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***TNF-α* promoter polymorphisms and risk of recurrence in patients with squamous cell carcinomas of the nonoropharynx**

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Abstract

Functional polymorphisms of *TNF-α* may play a critical role in the regulation of immune and inflammatory responses, and could affect transcriptional levels of the *TNF-α* gene and thus contribute to carcinogenesis and outcomes of cancer patients. In a cohort study, we explored the associations between *TNF-α* polymorphisms and risk of recurrence of squamous cell carcinoma of the nonoropharynx (SCCNO). We used log-rank test and multivariable Cox models to evaluate the associations between *TNF-α* polymorphisms and risk of recurrence. In overall comparisons, patients with the *TNF-α* -857 CC, *TNF-α* -863 CC, and *TNF-α* -1031 TT genotypes had significantly worse disease-free survival (log-rank, $P = .014$, log-rank, $P = .020$, and log-rank, $P = .002$, respectively) and higher risk of disease recurrence than patients with the corresponding variant genotypes, respectively (hazard ratio [HR], 1.4, 95% CI, 1.1-1.9, HR, 1.4, 95% CI, 1.0-1.8, and HR, 1.6, 95% CI, 1.2-2.2, respectively). However, no significant association was detected for the *TNF-α* -308 polymorphism. Moreover, in further stratified analyses based on smoking status and treatment, we found that the associations of the *TNF-α* -857, *TNF-α* -863, and *TNF-α* -1031 polymorphisms with risk of recurrence were more pronounced in smokers and patients treated with chemo-radiation. Our findings support a significant role of the *TNF-α* -857, *TNF-α* -863, and *TNF-α* -1031 polymorphisms in recurrence of SCCNO, especially in smokers and patients treated with chemo-radiation. Future prospective studies with larger sample size are needed to confirm these findings.

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Keywords

TNF- α variant; recurrence; genetic polymorphisms; biomarkers; head and neck cancer; squamous cell carcinomas of the nonoropharynx

Introduction

Squamous cell carcinoma of the head and neck (SCCHN), which includes tumors at HPV-related sites of SCCHN, such as the SCC of oropharynx (SCCOP), and non-HPV-related sites of SCCHN, such as the SCC of nonoropharynx (SCCNOP), is the sixth most common cancer worldwide.¹ The SCCNOP arise mainly from oral cavity (e.g., oral tongue, mandibular gingival, buccal, maxillary gingival, floor of mouth, hard palate, retromolar trigone), hypopharynx (e.g., pyriform sinus, post cricoid area, hypopharyngeal wall), and larynx (e.g., glottis, supraglottis). Although diagnostic and therapeutic approaches to SCCHN have improved over the past 2 decades, the prognosis for patients with SCCHN patients has not significantly improved. One of the major reasons for the poor prognosis of patients with SCCHN is the high recurrence rate.² Recurrence of SCCHN is associated with dramatically decreased overall survival.³⁻⁵ However, the recurrence rate differs markedly among patients with SCCHN who had similar clinical and pathological features at diagnosis and received similar treatments, suggesting that there may be inter-individual variation in genetic susceptibility to recurrence of SCCHN. Therefore, identification of factors that accurately predict recurrence in patients with this disease is the first step toward identifying at-risk subgroups that could benefit from secondary prevention programs and from more intensive surveillance to facilitate early detection and treatment of recurrence.

It is increasingly recognized that factors related to inflammation are associated with tumorigenesis and progression of SCCHN;⁶⁻⁸ however, no biomarkers for SCCHN recurrence have been well established. *TNF- α* , a multifunctional proinflammatory cytokine mainly produced by macrophages in response to inflammation, may be a potentially attractive biomarker for SCCHN recurrence. *TNF- α* plays a critical role in the initiation and regulation of immune and inflammatory responses and is pivotal in recurrent inflammatory reactions encountered in cancer therapy.⁹⁻¹⁰ Therapeutic approaches for SCCHN, including surgery, chemotherapy, and radiotherapy, can induce the proinflammatory effect of *TNF- α* . Single-nucleotide polymorphisms (SNPs) within the promoter of the *TNF- α* gene can cause interindividual differences in immune and inflammatory responses. Thus, *TNF- α* promoter polymorphisms may modulate the susceptibility of individuals to immune and inflammatory effects of cancer therapy and subsequently lead to variation in treatment outcome.

To date, hundreds of SNPs of *TNF- α* have been identified. Notably, 4 polymorphisms within the promoter region of *TNF- α* appear to have an effect on the transcriptional level of *TNF- α* and thus are considered functional SNPs: a G to A substitution at position -308 (rs1800629), a C to T substitution at position -857 (rs1799724), a C to A substitution at position -863 (rs1800630), and a T to C substitution at position -1031 (rs1799964).¹¹⁻¹³ These 4 polymorphisms have been well described and reported to be potential risk factors for numerous cancers,¹⁴⁻¹⁷ including SCCNOP.⁶⁻⁸ However, few large studies have

explored the associations between *TNF- α* promoter polymorphisms and risk of recurrence of SCCNOP. Because of the critical role of *TNF- α* in the regulation of immune and inflammatory responses, we hypothesized that the above-mentioned *TNF- α* polymorphisms modulate the risk of recurrence in patients with SCCNOP.

Materials and Methods

Study Subjects

A total of 978 patients with newly diagnosed, previously untreated, histopathologically confirmed index SCCNOP were consecutively enrolled in this study from May 1995 through April 2008 at our institution as part of an ongoing molecular epidemiologic study, which has been described previously.¹⁸ All subjects were recruited regardless of age, sex, ethnicity, or clinical stage except that patients with distant metastases at presentation were excluded. Before enrollment, all participants signed an informed consent form that was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center. Approximately 95% of contacted patients consented to enrollment in the study. At the first presentation to our institution, we collected information related to demographics, epidemiologic risk factors, and clinical characteristics, as well as blood samples for genotyping. Because of insufficient data on follow-up and treatment or unavailable blood samples for genotyping, 183 enrolled participants were ultimately excluded. Therefore, our final analysis included 795 patients with previously untreated incident SCCNOP.

We followed and monitored patients with SCCNOP through their treatment and post-treatment course through regularly scheduled clinical and radiographic examinations. Patients were considered disease free if there was no disease documented at the date of the last visit with the head and neck surgeon, head and neck radiation oncologist, or head and neck medical oncologist. There were no universal standards for imaging. Typically patients had either routine serial imaging, or follow-up imaging on the basis of symptoms or findings on physical examinations. Patients were considered to have experienced disease recurrence if they had appearance of a new lesion of the same histologic subtype as the original SCCHN lesion verified by biopsy (incisional, excisional, or needle biopsy), reappearance of any lesion that had disappeared, or development of tumor-related symptoms. Specifically, distant recurrences were defined as recurrences within other organ systems which occurred because of tumor metastases through the bloodstream (e.g. lung, esophagus, gastrointestinal, bone, brain). Regional recurrences were defined as recurrences in the regional lymph nodes which commonly drained the area of the primary tumor. For SCCHN, the level 1-7 lymph node basins had been well defined, and recurrences within lymph nodes in these levels were classified as regional recurrences. Local recurrences were defined as those recurrences which developed in the mucosa or soft tissue directly in the vicinity (same or adjacent site) of the primary tumor (e.g. in the oral cavity, oropharynx, larynx, or hypopharynx). In this study, we used these strict criteria to distinguish between local recurrences and second primary tumors. We classified lesions as second primary malignancies if they had different histopathology, or occurred > 5 years following treatment for the primary tumor, or clearly separated by > 2cm of normal epithelium based on clinical and radiographic assessment.

Therefore, patients who had the same histopathology, and occurred less than 5 years following treatment for primary tumor, and were not separated by > 2cm of normal epithelium were classified as local recurrences.

Clinical data, including stage of the index tumor at presentation, site of the index tumor, and treatment, were obtained at initial presentation and through follow-up examinations. Index cancer stage was dichotomized into early stage (clinical stage I and II) and late stage (clinical stage III and IV). We divided treatment into 4 categories: surgery only, surgery plus radiotherapy and/or chemotherapy, radiotherapy only, and radiotherapy plus chemotherapy. We subsequently dichotomized treatment into treatment with DNA damaging agents (radiation and/or chemotherapy) and treatment without such agents (surgery only). At presentation, all patients also provided epidemiologic data, including alcohol drinking and smoking status.

TNF- α Genotyping

Genomic DNA was extracted from the buffy coat of whole blood samples using the Qiagen DNA Blood Mini Kit (Qiagen Inc.) per the manufacturer's instructions and *TNF- α* genotyping was performed as previously described¹⁵. The PCR-restriction fragment length polymorphism analysis was used for *TNF- α* genotyping. For each of four SNPs, The PCR was performed in 10- μ L volumes containing approximately 20ng of genomic DNA, 0.1mM each deoxyribonucleotide triphosphate, 10 $\times$ PCR buffer (50 mM KCl, 10 mM Tris HCl, and 0.1% Triton X-100), 1.5 mM MgCl₂, 0.35 U Taq polymerase (Sigma-Aldrich Biotechnology, St. Louis, Mo), and 2 pmol of each of the 8 primers. The PCR cycles were as follows: 3 minutes of initial denaturation at 95°C, 35 cycles of 30 seconds at 95°C, annealing at the indicated temperature (supplementary table) for 30 seconds, 30 seconds at 72°C, and a single final 5-minute extension at 72°C. PCR fragments were digested with indicated restriction enzymes (supplementary table) at 37°C (55°C for *TNF- α* -863) overnight (New England Biolabs, Beverly, MA), and separated on a 3% of AH high resolution gel (for *TNF- α* -308 and *TNF- α* -863), 3% of Metaphor agarose gel (for *TNF- α* -857), and 2% of AH agarose gel (for *TNF- α* -1031) containing ethidium bromide, respectively. For *TNF- α* -308, the 80-base pair (bp) PCR products were digested with Sty I; the A allele was uncut, and the G allele was cut into 58-bp and 22-bp bands. For *TNF- α* -857, 100-bp PCR products were digested with Ase I; the C allele was uncut, and T allele was cut into 78-bp and 22-bp bands. For *TNF- α* -863, 86-bp PCR products were digested with Bsl I; the A allele was uncut, and the C allele was cut into 64-bp and 22-bp bands. For *TNF- α* -1031, 247-bp PCR products were digested with Bbs I; the T allele was uncut, and C allele was cut into 127-bp and 120-bp bands. Positive and negative controls were used in each genotyping assay, and 10% of samples were randomly selected and assayed in duplicates. The concordance between duplicates was 100%.

Tumor HPV16 detection

Paraffin-embedded tissue biopsies or specimens from study patients with tissues available were used to extract DNA for tumor HPV16/18 detection using the specific PCR and in situ hybridization methods as described previously.¹⁹ For quality control, a subset of samples

were re-assayed for tumor HPV16/18 status. The results of the re-run samples were 100% concordant with the original results.

Statistical Analysis

We first compared the mean age and follow-up time for patients with and without recurrence using Student's *t* test. Differences in ethnicity, sex, smoking status, alcohol use, tumor stage, comorbidity, treatment, genotype distributions, and allele frequencies between patients with and without recurrence were evaluated using the chi-squared test. The primary endpoint of the study was recurrence. Time to event was calculated from the date of diagnosis of the index of SCCHN to the date of clinically detectable recurrence (local, regional, or distant). Patients who were not known to have had an event at the date of last contact and patients who were lost to follow-up or died of other/unknown cause were censored. The associations between individual epidemiologic risk factors, clinical characteristics (including stage, comorbidity, and treatment variables), and time to recurrence were initially assessed using univariate Cox proportional hazards regression models. The data were consistent with the assumption of the Cox proportional hazards regression model according to examination of Kaplan-Meier survival curves and log-minus-log survival plots. The associations between various variables and disease-free survival (DFS) were evaluated using the log-rank test. Associations between *TNF*- α polymorphisms and risk of recurrence were quantified by calculating hazard ratios (HRs) and their 95% CIs. The Cox model included adjustment for potential confounders including age, sex, ethnicity, smoking, alcohol use, tumor stage, comorbidity, and treatment. For all analyses, statistical significance was set at $P < 0.05$, and all tests were 2-sided. SAS software (version 9.2.3; SAS Institute) was used to perform all statistical analyses.

Results

The 795 patients in the study were followed from May 1995 through July 2011, during which time 200 patients had disease recurrence. The median follow-up time was 43.8 months (range 0.8 to 169.6 months) for all patients in the study, 52.6 months for the patients without recurrence, and 17.6 months for the patients with recurrence. Among the 200 patients with recurrence, 57 (28.5%) had only distant recurrence, 56 (28.0%) had only local recurrence, 40 (20.0%) had only regional recurrence, and 47 (23.5%) had recurrence of more than 1 type. Additionally, the determination of tumor HPV status by specific PCR indicated that the HPV DNA tumor prevalence was 11.2% (chiefly HPV16 and 18) in 401 of 795 SCCNOP patients whose tumor specimens were available; in contrast, the tumor HPV positivity was up to 80.2% for our SCCOP patients (N= 495)¹⁹.

Patient, tumor, and treatment characteristics and 5-year actuarial recurrence rates overall and by patient subgroup are presented in Table 1. Compared with the recurrence-free group, patients with recurrence were more likely to be men ($P = .003$), patients with advanced stage ($P < .0001$), and patients treated with radiation and/or chemotherapy with or without surgery ($P < .0001$). The differences in recurrence were borderline statistically between ever smokers and never smokers and ever drinkers and never drinkers ($P = .066$ for smoking and $P = .057$ for alcohol users). However, we did not observe significant differences between

patients with and without recurrence with regard to other characteristics, including age ($P = .513$), ethnicity ($P = .920$), and comorbidity ($P = .767$). The univariate Kaplan-Meier analyses showed that sex, smoking, index cancer stage, and treatment were significantly associated with DFS (all log-rank P values $< .05$).

Table 2 shows the genotype distributions of the 4 *TNF- α* SNPs, 5-year actuarial recurrence rates by genotype, and results of univariate and multivariable analysis of the associations between the 4 SNPs and risk of recurrence. As shown in Figure 1, Kaplan-Meier analyses showed that patients with the *TNF- α* -857 CC, *TNF- α* -863 CC, and *TNF- α* -1031 TT genotypes had significantly worse DFS than patients with the corresponding variant genotypes (log-rank, $P = .014$, log-rank, $P = .020$, and log-rank, $P = .002$, respectively). However, no significant difference in DFS was observed for the *TNF- α* -308 polymorphism (log-rank, $P = .591$).

The associations between the 4 *TNF- α* SNPs and recurrence risk in SCCNOP patients were estimated using multivariable Cox proportional hazards regression analysis, with adjustment for potential confounders, including age, sex, ethnicity, smoking and alcohol use status, stage, comorbidity, and treatment. As shown in Table 2, patients with the *TNF- α* -857 CC, *TNF- α* -863 CC, and *TNF- α* -1031 TT genotypes had a significantly higher risk of disease recurrence than those with the corresponding variant genotypes (HR, 1.4, 95% CI, 1.1-1.9, HR, 1.4, 95% CI, 1.0-1.8, and HR, 1.6, 95% CI, 1.2-2.2, respectively); however, no significant association was found for the *TNF- α* -308 polymorphism (HR, 1.1, 95% CI, 0.8-1.4). To further investigate this association, we evaluated the combined risk alleles and risk genotypes of the four polymorphisms on risk of recurrence in SCCNOP patients. We found that the distributions of risk alleles and risk genotypes of all four polymorphisms between patients with recurrence and those without recurrence were statistically significant ($p = 0.002$ for the combined risk alleles and $p = 0.006$ for the combined risk genotypes), but we did not find significantly higher risk of the combined risk alleles or risk genotypes of the four polymorphisms on risk of recurrence in SCCNOP patients. However, we did find that patients with all 8 risk alleles of the four polymorphisms had only 1.5-fold risk compared with those without risk alleles (aHR, 2.0, 95% CI, 1.0-3.2), while patients with all four risk genotypes of the four polymorphisms had 2.5-fold risk compared with those without risk genotypes (aHR, 2.5, 95% CI, 1.3-4.8).

We further evaluated the associations between *TNF- α* polymorphisms and risk of recurrence in patients stratified by smoking status and treatment. As shown in Table 3, for smokers, patients with the *TNF- α* -857 CC, *TNF- α* -863 CC, and *TNF- α* -1031 TT genotypes had a significantly higher risk of disease recurrence than patients with the corresponding variant genotypes (HR, 1.5, 95% CI, 1.1-2.1, HR, 1.8, 95% CI, 1.3-2.5, and HR, 1.5, 95% CI, 1.1-2.1, respectively), but no significant association was found for the *TNF- α* -308 polymorphism (HR, 1.1, 95% CI, 0.8-1.5). For non-smokers, there was no significant association between any of the 4 *TNF- α* SNPs and risk of disease recurrence (HR, 0.7, 95% CI, 0.4-1.4 for *TNF- α* -308, HR, 1.1, 95% CI, 0.6-2.1 for *TNF- α* -857, HR, 1.2, 95% CI, 0.9-1.8 for *TNF- α* -863, and HR, 1.9, 95% CI, 0.8-4.6 for *TNF- α* -1031 polymorphism, respectively). The results were quite similar when recurrence risk was stratified by treatment. As shown in Table 4, for patients treated with radiation and/or chemotherapy with

or without surgery, individuals who carried the *TNF-α* -857 CC, *TNF-α* -863 CC, or *TNF-α* -1031 TT genotype had a significantly higher risk of disease recurrence than those with the corresponding variant genotypes (HR, 1.5, 95% CI, 1.1-2.1, HR, 1.4, 95% CI, 1.0-1.9, and HR, 1.5, 95% CI, 1.0-2.1, respectively), while no significant association was observed for the *TNF-α* -308 polymorphism (HR, 1.2, 95% CI, 0.8-1.6). However, for patients treated with surgery only, there was no significant association between any of the 4 *TNF-α* SNPs and risk of recurrence (HR, 1.0, 95% CI, 0.3-1.3 for *TNF-α* -308, HR, 1.2, 95% CI, 0.6-2.4 for *TNF-α* -857, HR, 1.0, 95% CI, 0.5-2.1 for *TNF-α* -863, and HR, 2.0, 95% CI, 0.9-4.4 for *TNF-α* -1031 polymorphism, respectively). Although the *TNF-α* -1031 TT associated with increased risk of recurrence for both smokers and non-smokers and both DNA damage treatment and or not, the association were not statistically significant for patients who were non-smokers or those treated with surgery only (without DNA damage treatment). These associations from such stratified analyses could be either real or biased estimates due to small sample sizes in each stratum. Larger studies are needed to validate these findings.

Discussion

In this study, we investigated the associations of *TNF-α* polymorphisms with recurrence among patients with SCCHN at nonoropharynx sites and found that the *TNF-α* -857 CC, *TNF-α* -863 CC, and *TNF-α* -1031 TT genotypes were significantly associated with increased risk of recurrence. Furthermore, we found that the associations were more pronounced in smokers than in nonsmokers and in patients treated with radiation and/or chemotherapy with or without surgery than in those treated with surgery alone. These results suggest that the *TNF-α* -857, *TNF-α* -863 and *TNF-α* -1031 polymorphisms predict recurrence of SCCNOP, particularly in some subgroups, which may help physicians to develop individualized treatment plans that would lead to improved survival and quality of life for patients with this disease.

If the associations between these polymorphisms and SCCNOP recurrence are confirmed, the clinicians could use these functional polymorphisms as biomarkers to identify an important subgroup of patients who are at high risk of recurrence. It is likely that future targeted therapies will be dictated (in part) by genotypes of identified significant SNPs as well as individualized within the SCCNOP subgroup. Furthermore, it may be possible to intensify treatment in patients who have the adverse genotypes before treatment begins, add adjuvant therapy for those with such adverse genotypes immediately after treatment, and intensify workup for treatable recurrent disease in those who are found to have high risk of recurrence in follow-up. However, these potential therapeutic options would each have to be tested in clinical trials. While local or distant recurrent SCCNOP is almost always ultimately fatal, it is hoped that the earlier recurrent disease is detected the better opportunity to successfully treat such disease. Ultimately, the data from such a study as proposed here would allow improved prognostication, facilitate more selective use of systemic therapy, and hopefully improve outcomes.

Although it is still unclear how these *TNF-α* polymorphisms influence the risk of recurrence of SCCNOP, these *TNF-α* promoter polymorphisms could result in altered expression of *TNF-α* which may affect the regulation of immune and inflammatory responses and

subsequently responses of cancer patients to treatment.¹⁹⁻²² To date, few studies have investigated the associations between *TNF-α* polymorphisms and recurrence of SCCNOP; however, studies on associations between *TNF-α* polymorphisms and cancer risk and clinical outcomes have been reported. For example, patients with the *TNF-α* -308 AA genotype had relatively low DFS and/or overall survival than patients with the *TNF-α* -308 GG genotype or *TNF-α* -308 AG genotype in breast or pancreatic carcinomas,^{23, 24} while no such associations were detected in several other types of human cancers,²⁵⁻³⁰ which is consistent with our results for SCCNOP in the current study. However, in SCCHN, the patients with the *TNF-α* -308 GA genotype had significantly worse overall survival than those with the *TNF-α* -308 AA genotype.³¹

Several studies also have reported the associations of the *TNF-α* -857, -863, and -1031 polymorphisms with prognosis of cancer patients. In SCCHN, the *TNF-α* -857 CT or TT genotype was significantly associated with better overall survival,³² similar to the results from the current study, while the significant association between *TNF-α* -857 polymorphism and patient outcome was not observed in bladder cancer.²⁶ Although no significant association was found between the *TNF-α* -863 polymorphism and clinical outcomes in patients with bladder cancer or Hodgkin lymphoma,³³ we found that patients with the *TNF-α* -863 CC genotype had a significantly higher risk of recurrence of SCCNOP than patients with the *TNF-α* -863 CA or AA genotypes in the current study. The inconsistent results were also observed for *TNF-α* -1031 polymorphism. A significant association was observed between the *TNF-α* -1031 CC genotype and a reduced risk of recurrence in bladder cancer but not in lung cancer.^{25,26} In contrast, the *TNF-α* -1031 TT genotype conferred an increased risk of recurrence of SCCNOP in the current study.

The exact mechanism for these seemingly conflicting results remains unknown. *TNF-α* promoter polymorphisms may play different roles in the prognosis of patients with different types of cancers. In addition, other factors, such as genetic background, environmental factors, treatments, stage, or study-design factors (e.g., sample size, adequacy of adjustment for other confounding factors, and specific population studied), may influence the impact of these SNPs on prognosis of cancers. In addition, other molecular pathways (e.g., cell cycle control), other inflammatory cytokines (e.g., IL-10), and/or interactions between functional variants of these SNPs and therapeutic agents may also contribute to recurrence of SCCNOP. However, these hypotheses need to be tested in future studies.

Notably, we found significant associations between *TNF-α* polymorphisms and disease recurrence in ever-smokers but not never-smokers. This finding can be explained by the direct effect of tobacco smoking on both pro-inflammatory and immunosuppressive responses,³⁴⁻³⁶ which will worsen the host response to treatment and thus increase the risk of disease recurrence. Furthermore, smoking also has an indirect effect on immune and inflammatory responses.³⁷ It has been reported that smoking can affect the expression and secretion of pro-inflammatory cytokines, such as *TNF-α*, but not anti-inflammatory cytokines. Additionally, smoking-induced *TNF-α* production could adversely influence the function of human NK cells, thereby contributing to a higher risk of recurrence.³⁸ Compared with never smoker patients, the smokers patients are more likely to have somatic mutations,

such as p53 mutations, and such mutations may contribute to poor response to cancer treatment, leading to increased risk of disease recurrence.

Stratified analyses also showed that the associations of *TNF-α* polymorphisms with the risk of recurrence in SCCNOP were more pronounced in patients treated with chemotherapy and/or radiotherapy. This finding is biologically plausible for several reasons. First, radiotherapy/chemotherapy could induce the secretion of some major cytokines, including *TNF-α*.³⁹ It has already been demonstrated that radiation-induced *TNF-α* production was attributed to Tristetraprolin inactivation, which was caused by p38-mediated phosphorylation and proteasome-mediated degradation.⁴⁰ Second, *TNF-α* polymorphisms, which could lead to altered expression of *TNF-α*, might act synergistically with chemoradiation to affect *TNF-α* production and such altered levels of *TNF-α* may affect the regulation of immune and inflammatory responses, thus subsequently affecting susceptibility to cancer recurrence. Third, the anticancer properties of *TNF-α* might act synergistically with radiation to induce apoptosis of tumor cells through binding to cell surface receptors or promote tumor lysis by activating the antitumor T-cell response,⁴¹ leading to a lower proportion of patients with disease. Fourth, since all these patients are SCCNOP, who may be more likely to have somatic genetic changes, the patients with these putatively functional variants of *TNF-α* might enable many tumor cells to escape from the immune system and apoptotic response, leading to less sensitivity to chemoradiotherapy, subsequently leading to the increased risk of disease recurrence. However, all these hypotheses need to be tested in future studies.

Several limitations of the current study should be addressed. First, because most of the patients in this study were non-Hispanic whites, our results may not be generalizable to other ethnic groups. Second, all subjects prospectively completed a standardized epidemiologic questionnaire at enrollment that included demographics, exposure, and clinical data, however, clinical outcomes, including recurrence of SCCNOP, were collected retrospectively, without a strictly defined screening or follow-up regimen. Thus, future prospective studies with larger sample size are needed. Third, because of the hospital-based and retrospective nature of the parent study, there may have been a selection bias and confounding bias for the study patients. Finally, because of the small sample size and the limited number of outcome events in some strata, our statistically significant results might have occurred by chance. Larger studies are warranted to confirm our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

<i>TNF-α</i>	<i>tumor necrosis factor-alpha</i>
SCCHN	squamous cell carcinoma of the head and neck
HR	hazard ratio
CI	confidence interval
PCR	polymerase chain reaction
SNPs	single nucleotide polymorphisms
SCCNOP	squamous cell carcinomas of the nonoropharynx

Novelty and impact statements

TNF- α promoter variants modify the recurrence risk of SCCNOP, and may be a marker of genetic susceptibility to recurrence of SCCNOP, particularly in patients who are smokers and those with chemoradiotherapy

Figure 1. Disease-free survival estimates for patients with SCCNOP by *TNF-α* genotypes

Table 1
Characteristics of patients with SCCNOP (N = 795)

Characteristic	No. (%) of patients	No. of patients with recurrence	5-year recurrence rate	<i>P</i> ^a
No. of patients	795 (100)	200	0.28	
Age				
57 years	342 (43.0)	90	0.27	0.669
> 57 years	453 (57.0)	110	0.29	
Sex				
Male	541 (68.0)	153	0.31	0.006
Female	254 (32.0)	47	0.22	
Ethnicity				
Non-Hispanic white	634 (79.8)	159	0.28	0.888
Other	161 (20.2)	41	0.26	
Smoking				
Never	189 (23.8)	38	0.22	0.017
Ever	606 (76.2)	162	0.30	
Alcohol use				
Never	216 (27.2)	44	0.23	0.110
Ever	579 (72.8)	156	0.29	
Index cancer stage				
I or II	323 (40.7)	46	0.18	< 0.001
III or IV	471 (59.3)	154	0.35	
Comorbidity				
None or mild	673 (84.7)	168	0.28	0.275
Moderate to severe	122 (15.3)	32	0.35	
Treatment ^b				
S only	265 (33.3)	36	0.16	< 0.001
XC/XS/XCS	530 (66.7)	164	0.34	

^a *P*: log-rank test for disease-free survival between the 2 groups.

^b S, surgery; X, radiotherapy; C, chemotherapy.

Table 2
Associations between *TNF- α* genotypes and recurrence in patients with SCCNOP (N = 795)

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank <i>P</i> value	Adjusted HR ^a , (95% CI)
<i>TNF-α</i> -308 (rs1800629)				
GA / AA ^b	107/438	0.27	0.591	1.0
GG	93/357	0.29		1.1 (0.8-1.4)
<i>TNF-α</i> -857 (rs1799724)				
CT / TT ^b	68/328	0.22	0.014	1.0
CC	132/467	0.32		1.4 (1.1-1.9)
<i>TNF-α</i> -863 (rs1800630)				
CA / AA ^b	82/391	0.24	0.020	1.0
CC	118/404	0.32		1.4 (1.0-1.8)
<i>TNF-α</i> -1031 (rs1799964)				
CT / CC ^b	54/302	0.21	0.002	1.0
TT	146/493	0.33		1.6 (1.2-2.2)

^a Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

^b Reference group.

Table 3
Associations between *TNF- α* genotypes and recurrence in patients with SCCNOP stratified by smoking status

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank <i>P</i> value	Adjusted HR ^a , (95% CI)
Ever smoker patients				
<i>TNF-α</i> -308 (rs1800629)				
GA / AA ^b	83/333	0.28	0.377	1.0
GG	79/273	0.31		1.1 (0.8-1.5)
<i>TNF-α</i> -857 (rs1799724)				
CT / TT ^b	51/239	0.23	0.019	1.0
CC	111/367	0.35		1.5 (1.1-2.1)
<i>TNF-α</i> -863 (rs1800630)				
CA / AA ^b	56/296	0.22	0.0002	1.0
CC	106/310	0.37		1.8 (1.3-2.5)
<i>TNF-α</i> -1031 (rs1799964)				
CT / CC ^b	47/228	0.25	0.028	1.0
TT	115/378	0.33		1.5 (1.1-2.1)
Never smoker patients				
<i>TNF-α</i> -308 (rs1800629)				
GA / AA ^b	24/405	0.25	0.340	1.0
GG	14/84	0.22		0.7 (0.4-1.4)
<i>TNF-α</i> -857 (rs1799724)				
CT / TT ^b	17/89	0.22	0.725	1.0
CC	21/100	0.24		1.1 (0.6-2.1)
<i>TNF-α</i> -863 (rs1800630)				
CA / AA ^b	20/95	0.28	0.122	1.0
CC	18/94	0.22		1.2 (0.9-1.8)
<i>TNF-α</i> -1031 (rs1799964)				
CT / CC ^b	7/74	0.10	0.014	1.0
TT	31/115	0.29		1.9 (0.8-4.6)

^a Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

^b Reference group.

Table 4
Associations between *TNF- α* genotypes and recurrence in patients with SCCNOP stratified by treatment

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank <i>P</i> value	Adjusted HR ^a , (95% CI)
Patients with surgery only				
<i>TNF-α</i> -308 (rs1800629)				
GA / AA ^b	23/146	0.20	0.237	1.0
GG	13/119	0.18		1.0 (0.3-1.3)
<i>TNF-α</i> -857 (rs1799724)				
CT / TT ^b	13/114	0.16	0.342	1.0
CC	23/151	0.19		1.2 (0.6-2.4)
<i>TNF-α</i> -863 (rs1800630)				
CA / AA ^b	17/137	0.16	0.736	1.0
CC	19/128	0.20		1.0 (0.5-2.1)
<i>TNF-α</i> -1031 (rs1799964)				
CT / CC ^b	10/119	0.15	0.051	1.0
TT	26/146	0.20		2.0 (0.9-4.4)
Patients with chemoradiation				
<i>TNF-α</i> -308 (rs1800629)				
GA / AA ^b	84/292	0.31	0.213	1.0
GG	80/238	0.36		1.2 (0.8-1.6)
<i>TNF-α</i> -857 (rs1799724)				
CT / TT ^b	55/214	0.25	0.025	1.0
CC	109/316	0.38		1.5 (1.1-2.1)
<i>TNF-α</i> -863 (rs1800630)				
CA / AA ^b	65/254	0.26	0.020	1.0
CC	99/276	0.38		1.4 (1.0-1.9)
<i>TNF-α</i> -1031 (rs1799964)				
CT / CC ^b	44/183	0.26	0.043	1.0
TT	120/347	0.36		1.5 (1.0-2.1)

^a Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

^b Reference group.