The Six-Nucleotide Deletion/Insertion Variant in the CASP8 Promoter Region Is Inversely Associated with Risk of Squamous Cell Carcinoma of the Head and Neck

Chunying Li1, Jiachun Lu1, Zhensheng Liu1, Li-E. Wang1, Hui Zhao1, Adel K. El-Naggar2, Erich M. Sturgis1,3, and Qingyi Wei1

Abstract

Caspase 8 (CASP8) is an apoptosis-related cysteine peptidase involved in the death receptor pathway and likely in the mitochondrial pathway. A CASP8 promoter region six-nucleotide deletion/insertion (−652 6N ins/del) variant and a coding region D302H polymorphism are reportedly important in cancer development, but no reported study has assessed the associations of these genetic variations with risk of head and neck cancer. In a hospital-based study of non-Hispanic whites, we genotyped CASP8 −652 6N del and 302H variants in 1,023 patients with squamous cell carcinoma of the head and neck (SCCHN) and 1,052 cancer-free controls. Crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression models. The CASP8 −652 6N del variant genotypes or haplotypes were inversely associated with SCCHN risk (adjusted OR, 0.70; 95% CI, 0.57-0.85 for the ins/del + del/del genotypes compared with the ins/ins genotype; adjusted OR, 0.73; 95% CI, 0.55-0.97 for the del-D haplotype compared with the ins-D haplotype). Furthermore, the number of the CASP8 −652 6N del (but not 302H) variant allele tended to correlate with increased levels of camptothecin-induced p53-mediated apoptosis in T lymphocytes from 170 cancer-free controls. We concluded that the CASP8 −652 6N del variant allele may contribute to the risk of developing SCCHN in non-Hispanic white populations. Further validation by population-based case-control studies and rigorous mechanistic studies is warranted. Cancer Prev Res; 3(2); 246–53. ©2010 AACR.

Introduction

Squamous cell carcinoma of the head and neck (SCCHN), which includes cancers of the oral cavity, pharynx, and larynx, is the eighth leading cause of cancer-related death worldwide (1). In 2008, an estimated 47,560 new cases of SCCHN were diagnosed in the United States (2). Although smoking and alcohol use are major causes of SCCHN (3), only a small fraction of smokers and drinkers develop SCCHN. This suggests that susceptibility to SCCHN exists in the general population (4, 5). Recently, investigators have sought to determine whether genetic variations or polymorphisms in genes regulating apoptosis, such as CASP8, may confer such a genetic susceptibility to cancer (6, 7).

CASP8 encodes caspase 8, a cysteine peptidase in the FAS/FASLG-mediated apoptosis pathway that can activate various cellular proteases or proteins, leading to apoptosis (8, 9). CASP8 is located on chromosome 2q33-34, harboring 10 exons that span ∼30 kb (10). At least 168 single nucleotide polymorphisms (SNP), mostly rare or nonfunctional, have been reported for CASP8. Several studies have evaluated the associations between some of these CASP8 SNPs and risk of various cancers (11–15). At least two common, potentially functional SNPs, the CASP8 promoter region six-nucleotide insertion/deletion (−652 6N ins/del, rs3834129) and the coding region D302H (rs1045485), are thought to be important in cancer etiology.

Studies have shown that the CASP8 −652 6N ins/del and D302H polymorphisms might cause dysregulated apoptosis and thus carcinogenesis (11, 12, 14, 15). It was shown that the 6N del-containing promoter was associated with lower luciferase expression than the 6N ins-containing promoter, that the 6N del allele had an effect on binding of transcriptional factor Sp1, and that the 6N del allele had an effect on activation-induced cell death of tumor-related T lymphocytes (14). Further association analysis found that this minor, potentially functional −652 6N del allele was inversely associated with risk of several types of cancer in Chinese populations (14), which were not supported by the results from some European studies of Caucasian populations (16–18). Some studies have also reported that the CASP8 302H variant genotypes were also inversely
associated with the risk of developing breast cancer (15, 19), glioma (11, 12), other brain tumors (12), and lymphoma (12). However, to the best of our knowledge, no reported study has investigated the role of these two CASP8 polymorphisms in the development of SCCHN. Given that CASP8 plays a role in carcinogenesis, we hypothesized that these two reportedly significant polymorphisms in CASP8 are associated with risk of SCCHN and tested this hypothesis in a case-control study of 1,023 patients with SCCHN and 1,052 cancer-free control subjects, frequency-matched by age and sex.

Subjects and Methods

Study subjects
Our study population has been described previously (6, 7, 20). In brief, non-Hispanic white patients with newly diagnosed and untreated SCCHN were recruited between October 1999 and September 2006 among those who were referred to The University of Texas M.D. Anderson Cancer Center. Inclusion conditions were U.S. residents, age of 18 y or greater, no history of prior cancers except for nonmelanoma skin carcinomas, agreement to donate a blood sample, and tumor-node-metastasis stage 1 to IVB (patients with distant metastases were excluded). There were no restrictions on age or tumor stage. Approximately 95% of the patients we approached for recruitment agreed to participate in the study. Cancer-free control subjects were recruited during the same period from self-reported cancer-free visitors to M.D. Anderson Cancer Center who were not seeking medical care but instead were accompanying patients on outpatient clinic visits. Approximately 90% of the control subjects we approached agreed to participate. The controls were not blood-related to the patients or to each other and were frequency-matched to the patients by age (±5 y) and sex. After obtaining informed consent, we interviewed each eligible participant in person to obtain data on age, sex, ethnicity, tobacco smoking history, alcohol use, and other risk factors for SCCHN. At the end of the interview, a sample of blood (30 mL) was drawn into a heparinized tube from each subject. The research protocols were approved by the M.D. Anderson Institutional Review Board.

Genotype analysis
For each subject, we centrifuged 1 to 2 mL of whole blood to obtain a leukocyte cell pellet from the buffy coat. Genomic DNA was extracted from the cell pellet using a QIAamp DNA blood mini kit (Qiagen). DNA purity and concentration were determined by spectrophotometric measurement of absorbance of UV light at 260 and 280 nm. We used the previously described primers (14) for the CASP8 −652 6N ins/del polymorphism (rs3834129−/CTTACT; NM_001228.4) to amplify the fragments containing the CASP8 −652 6N ins/del variant site and the BstUI restriction enzyme (New England Biolabs) to interrogate the genotypes. We also used the previously reported primers (21) to amplify the target fragments of the CASP8 codon D302H G > C (rs1045485: G4C; NM_001228.4) and the BstUI restriction enzyme (New England Biolabs) to identify the CASP8 D302H G > C genotypes. Approximately 10% of the samples were randomly selected for genotype confirmation, and the results were 100% concordant.

Detection of camptothecin-induced apoptosis in T-lymphocytes
To test the functionality of CASP8 variant alleles, we used camptothecin (500 nmol/L, C9911; Sigma-Aldrich, Inc.) to selectively induce apoptosis of T-lymphocytes of the available samples from the controls. The baseline or spontaneous apoptosis index was obtained from the same individual’s samples that were not incubated with camptothecin but were otherwise processed in parallel with those treated by camptothecin. Although we did not sort the cells, it is likely that the S phase peripheral lymphocytes as the surrogates for the target cells were inhibited by camptothecin, a topoisomerase I inhibitor (22), that has been shown to induce apoptosis in melanocytes (23), oral squamous cell carcinoma cells (24), and cells of the immune system (25). In our hands, lymphocytes treated with 112.5 μg/mL of phytohemagglutinin (Murex Biotech Limited) could tolerate high doses (>1,000 nmol/L) of camptothecin without cell death events other than apoptosis (data not shown). We detected apoptotic cells with an APO-Brdu kit (Phoenix Flow Systems), which contains apoptosis-positive and -negative cells as the assay controls and uses a two-color staining method for labeling DNA breaks and total cellular DNA. The kit includes washing, reaction, and rinsing buffers for processing each step of the assay; terminal deoxynucleotidyl transferase enzyme, bromodeoxyuridine triphosphate, and fluorescein-labeled anti-bromodeoxyuridine antibody for labeling DNA; and propidium iodide/RNase A solution for counterstaining the total DNA. The ratio of apoptotic cells (~1 × 10⁷) was measured by flow cytometry (Epics Profile II Flow Cytometer, Beckman Coulter, Inc.) according to the instructions of the manufacturer. Specific apoptosis (the apoptotic index) was determined by the formula [100 × (experimental apoptosis – spontaneous apoptosis)/100 – spontaneous apoptosis] (26). However, the phenotype assays were done only once with the samples available but without rigorous quality control that demands repeated sample collections and repeated assays on the same samples. We did not have such opportunities in this study. Therefore, this genotype-phenotype correlation study was preliminary in nature, and more rigorous quality control issues should be addressed before it can be used in large-scale population studies.

Statistical analysis
We used the χ² tests to compare the distributions of demographic variables and selected risk factors between the patients and controls, whereas we used Student’s t test, one-way ANOVA, and Pearson correlation for the apoptosis index. The Hardy-Weinberg equilibrium
\[ p^2 + 2pq + q^2 = 1, \]

where \( p \) is the frequency of the variant allele and \( q = 1 - p \) was tested by a goodness-of-fit \( \chi^2 \) test to compare the observed genotype frequencies with expected genotype frequencies under the Hardy-Weinberg equilibrium in the controls. The association between case-control status and each individual SNP, measured by odds ratios (OR) and 95% confidence intervals (CI), were estimated using an unconditional logistic regression model, both with and without adjustments for age, sex, smoking status, and alcohol use. Because the estimates for crude and adjusted ORs were similar, only adjusted ORs were presented. Logistic regression analysis was also used for both stratification by covariates and trend test. We also calculated the linkage disequilibrium for the \text{CASP8} \ -652 \ 6N \ ins/del and codon D302H G > C SNPs. We reconstructed haplotypes for each subject on the basis of the observed \text{CASP8} \ -652 \ 6N \ ins/del and codon D302H genotypes by using the PHASE program (version 2), as previously described (21), in which each individual was assigned a pair of haplotypes that had the highest estimated probability given the individual’s unphased genotypes. The global omnibus test for case-control differences in the frequencies of all haplotypes was done using Pearson’s \( \chi^2 \) test. All analyses were done using Statistical Analysis System software (version 9.1, SAS Institute, Inc.). All statistical tests were two-sided, and \( P < 0.05 \) was considered statistically significant.

**Results**

**Characteristics of the study population**

Because only a few subjects recruited for this study at M.D. Anderson were from a minority group (each <10%), the final analysis included only non-Hispanic whites. The baseline characteristics of the cases and controls are summarized in Table 1. The final analysis included 1,023 subjects. The baseline characteristics of the cases and controls are summarized in Table 1. The final analysis included 1,023 subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n = 1,023), %</th>
<th>Controls (n = 1,052), %</th>
<th>( P^* )</th>
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<td>( &gt; 56 )</td>
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<td>T2</td>
<td>373</td>
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</tr>
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<td>T4</td>
<td>190</td>
<td>18.6</td>
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</tr>
<tr>
<td>Lymph node metastasis (N)§</td>
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</tr>
<tr>
<td>N0</td>
<td>375</td>
<td>36.6</td>
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</tr>
<tr>
<td>N1</td>
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</tr>
<tr>
<td>N2</td>
<td>455</td>
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</tr>
<tr>
<td>N3</td>
<td>44</td>
<td>4.3</td>
<td></td>
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</table>

* \( P \) values for a two-sided \( \chi^2 \) test.
† Included both oropharyngeal and hypopharyngeal cancer cases.
‡ The extent of the primary T1, tumor \( \leq 2 \) cm at the greatest dimension; T2 to T4, increasing greatest dimensions.
§ Regional lymph node involvement. N0, no regional lymph nodes involved; N1 to N3, increasing involvement of regional lymph nodes.
patients newly diagnosed with SCCHN and 1,052 frequency-matched controls by age and sex. Therefore, there was no difference in age (by median age of 56 years) and sex distributions between cases and controls (P = 0.785 and 0.782, respectively). However, the cases were more likely than the controls to be smokers and drinkers (P < 0.001 for both smoking and alcohol use). Of the 1,023 cases, 295 (28.8%) had cancers of the oral cavity, 564 (55.1%) had cancers of the pharynx, and 164 (16.1%) had cancers of the larynx (Table 1).

**Distribution of genotypes and risk estimates**

We assessed the associations between the two CASP8 SNPs and the risk of SCCHN. As shown in Table 2, the observed genotype frequencies were in agreement with those expected from the Hardy-Weinberg equilibrium for the control groups (P = 0.324 for the CASP8 –652 6N genotypes and P = 0.522 for the CASP8 D302H genotypes). The frequency of the CASP8 –652 6N variant del allele was not significantly lower among the cases than among the controls (0.473 and 0.498, respectively; P = 0.107), and the frequency of the D302H variant H allele was not significantly higher among the cases than among the controls (0.144 and 0.136; respectively; P = 0.444). However, there was a significant difference in the distributions of the genotypes for CASP8 –652 6N ins/del (P = 0.002) between the cases and controls.

When we used the ins/ins genotype as the reference group, we found that the ins/del genotype was associated with a significantly reduced SCCHN risk (adjusted OR, 0.65; 95% CI, 0.52-0.81 with adjustments for age, sex, smoking status, and alcohol use), but the del/del genotype was associated with a borderline reduced SCCHN risk (adjusted OR, 0.80; 95% CI, 0.62-1.03). Because the

<table>
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<tr>
<th>Variables</th>
<th>Cases</th>
<th>Controls*</th>
<th>P</th>
<th>Adjusted OR (95% CI)†</th>
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<td>Genotypes</td>
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<td></td>
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<tr>
<td>Total no. subjects</td>
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<td>1,052</td>
<td>0.002‡</td>
<td></td>
</tr>
<tr>
<td>Total no. alleles</td>
<td>2,046</td>
<td>2,104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CASP8 –652 6N (AGTAAG/-)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ins/ins</td>
<td>311 (30.4)</td>
<td>257 (24.4)</td>
<td>1.00 (ref.)</td>
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<tr>
<td>ins/del</td>
<td>456 (44.6)</td>
<td>542 (51.5)</td>
<td>0.65 (0.52-0.81)</td>
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<tr>
<td>del/del</td>
<td>256 (25.0)</td>
<td>253 (24.1)</td>
<td>0.80 (0.62-1.03)</td>
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<tr>
<td>Trend test</td>
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<td></td>
<td></td>
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<tr>
<td>ins/del + del/del</td>
<td>712 (69.6)</td>
<td>795 (75.6)</td>
<td>0.70 (0.57-0.85)</td>
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<tr>
<td>del allele</td>
<td>0.473</td>
<td>0.498</td>
<td>0.107§</td>
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<tr>
<td>CASP8 D302H</td>
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<tr>
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<td>745 (72.8)</td>
<td>783 (74.4)</td>
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<tr>
<td>DH</td>
<td>261 (25.5)</td>
<td>252 (24.0)</td>
<td>1.07 (0.87-1.31)</td>
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<tr>
<td>HH</td>
<td>17 (1.7)</td>
<td>17 (1.6)</td>
<td>1.00 (0.50-2.00)</td>
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<tr>
<td>Trend test</td>
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<tr>
<td>DH + HH</td>
<td>278 (27.2)</td>
<td>269 (25.6)</td>
<td>1.06 (0.87-1.30)</td>
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<td>H allele</td>
<td>0.144</td>
<td>0.136</td>
<td>0.444§</td>
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<td>Haplotypes‡</td>
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<td>Total no. of haplotypes</td>
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<td>972 (46.2)</td>
<td>1.00 (ref.)</td>
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<td>del - D</td>
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<td>846 (40.2)</td>
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<td>del - H</td>
<td>217 (10.6)</td>
<td>202 (9.6)</td>
<td>1.00 (0.64-1.58)</td>
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<tr>
<td>ins - H</td>
<td>80 (3.9)</td>
<td>84 (4.0)</td>
<td>0.84 (0.37-1.93)</td>
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</table>

*The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium (p^2 + 2pq + q^2 = 1) (χ^2 = 0.97, P = 0.324 for CASP8 –652 6N ins/del; χ^2 = 0.41, P = 0.522 for CASP8 D302H).
† ORs and CIs were adjusted in a logistic regression model that included age, sex, smoking status, and alcohol use with P values for a two-sided χ^2 test for either genotype distribution or allele frequency.
‡ χ^2 tests for differences in distribution of genotype frequencies between cases and controls.
§ χ^2 tests for differences in allele frequencies between cases and controls.
ǁ Haplotypes constructed in the order of variant alleles of CASP8 –652 ins/del and D302H.
¶ Chi tests for differences in haplotype frequencies between cases and controls.
−652 6N del allele seemed to be more likely dominant than recessive in predicting risk of SCCHN, we combined the variant genotypes (i.e., heterozygotes and variant homozygotes) together as one group for further comparison. Compared with the 6N ins/ins genotype, the 6N del variant genotypes (ins/del + del/del) were associated with a significantly reduced risk of SCCHN (adjusted OR, 0.70; 95% CI, 0.57-0.85); in contrast, compared with the 302DD genotype, the variant 302H genotypes (DH + HH) were not associated with significantly altered risk of SCCHN (adjusted OR, 1.06; 95% CI, 0.87-1.30; Table 2).

**CASP8 haplotypes and SCCHN risk**

Because our study had an adequate sample size and study power for proper evaluation of the main effects of CASP8 haplotypes, we also assessed the association between CASP8 haplotypes and SCCHN risk. We found that the linkage disequilibrium between the −652 6N del and 302H (C) variant alleles was incomplete in all control subjects (D’ = 0.415, r² = 0.027), suggesting that variant haplotypes may play a role in cancer risk. However, the overall distributions of the CASP8 −652 6N del and 302H variant-reconstructed haplotypes between the SCCHN cases and controls were not significantly different (P = 0.121). Nevertheless, when the most common haplotype, ins/D, was used as the reference, a significantly lower SCCHN risk was associated with the del allele (adjusted OR, 0.73; 95% CI, 0.55-0.97 for the del/D haplotype) but not for the H allele (adjusted OR, 0.84; 95% CI, 0.37-1.93 for the ins/H haplotype and adjusted OR, 1.00; 95% CI, 0.64-1.58 for the del/H haplotype; Table 2), suggesting that CASP8 −652 6N del was indeed a significant contributor to the haplotype effects.

### Table 3. Stratification analysis of the CASP8 −652 ins/del genotypes by selected variables in squamous cell carcinoma of the head and neck cases and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n = 1,023)</th>
<th>Controls (n = 1,052)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ins/ins, no. (%)</td>
<td>ins/del + del/del, no. (%)</td>
<td>ins/ins, no. (%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;56</td>
<td>151 (29.9)</td>
<td>354 (70.1)</td>
<td>128 (25.0)</td>
</tr>
<tr>
<td>≥56</td>
<td>160 (30.9)</td>
<td>358 (69.1)</td>
<td>129 (23.9)</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
<td>231 (29.2)</td>
<td>559 (70.8)</td>
<td>193 (23.9)</td>
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<tr>
<td>Female</td>
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<td>153 (65.7)</td>
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<td>Never</td>
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<td>Ever</td>
<td>222 (29.6)</td>
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<td>89 (32.5)</td>
<td>185 (67.5)</td>
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<td>Larynx</td>
<td>53 (32.3)</td>
<td>111 (67.7)</td>
<td>257 (24.4)</td>
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</table>

*ORs and CIs were adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.
†Included both oropharyngeal and hypopharyngeal cancer cases.

Stratification analysis of SCCHN risk and CASP8 polymorphisms

As shown in Table 3, the stratified analysis showed that the inverse association of CASP8 ins/del + del/del genotypes with SHNCC risk remained significant among subjects with the following characteristics: younger than the median age of 56 years of the controls (adjusted OR, 0.74; 95% CI, 0.56-0.99), older than the median age of 56 years of the controls (adjusted OR, 0.66; 95% CI, 0.50-0.88), male (adjusted OR, 0.73; 95% CI, 0.58-0.92), female (adjusted OR, 0.66; 95% CI, 0.50-0.88), current or former smoker (adjusted OR, 0.66; 95% CI, 0.50-0.85), current or former drinker (adjusted OR, 0.69; 95% CI, 0.54-0.89), tumor site of pharynx (adjusted OR, 0.69; 95% CI, 0.54-0.87), and tumor site of larynx (adjusted OR, 0.59; 95% CI, 0.41-0.87). The differences in these stratum-specific ORs might be the result of fewer observations in some strata, or the differences might indicate that some subgroups are more susceptible to SCCHN. However, there were no differences in the risk for subgroups of patients with different stages, tumor sizes, and degrees of regional lymph node involvement (data not shown). Further homogeneity tests suggested that there were no significant differences in the ORs associated with CASP8 −652 6N between these different strata. As a result,
we did not find any gene-gene or gene-environment inter-
actions in risk associated with the CASP8 −652 6N variant
and by selected stratification factors nor by tumor subsite
(data not shown).

CASP8 genotypes and T-cell apoptosis
To further provide some mechanistic insight into the
functionality of the studied CASP8 SNPs, we performed
camptothecin-induced apoptosis assays for blood samples
from 170 cancer-free controls. No differences in the assay
measurements by dichotomized groups of age (median of
56 years), sex (male versus female), smoking status (ever
versus never), and alcohol use (ever versus never; data not
shown). However, there seemed to be a weak statistical
correlation ($r = 0.250$, $P = 0.001$) between increasing ap-
optotic index and increasing age (in years) in these 170
subjects. Furthermore, there was no difference in the
means of the apoptotic indices by the CASP8 D302H
G > C genotypes (66.1%, 65.0%, and 71.5% for the GG,
GC, and CC genotypes, respectively; $P = 0.951$; Fig. 1B).
However, the apoptotic index seemed to increase as the
number of CASP8 −652 6N del allele increased (61.0%,
66.3%, and 69.9% for the II, ID, and DD genotypes, re-
spectively; $P = 0.109$).

Discussion

In this study of a non-Hispanic white population, we
found that the CASP8 −652 6N del variant genotypes
(ins/del, del/del, and the combined ins/del + del/del) were
associated with a significantly lower risk of SCCHN, com-
pared with the ins/ins genotype. However, hap-
loptypoe analysis suggested that the del allele, in the pres-
ence 302D (Del-D haplotype of −652 6N/D302H), but
not the 302H, allele (i.e., Del-H, which had fewer observa-
tions), was also associated with a significantly lower
SCCHN risk, compared with the ins-D haplotype, regard-
less of tumor sites or tumor-related clinical characteristics.
Furthermore, the apoptotic index seemed to increase as
the number of the CASP8 −652 6N del allele increased,
which suggests that the del allele may be functional. To
the best of our knowledge, this is the first association
study of these two CASP8 SNPs and risk of SCCHN.

Several studies have investigated the association be-
tween the CASP8 −652 6N polymorphism and risk of can-
cers other than SCCHN. The earliest Chinese study,
comparing patients with several types of cancer and
cancer-free controls, found that CASP8 −652 6N del vari-
ant genotypes were associated with significantly reduced
risks of lung, breast, esophageal, gastric, colorectal, and
cervical cancers (14). However, a later, larger multicenter
study of 7,753 patients with breast cancer and 7,921 con-
trols indicated that the CASP8 −652 6N del variant had no
significant effect on breast cancer risk in Europeans (17).
An Italian study of 580 patients with familial breast cancer
and 406 controls also found no association between the
CASP8 −652 6N del variant genotypes and breast cancer
risk, but the researchers observed that the −652 6N del
polymorphism of CASP8 was associated with age at diag-
nosis (18). However, we did not observe an association
between the del allele and age of the disease onset. A more
recent study of 7,161 patients with breast, prostate, or co-
lorectal cancer and 6,266 controls from different ancestries
found that −652 6N del variant genotypes were not asso-
ciated with the risk of breast, colorectal, or prostate cancer
in populations with Asian, European, or African origin

Fig. 1. Graphic presentation of apoptotic index by age and CASP8 genotype. A, scatter plot for correlation between apoptotic index (%) and age of
170 cancer-free subjects. The apoptotic index increases as age increases. B, boxplot for apoptotic index by CASP8 D302H genotypes. There is no trend for
decreased apoptotic index as the number of the variant C allele increases. C, boxplot for apoptotic index by CASP8 −652 6N genotypes. There is an
insignificant trend of increased apoptotic index as the number of the D (deletion) alleles increase, which is consistent with its observed inverse association,
assuming the lymphocytes serve as the surrogates for target cells.
In a more recent study of 805 non-Hispanic white patients with cutaneous melanoma and 835 cancer-free, age-, sex-, and ethnicity-matched controls, we found that CASP8 −652 6N del variant genotypes were associated with a significantly reduced risk of melanoma (21), which inspired us to conduct this SCCHN study in a complete different study population. Once again, we observed a similar association between CASP8 −652 6N del variant genotypes and reduced risk of SCCHN.

The inconsistent results among these reported studies indicated that the association between −652 6N del variant genotypes and cancer risk may be related to sample sizes, ethnicity of the study populations, or cancer types. Therefore, whether or not the reported association of CASP8 −652 6N SNP with cancer risk is cancer-specific needs to be validated in additional studies of cancers at different sites either in additional groups of homogenous ethnicity or in more ethnically diverse groups. Nevertheless, the possible phenotype association with the del allele, if validated by others, may provide further support for the notion that a reduced risk of SCCHN associated with the CASP8 −652 6N del variant genotypes may be biologically plausible.

Apoptosis is an important physiologic mechanism, eliminating cells with unreparable damage to DNA and thus sustaining homeostasis (27, 28). Apoptosis occurs through two mechanisms: the death receptor FAS/FASLG (also called extrinsic) pathway and the mitochondrial (DNA damage–induced and p53-mediated, also called intrinsic) pathway (29). Alterations in cellular apoptotic potential or resistance to apoptosis may be caused by genetic variations in apoptosis-regulating genes. The CASP8 promoter −652 6N del allele has been shown to have a substantial effect on promoter activity of the CASP8 gene because it destroys a binding element for stimulatory protein 1 (Sp1), thus resulting in lower apoptosis reactivity of T lymphocytes upon being stimulated by cancer cells or phytohemagglutinin in an ex vivo model (14); alternatively, reduced apoptosis of the cells involved in the antitumor process may also lead to protection against cancer. Although tobacco-induced DNA damage is a major risk factor for SCCHN (30), the immune surveillance should also provide common protection against tobacco-induced epithelial carcinogenesis and subsequent tumor progression. In this sense, our finding of an inverse association between the −652 6N del variant and SCCHN risk in Caucasian populations from the United States was consistent with results observed in Chinese populations (14).

Because the 302D > H change localizes to the external surface of the expressed protein, it is conceivable that it may influence autoprocessing of procaspase-8 molecules or CASP8 interactions with the antiapoptotic FADD-like apoptosis regulator (31). The published studies of association between the CASP8 D302H polymorphism and different cancers have generated inconsistent results. A study of 3,191 patients with breast cancer and 3,258 cancer-free controls from the United Kingdom found that CASP8 302H variant genotypes were associated with a significantly reduced breast cancer risk (15). Later, a small study of 216 patients with colon cancer and 255 controls from the United States found no association between the CASP8 D302H polymorphism alone and colon cancer risk but did find an association between combined CASP8 302H and glutathione-S-transferase T1-null genotypes and colon cancer risk (32). Similarly, a German study of 511 patients with familial breast cancer and 547 controls found no association between the CASP8 D302H polymorphism alone and familial breast cancer risk but did find an association between the combined CASP8 D302H and CASP10 V410I genotypes and familial breast cancer risk (33). One large study using pooling analyses of 14 studies within the Breast Cancer Consortium has confirmed the main effect of the CASP8 D302H genotype on breast cancer (19), whereas others found that the 302H allele was associated with increased glioma risk (11) or decreased risk of meningioma and marginal zone B-cell lymphoma (12). However, we did not find an association between the CASP8 D302H variant genotypes and SCCHN risk in our present study. Instead, we found that the del-D haplotype of −652 6N/D302H was inversely associated with SCCHN risk. To date, no published studies have evaluated the roles of the CASP8 −652 6N/D302H haplotypes in cancer risk. Further validation of our findings by population-based case-control studies and rigorous mechanistic studies is warranted.

Caspase 8 is mainly involved in the FAS-FAS ligand mediated extrinsic (death receptor) pathway (8, 9), but it is also postulated that caspase 8 may interact with the BID protein to influence the intrinsic (mitochondrial) pathway, which acts through the caspases (34). The latter mechanism is responsible for camptothecin-induced and p53-mediated apoptosis because the inhibition of topoisomerase I by camptothecin leads to increased levels of DNA strand breaks, a strong apoptotic signal (22). Interestingly, we found that the apoptotic index seemed to increase as the number of the CASP8 −652 6N del allele increased, but the same trend was not seen with the CASP8 302H variant allele. The trend is consistent with the finding of an inverse association between the CASP8 −652 6N del allele and SCCHN, reflecting a protective effect in those cells that have DNA damage but undergo apoptosis that lead to a reduced probability of carcinogenesis. Although our experiments were done in peripheral lymphocytes, the results suggest that the CASP8 −652 6N del allele may have enhanced the apoptotic potential of the target tissues through the mitochondrial pathway in response to DNA damage (24), a hypothesis that should be further tested in the target tissue. However, these results from a small sample are preliminary, and additional studies are needed to substantiate our findings.

Therefore, it is important to conduct further analyses that comprehensively cover this genetic region to better understand which region of the gene might be the one harboring a “causal” variant. Additional analyses that incorporate other relevant genes in this pathway are needed to fully understand the role of genetic variation in apoptosis and SCCHN. We did not observe any statistical differences in...
the apoptosis index by dichotomized groups of age (by the median), sex, smoking status, and alcohol use, and the pilot study was not powered to detect additional confounders that were either not available or not measured such as antioxidant/oxidant intake or exposure. Also, the use of available control samples for the apoptosis assays may cause possible selection biases. In the future, well-designed phenotype-genotype association studies with information on all possible confounders should be done to validate our observed association and identify the true disease-causing variants and their functionality.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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The Six-Nucleotide Deletion/Insertion Variant in the \textit{CASP8} Promoter Region Is Inversely Associated with Risk of Squamous Cell Carcinoma of the Head and Neck

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