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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*=.0415 and .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 ratios, 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 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 programed 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.[42–44] 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'-AAACCACCG-GAAGGAACCAT-3' and reverse 5'-GCCAGCTTGGAAGT-CATGT-3',[45] while for GAPDH the primers were forward 5'-GAAATCCCATCACCATCTTCCAGG-3' and reverse 5'-GAGCCCCAGCCTTCTCCATG-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-captoethanol) 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

Table 1

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

	Controls (n=352)			Cases (n=176)			
Characteristics	n	%	Mean (SD)	n	%	Mean (SD)	*P* value*
Age (y)			48.7 (10.8)			49.3 (9.4)	
	0.7138						
Gender							1.0000
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.

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 betel 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*=.0415 and .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*=.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*=.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 5). 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

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'-TCATCCATGATCTTGTTCTA-3'	*Mfe*I	T: 524
	Reverse 5'-GGAAAACGCTGTAGGTCAGA-3'		A: 449+75
IL-8+781	Forward 5'-CTCTAACTCTTTATATAGGA-3'	*Eco*RI	T: 203
	Reverse 5'-GATTGATTTTATCAACAGGC-3'		C: 184+19
IL-8+1633	Forward 5'-CTGATGGAAGAGAGCTCTGT-3'	*Nla*III	T: 397
	Reverse 5'-TGTTAGAAATGCTCTATATT-3'		C: 234+163
IL-8+2767	Forward 5'-CCAGTTAAATTTTCATTTCA-3'	*Bsf*Z171	A: 222
	Reverse 5'-CAACCAGCAAGAAATTACTA-3'		T: 198+24

Table 3

Distribution of *interleukin-8* genotypes among the nasopharyngeal carcinoma patients and non-cancer healthy control subjects.

	Controls		Patients				
	n	%	n	%	OR (95% CI)*	aOR (95% CI)*	*P* value†
IL-8 -251							
TT	121	34.4	80	45.5	1.00 (Reference)	1.00 (Reference)	
TA	158	44.9	69	39.2	0.66 (0.44–0.99)*	0.61 (0.47–0.93)*	.0415*
AA	73	20.7	27	15.3	0.56 (0.33–0.94)*	0.52 (0.37–0.91)*	.0289*
P_{trend}							.0394*
Carrier comparison							
TT+TA	279	79.3	149	84.7	1.00 (Reference)	1.00 (Reference)	
AA	73	20.7	27	15.3	0.69 (0.43–1.12)	0.67 (0.41–1.14)	.1356
TT	121	34.4	80	45.5	1.00 (Reference)	1.00 (Reference)	
TA+AA	231	65.6	96	54.5	0.63 (0.43–0.91)*	0.60 (0.48–0.88)*	.0134*
IL-8+781							
CC	132	37.5	79	44.9	1.00 (Reference)	1.00 (Reference)	
CT	164	46.6	74	42.0	0.75 (0.51–1.11)	0.77 (0.46–1.09)	.1566
TT	56	15.9	23	13.1	0.69 (0.39–1.20)	0.66 (0.41–1.18)	.1861
P_{trend}							.2500
Carrier comparison							
CC+CT	296	84.1	153	86.9	1.00 (Reference)	1.00 (Reference)	
TT	56	15.9	30	13.1	1.04 (0.64–1.68)	1.01 (0.59–1.63)	.8849
CC	132	37.5	79	44.9	1.00 (Reference)	1.00 (Reference)	
CT+TT	231	62.5	97	55.1	0.70 (0.49–1.01)	0.75 (0.53–1.09)	.0573
IL-8+1633							
CC	121	34.4	58	33.0	1.00 (Reference)	1.00 (Reference)	
CT	158	44.9	83	47.2	1.10 (0.73–1.65)	1.04 (0.69–1.53)	.6619
TT	73	20.7	35	19.8	1.00 (0.60–1.67)	0.97 (0.62–1.62)	.9993
P_{trend}							.8850
Carrier comparison							
CC+CT	279	79.3	141	80.2	1.00 (Reference)	1.00 (Reference)	
TT	73	20.7	35	19.8	0.95 (0.60–1.49)	0.93 (0.56–1.42)	.8190
CC	121	34.4	58	33.0	1.00 (Reference)	1.00 (Reference)	
CT+TT	231	65.6	118	67.0	1.07 (0.73–1.56)	1.02 (0.71–1.51)	.7452
IL-8+2767							
AA	128	36.4	60	34.1	1.00 (Reference)	1.00 (Reference)	
AT	149	42.3	84	47.7	1.20 (0.80–1.81)	1.14 (0.83–1.73)	.3738
TT	75	21.3	32	18.2	0.91 (0.54–1.52)	0.95 (0.52–1.44)	.7203
P_{trend}							.4699
Carrier comparison							
AA+AT	277	78.7	144	81.8	1.00 (Reference)	1.00 (Reference)	
TT	75	21.3	32	18.2	0.82 (0.52–1.30)	0.85 (0.57–1.24)	.3997
AA	128	36.4	60	34.1	1.00 (Reference)	1.00 (Reference)	
AT+TT	224	63.6	116	65.9	1.10 (0.76–1.62)	1.03 (0.77–1.56)	.6072

CI = confidence interval.

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

† Statistically identified as significant based on Chi-square test without Yates' correction.

Table 4

Allelic 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

CI = 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.

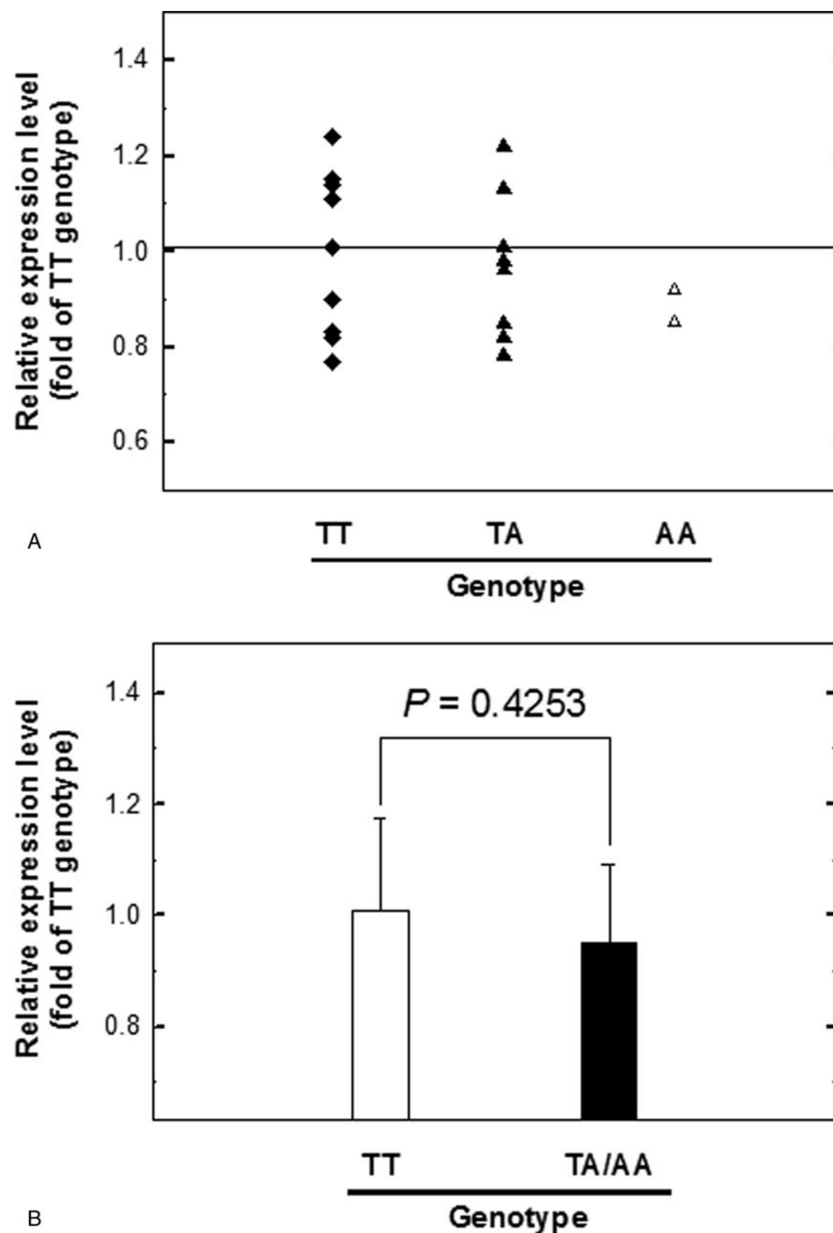

Figure 1. Analysis of *IL-8* mRNA expression levels among nasopharyngeal carcinoma (NPC) patients. (A) Quantitative RT-PCR of NPC tissue samples for the three genotypes of *IL-8* promoter T–251A was performed. GAPDH was used as an internal quantitative control. Fold changes were normalized using the levels of GAPDH expression, and each assay was performed at least in triplicate. (B) The TA and AA groups were combined and compared with the TT group. GAPDH= glyceraldehyde.

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* − 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

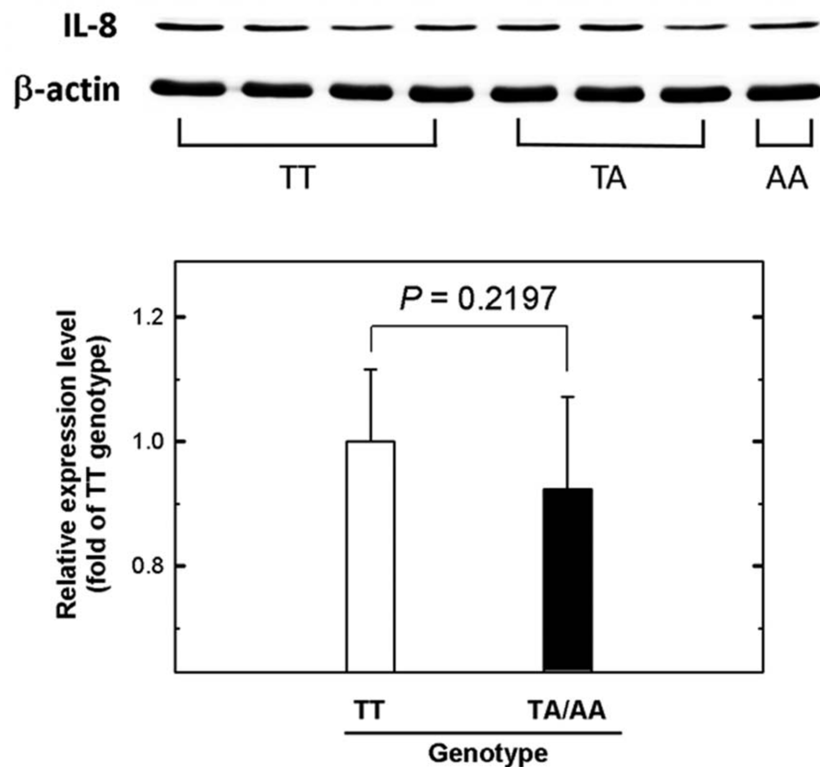

Figure 2. The expression levels of *IL-8* in nasopharyngeal carcinoma (NPC) tissues from patients with different *IL-8* promoter T–251A genotypes. (A) Western blotting analysis of *IL-8* expression in tumor tissues from cases with TT, TA, and AA *IL-8* promoter T–251A genotypes. (B) Quantification of the Western blotting data from (A). β-actin was used as the loading control. Data were averaged from at least 3 repeat analyses of the tissues of each group, with 15 μg total sample protein for each lane.

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=.0336) and smokers (P=.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.

	Non-smokers			Smokers		
Genotypes	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.

Table 6

Distribution of *interleukin-8* genotypes among the EBV positive and negative nasopharyngeal carcinoma patients.

	EBV positive		EBV negative				
	n	%	n	%	OR (95% CI)*	aOR (95% CI)*	*P* value†
IL-8 -251							
TT	27	46.6%	36	41.4	1.00 (Reference)	1.00 (Reference)	
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_{trend}							.8269
Carrier comparison							
TT+TA	49	84.5%	72	82.8	1.00 (Reference)	1.00 (Reference)	
AA	9	15.5%	15	17.2	0.88 (0.36–2.17)	0.82 (0.40–2.01)	.7843
TT	27	46.6%	36	41.4	1.00 (Reference)	1.00 (Reference)	
TA+AA	31	53.4%	51	58.6	0.81 (0.41–1.58)	0.78 (0.46–1.49)	.5382

EBV=Epstein–Barr virus.
* Adjusted with age, gender, smoking, alcohol drinking and areca chewing habits.
† Statistically identified as significant based on Chi-square test without Yates' correction.

cancer risk and the results were heterogeneous.[37] Four studies have been published with regard to this SNP and risks of NPC, 1 in African population,[40] 2 in Chinese population,[38,39] and 1 in Europeans population.[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 publications may be 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 association of *IL-8* −251A/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 7

Distribution of *interleukin-8 (IL-8)* haplotypes among nasopharyngeal carcinoma patients and control subjects after stratified by smoking status.

	Overall			Non-smokers			Smokers		
Haplotypes	Controls	Cases	aOR (95% CI)*	Controls	Cases	aOR (95% CI)*	Controls	Cases	aOR (95% CI)*
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	0.95 (0.42–2.32)	13	8	0.91 (0.29–2.28)
TCCT	28	17	0.86 (0.45–1.78)	16	10	0.88 (0.47–2.08)	12	7	0.73 (0.34–2.17)
TTCA	27	14	0.79 (0.41–1.71)	15	8	0.82 (0.38–1.85)	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 (0.29–1.21)
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-value			**0.0221***			**0.0336***			**0.0117***
Total	352	176		202	103		150	73	

Haplotypes were composed of four polymorphic genotypes according to the sequences: T-251A, C+781T, C+1633T, and A+2767T.
CI=confidence interval.
* 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. Haplotypes that had a frequency of less than seventh rank were combined into the group of others.

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