investigated subject was prepared using the QIAamp Blood Mini Kit (Qiagen, Valencia, CA), further stored in –80°C and subject to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology as previously described.[12–44] The PCR cycling conditions were: one cycle at 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 10 minutes. The sequences of forward and reverse primers and the restriction enzymes for the investigated SNP are summarized in Table 2. The genotype analysis was performed by 2 researchers independently and blindly. About 5% of the samples for each SNP were randomly selected for direct sequencing and the results from PCR-RFLP and direct sequencing were 100% concordant.

2.3. Interleukin-8 mRNA expression pattern

To evaluate the correlation between IL-8 mRNA expression and IL-8 polymorphism, 20 surgically removed NPC tissue samples obtained from sites adjacent to tumors with different genotypes were subjected to extraction of the total RNA using Trizol Reagent (Invitrogen, Carlsbad, CA). Total RNA was measured by real-time quantitative RT-PCR using an FTC-3000 real-time quantitative PCR instrument (Funglyn Biotech Inc., Canada). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal quantitative control. The primers used for amplification of IL-8 mRNA were forward 5’-AAACCACCGGAGGGACCATG-3’ and reverse 5’-GCCACGGTGGAGT-CATGT-3’, while for GAPDH the primers were forward 5’-GAAATCCCATCACCCATCCGAGG-3’ and reverse 5’-GAGCCCCAGCCCTTCCATCATCTG-3’. Fold changes were normalized using the levels of GAPDH expression, and each assay was done at least in triplicate as previously published.[12,46]

2.4. Western blotting analysis

The NPC specimens were homogenized in radio immunoprecipitation assay (RIPA) lysis buffer (Upstate Biotechnology Inc., Lake Placid, NY), the homogenates were centrifuged at 10,000 X g for 30 minutes at 4°C, and the supernatants were used for Western blotting. Samples were denatured by heating at 95°C for 10 minutes, were separated on a 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel, and were then transferred to a nitrocellulose membrane (BioRad Laboratories, Hercules, CA). The membrane was blocked with 5% non-fat milk and incubated over-night at 4°C with mouse monoclonal anti-human IL-8 antibody (1:1000; BD Transduction Laboratories; BD Biosciences, Franklin Lakes, NJ), and then with the corresponding horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (Chemicon, Temecula, CA) for 1 hour at room temperature. After reaction with enhanced chemiluminescence (ECL) solution (Amersham, Arlington Heights, IL), bound antibody was visualized using a chemiluminescence imaging system (Syngene, Cambridge, UK). Finally, the blots were incubated at 56°C for 18 minutes in stripping buffer (0.0626 M Tris–HCl, pH 6.7, 2% SDS, 0.1 M mer-captopropanol) and re-probed with a mono-clonal mouse anti-β-actin antibody (Sigma, St. Louis, MO) as the loading control. The optical density of each specific band was measured using a computer-assisted imaging analysis system (GeneTools Match software; Syngene).

2.5. Statistical analyses

All 352 controls and 176 NPC cases with genotypic and clinical data were analyzed. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of IL-8 SNPs in the control subjects from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s Chi-square test was used to compare the distribution of the IL-8 genotypes between cases and controls. The comparison of the age between control and case group was performed by Student’s t test. The associations between the IL-8 polymorphisms and NPC risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis with the adjustment for possible confounders. The STATA program was used for haplotype analysis. Any P < .05 was considered statistically significant.

3. Results

3.1. Comparisons of basic characters between the case and control groups

The frequency distributions of age, gender, personal behavioral habits for the 176 NPC patients, and 352 non-cancer controls are
summarized in Table 1. The cases and controls were matched on
age and gender. There were no significant differences between
the cases and controls in the distributions of personal behavioral
habits including smoking, alcohol drinking, and areca quid
chewing status (Table 1).

### 3.2. Association analysis of IL-8 genotypes and NPC risk

The distributions of the IL-8 promoter T→251A, C+781T, C+1633T, and A+2767T genotypes among the cases and controls
are presented and statistically analyzed in Table 2. The genotypes
of IL-8 promoter T→251A SNP were differently distributed
between cases and controls (P for trend= .0394) (Table 3 top
panel). In detail, the IL-8 promoter T→251A heterozygous TA
and homozygous AA variant genotypes were associated with
decreased NPC risks (OR=0.66 and 0.56, 95% CI=0.44–0.99
and 0.33–0.94, P=0.0415 and 0.0289, respectively) (Table 3 top
panel). In the dominant model, there was a significant association
between the variant genotypes (TA+AA) and NPC risk (OR=0.63,
95% CI=0.43–0.91, P=0.0134). The significant findings were
still observed after adjusting for the potential confounders
including age, gender, smoking, alcohol drinking, and areca
chewing habits (Table 3 top panel). No significant associations
were observed for the other 3 investigated SNPs Table 3.

We also performed allelic analysis. Supporting the findings in
Table 3, the results showed that the variant allele A was 34.9% in
the NPC patient group, significantly much lower than that
(43.2%) in the control group (adjusted OR=0.75, 95% CI=0.59–0.94,
P=0.0101). Again, the other 3 SNPs were not significantly associated with NPC risks (Table 4).

### 3.3. Stratified analysis of IL-8 genotypes by environmental factors

We then performed stratified analyses of IL-8 genotypes with
NPC risks by potential environmental risk factors, including
cigarette smoking, alcohol drinking, and areca chewing habits.
The adjusted ORs for carriers with genotype of TA or AA at IL-8
promoter T→251A were 0.68 and 0.71 among non-smokers
(95% CI=0.41–1.12 and 0.43–1.33, respectively), and were
0.71 and 0.42 among smokers (95% CI=0.43–1.23 and 0.19–
0.93, respectively) (Table 3). The interaction analysis did not
show a significant interaction. Likewise, there was no significant
interaction between IL-8 T→251A genotypes and alcohol
drinking or areca chewing habits in modulating NPC risks (data
not shown).

### 3.4. Correlation between IL-8 T→251A genotype and the
expression levels of IL-8 mRNA and proteins

Finally, 20 surgically removed NPC tissue samples were collected
from sites adjacent to tumors for this analysis. Among these
tissues, 10 were of IL-8 T→251A genotype TT, 8 were of TA,
and 2 were of AA. The mRNA expression levels of IL-8 in these
patients were examined by real-time quantitative RT-PCR
(Fig. 1). The levels of IL-8 mRNA for the TA and AA genotypes
were 0.85- and 0.81-fold compared with those of the TT
genotype. Combining TA and AA genotypes and compared to TT
genotype, there was no significant difference in the mRNA levels
of IL-8 (P=.4253). We also examined the IL-8 protein
expression levels at the tumor sites of the same NPC patients
by Western blotting (Fig. 2A) and did not find significant

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### Table 1

Demographic characteristics of investigated 176 nasopharyngeal carcinoma patients and 352 non-cancer healthy controls.

| Characteristics | Controls (n = 352) | Cases (n = 176) | P value |
|-----------------|-------------------|----------------|---------|
| Age (y)         |                   |                |         |
|                 | 0.7138            | 1.0000         |         |
| Gender          |                   |                |         |
| Male            | 256 72.7          | 128 72.7       |         |
| Female          | 96 27.3           | 48 27.3        |         |
| Behavioral habits |                 |                |         |
| Cigarette smoker| 150 42.6          | 73 41.4        | .8519   |
| Alcohol drinker | 124 35.2          | 72 40.9        | .2150   |
| Areca chewer    | 115 32.7          | 54 30.7        | .6926   |

SD = standard deviation.

* Based on Chi-square test or Student’s t test.

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### Table 2

Summary of the primers, restriction enzymes and amplicon size after enzyme cutting for *interleukin-8* genotyping PCR-RFLP conditions.

| Polymorphic site | Primer sequences | Restriction enzyme | Amplicon size after cutting, bp |
|------------------|------------------|--------------------|-------------------------------|
| IL-8 -251        | Forward 5'-TCATCAGATGCTGGTCTA-3' | MfeI              | T: 524                        |
|                  | Reverse 5'-GAAAAAGCTGCTGCTAGA-3' |                 | A: 440 + 75                   |
| IL-8 +781        | Forward 5'-CTGAATCTTATAGGATCA-3' | EcoRI             | T: 203                        |
|                  | Reverse 5'-CGTACTTATACACGAACG-3' |                 | C: 184 + 19                   |
| IL-8 +1633       | Forward 5'-CTGAGGAGGACTCCTGT-3'  | NtflI             | T: 397                        |
|                  | Reverse 5'-CTTGAAGATGTCTATATT-3' |                 | C: 234 + 163                  |
| IL-8 +2767       | Forward 5'-CACCTTAAATATTCTATTC-3' | BstZ171I          | A: 222                        |
|                  | Reverse 5'-CAACAGGAAGAAGAATTACTA-3' |                 | T: 198 + 24                   |
### Table 4

Alloic frequency analysis for **interleukin-8 (IL-8)** polymorphisms and nasopharyngeal carcinoma.

| Allele          | Controls n (%) | Patients n (%) | aOR (95% CI) \(^*\) | P value \(^*\) |
|-----------------|----------------|----------------|---------------------|--------------|
| **IL-8 -251**   |                |                |                     |              |
| T               | 400 (56.8)     | 229 (65.1)     | 1.00 (Reference)    |              |
| A               | 304 (43.2)     | 123 (34.9)     | 0.75 (0.59–0.94)    | .0101 \(^*\) |
| **IL-8 +781**   |                |                |                     |              |
| C               | 428 (60.8)     | 232 (65.9)     | 1.00 (Reference)    |              |
| T               | 276 (39.2)     | 120 (34.1)     | 0.81 (0.64–1.06)    | .1056        |
| **IL-8 +1633**  |                |                |                     |              |
| C               | 400 (56.8)     | 199 (56.5)     | 1.00 (Reference)    |              |
| T               | 304 (43.2)     | 153 (43.5)     | 0.99 (0.79–1.31)    | .9300        |
| **IL-8 +2767**  |                |                |                     |              |
| A               | 405 (57.5)     | 204 (58.0)     | 1.00 (Reference)    |              |
| T               | 299 (42.5)     | 148 (42.0)     | 0.97 (0.77–1.24)    | .8949        |

\(^*\) Confidence interval.

\(^*\) The ORs were estimated with multivariate logistic regression analysis after adjusted with age, gender, smoking, alcohol drinking and areca chewing habits.

\(^*\) Statistically identified as significant based on Chi-square test without Yates' correction.
difference of IL-8 protein expression among different IL-8 T–251A genotypes either (P=.2197) (Fig. 2B).

3.5. Interaction of IL-8 T–251A genotype and the EBV infection status on NPC risk

EBV infection was reported to be associated with NPC development and clinical outcomes in Taiwan.[47–50] However, the early predictive rates were of very wide range in accuracy according to the detection methodology in the targets including LMP-1, EBNA-1, EBNA-2, which are still in development.[47,48,51] In our investigated population, 145 of 176 NPC patients have the complete records in their detectable plasma EBV DNA, and the distributions of their TT, AT, AA genotypes at IL-8 – 251 were 27 (46.6%), 22 (37.9%), 9 (15.5%) in EBV positive NPC patients and 36 (41.4%), 36 (41.4%), 15 (17.2%) in EBV negative NPC patients. The results showed that there was not a positive interaction between IL-8 – 251 genotypes and EBV infection (P=.8269). The control subjects were lacking of their EBV infection status that the effects of EBV infection on IL-8 T–251 genotypes as for early prediction of NPC could not be evaluated in this study (Table 6).

3.6. Haplotype of IL-8 genotypes and stratified analysis by environmental factors

We have performed the IL-8 T–251A-C+781T-C+1633T-A+2767T haplotype analysis, finding that the haplotypes of IL-8 T–251A-C+781T-C+1633T-A+2767T were differentially distributed between case and control groups (P=.0221). Among the
haplotypes for IL-8 T−251A-C+781T-C+1633T-A+2767T, the distributions of ATCA haplotype were significantly of lower percentage for the cases than the controls (adjusted OR = 0.47, 95% CI = 0.25–0.92). Furthermore, the haplotypes of IL-8 T−251A-C+781T-C+1633T-A+2767T were differentially distributed among the subgroups of non-smokers (P = 0.0336) and smokers (P = 0.0117). Most interesting, the smokers of ATCA haplotype for IL-8 T−251A-C+781T-C+1633T-A+2767T were of significantly lower risk of NPC (adjusted OR = 0.31, 95% CI = 0.14–0.93) (Table 7). There were no any significant differences in the cases while analyzing the interaction between status of alcohol drinking, betel quid chewing, and IL-8 T−251A-C+781T-C+1633T-A+2767T haplotypes (data not shown). The protective effects of IL-8−251A and +781T seemed to be additive for those carrying ATCA haplotype, especially among those smokers.

4. Discussion

In the current study, the role of IL-8 in NPC was evaluated from the levels of DNA, RNA, and protein. The contributions of IL-8 promoter T−251A, C+781T, C+1633T, and A+2767T SNPs to NPC risk were evaluated and the results showed that the TA and AA genotypes of T−251A were significantly associated with the risks of NPC. This study is the first to analyze the association between IL-8 T−251A genotype and NPC susceptibility.

It has been reported that chronic smoking and EBV infection contributed to the etiology of NPC development and decreased the survival rates of the patients,[52–54] but the detailed mechanisms are not clear. In this study, we found that IL-8 promoter T−251A genotypes were associated to NPC risk, suggesting that IL-8 may mediate the effect of infection and inflammation on NPC development. The IL-8 promoter T−251A SNP has been studied extensively previously in relation to

| Table 5 | Odds ratios for interleukin-8 (IL-8) promoter -251 genotype and nasopharyngeal carcinoma after stratified by smoking status. |
|---------|--------------------------------------------------------------------------------------------------|
| Genotypes | Non-smokers | Smokers |
|          | Controls | Cases | aOR (95% CI) | Controls | Cases | aOR (95% CI) |
| TT       | 68       | 45   | 1.00 (Reference) | 53       | 35   | 1.00 (Reference) |
| AT       | 91       | 38   | 0.68 (0.41–1.12) | 67       | 31   | 0.71 (0.43–1.23) |
| AA       | 43       | 20   | 0.71 (0.43–1.33) | 30       | 8    | 0.42 (0.19–0.93) |
| Total    | 202      | 103  |                 | 150      | 73   |                 |

CI = confidence interval.

* The ORs were estimated with multivariate logistic regression analysis after adjusted with age, gender, alcohol drinking and areca chewing habits.
cancer risk and the results were heterogeneous.\textsuperscript{137} Four studies have been published with regard to this SNP and risks of NPC, 1 in African population,\textsuperscript{40} 2 in Chinese population,\textsuperscript{38,39} and 1 in Europeans population.\textsuperscript{41} The meta-analysis of these 4 studies showed that the variant genotypes were associated with increased risks of NPC. The discrepancy of our results to the previous studies may have been attributed to different populations, different exposures, or the relative small sample sizes of all these publications. Future large validation studies are needed to clarify the role of IL-8 promoter T251A/T SNP with NPC risk in different populations.

One limitation of this study is that we defined ever smokers as those who smoked more than twice a week for at least 1 year. This is not a traditional definition, which may have obscured the true association of smoking and NPC risk and resulted in the lack of interaction between IL-8 genotypes and smoking in elevating NPC risk. Future larger studies with detailed smoking information are warranted to clarify the interaction between IL-8 genotypes and smoking status in modulating NPC risk.

The transcriptional and translational impacts of different genotypes at IL-8 promoter T–251A were investigated in this current study but no correlation between genotypes and gene expression was found. Also, the serum level of IL-8 was detected in 20 NPC patients, and the levels were not differentially distributed among patients of IL–8 – 251 TT, TA, and AA genotypes, similar to those at mRNA and protein levels. Further, we have stratified them according to the EBV infection, finding that there was neither correlation between IL–8 – 251 genotypes and IL–8 serum level nor no interaction between EBV infection status with IL–8 – 251 genotypes on IL–8 serum level. Only 20 samples from NPC patients may have limited our power to find significant correlations between genotypes and gene expression. In addition, the tissue samples from normal subjects were not available for analysis. Further investigations of IL–8 mRNA and/or protein expression in relation to genotypes are warranted. In addition, the enlargement of the sample size is encouraged in the future and may alter the current conclusion.

In conclusion, our study provided evidence that the TA and AA genotypes at IL–8 promoter T–251A SNP are associated with decreased risks of NPC in Taiwan, supporting a role of inflammation in the etiology of NPC. Importantly, the novel genomic biomarkers can add to the traditional methodology depending on the EBV infection in NPC risk and prognosis outcome prediction. It would be valuable to investigate additional SNPs in other inflammatory mediators followed by mechanistic study to understand the roles of inflammation in NPC pathogenesis.

### Table 6

| Distribution of interleukin-8 (IL-8) genotypes among the EBV positive and negative nasopharyngeal carcinoma patients. |
|---------------------------------------------------------------|
| **EBV positive** | **EBV negative** | **OR (95% CI)\textsuperscript{*}** | **aOR (95% CI)\textsuperscript{*}** | **P value\textsuperscript{*}** |
| IL–8 – 251 | | | | |
| TT | 27 | 46.6% | 36 | 41.4 | 1.00 (Reference) | 1.00 (Reference) | .8269 |
| TA | 22 | 37.9% | 36 | 41.4 | 0.81 (0.39–1.69) | 0.77 (0.43–1.59) | .5813 |
| AA | 9 | 15.5% | 15 | 17.2 | 0.80 (0.30–2.10) | 0.73 (0.42–1.96) | .6502 |
| \(P\text{value}\) | | | | | | | |
| Haplotype | Carrier comparison | TT + TA | 49 | 84.5% | 72 | 82.8 | 1.00 (Reference) | 1.00 (Reference) | .7843 |
| | AA | 9 | 15.5% | 15 | 17.2 | 0.88 (0.36–2.17) | 0.82 (0.40–2.01) | .1392 |
| | TT | 27 | 46.6% | 36 | 41.4 | 1.00 (Reference) | 1.00 (Reference) | .8269 |
| | TA + AA | 31 | 53.4% | 51 | 58.6 | 0.81 (0.41–1.58) | 0.78 (0.46–1.49) | .5382 |

\* Adjusted with age, gender, smoking, alcohol drinking and areca chewing habits.

\textsuperscript{*} Statistically identified as significant based on Chi-square test without Yates’ correction.

### Table 7

| Distribution of interleukin-8 (IL-8) haplotypes among nasopharyngeal carcinoma patients and control subjects after stratified by smoking status. |
|---------------------------------------------------------------|
| **Haplotypes** | **Controls** | **Cases** | **aOR (95% CI)\textsuperscript{*}** | **Non-smokers** | **Cases** | **aOR (95% CI)\textsuperscript{*}** | **Smokers** | **Cases** | **aOR (95% CI)\textsuperscript{*}** |
| | | | | | | | | | |
| TCCA | 39 | 26 | 1.00 (Reference) | 23 | 15 | 1.00 (Reference) | 16 | 11 | 1.00 (Reference) |
| ACCA | 32 | 12 | 0.53 (0.27–1.33) | 18 | 7 | 0.63 (0.33–1.86) | 14 | 5 | 0.48 (0.13–1.01) |
| TCTA | 30 | 19 | 0.97 (0.48–1.86) | 17 | 11 | 0.96 (0.42–2.32) | 13 | 8 | 0.91 (0.29–2.28) |
| TCCT | 28 | 17 | 0.86 (0.43–1.70) | 16 | 10 | 0.88 (0.47–2.08) | 12 | 7 | 0.73 (0.34–1.77) |
| TTCA | 27 | 14 | 0.70 (0.41–1.21) | 15 | 8 | 0.82 (0.38–1.65) | 12 | 6 | 0.75 (0.29–1.94) |
| ACTA | 26 | 9 | 0.51 (0.33–1.04) | 14 | 6 | 0.64 (0.31–1.13) | 12 | 3 | 0.36 (0.29–2.28) |
| ATCA | 25 | 8 | 0.47 (0.25–0.92) | 13 | 5 | 0.48 (0.20–1.03) | 12 | 3 | 0.31 (0.14–0.93) |
| Others | 145 | 71 | 0.69 (0.39–1.10) | 86 | 41 | 0.61 (0.36–1.03) | 59 | 30 | 0.71 (0.35–1.47) |
| \(P\text{value}\) | | | | | | | | | |
| Total | 352 | 176 | 202 | 103 | 0.0221\textsuperscript{*} | 0.0336\textsuperscript{*} | 0.0117\textsuperscript{*} | | |

\* The ORs were estimated with multivariate logistic regression analysis after adjusted with age, gender, alcohol drinking and areca chewing habits, using the most common haplotype, TCCA, as the reference.

\textsuperscript{*} Haplotypes were composed of four polymorphic genotypes according to the sequences: T–251A, C + 781T, C + 1633T, and A + 2767T.

\(\text{CI}\) = confidence interval
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