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The 6-nucleotide deletion variant in the *CASP8* promoter region is inversely associated with risk of squamous cell carcinoma of the head and neck

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Abstract

Caspase 8 is an apoptosis-related cysteine peptidase involved in the death receptor pathway and likely in the mitochondrial pathway. A *CASP8* promoter-region 6-nucleotide deletion/insertion (-652 6N ins/del) and a coding region D302H polymorphism are reportedly important in cancer development, but no reported study has assessed the associations of these 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 (ORs) and 95% confidence intervals (CIs) 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 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.

Keywords

apoptosis; cancer risk; caspase; cell death; genetic susceptibility

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 will be 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 (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 (SNPs), mostly rare or nonfunctional, have been reported for *CASP8* (<http://egp.gs.washington.edu/data/casp8/casp8.csnp.txt>). Several studies have evaluated the associations between some of these *CASP8* SNPs and cancer risk of various cancers (11–15). At least two common, potentially functional SNPs, the *CASP8* promoter-region 6-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 may 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 impact 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 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 1023 patients with SCCHN and 1052 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 years or greater, no history of prior cancers excepting non-melanoma skin carcinomas, agreement to donate a blood sample, and TNM stage I-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 each other and were frequency-matched to the patients by age (± 5 years) 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 1–2 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, Valencia, CA). DNA purity and concentration were determined by spectrophotometric measurement of absorbance of ultraviolet light at 260 nm 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 *BfaI* restriction enzyme (New England Biolabs, Beverly, MA) to interrogate the genotypes. We also used the previously report 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, Beverly, MA) 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 (CPT)-Induced Apoptosis in T-lymphocytes

To test the functionality of *CASP8* variant alleles, we used CPT (500 nM, C9911, Sigma-Aldrich, Inc., St. Louis, MO) 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 CPT but otherwise processed in parallel with those treated by CPT. 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 CPT, 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 phytohemagglutinin (Murex Biotech Limited, Dartford, UK) can tolerate high doses (> 1000 nM) of CPT without cell death events other than apoptosis (data not shown). We detected apoptotic cells with an APO-BrdU kit (Phoenix Flow Systems, San Diego, CA), 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 (TdT), bromodeoxyuridine triphosphate (BrdUTP), and fluorescein-labeled antiBrdU antibody for labeling DNA; and propidium iodide (PI)/RNase A solution for counterstaining the total DNA. The ratio of apoptotic cells (approximately 1×10^5) was measured by flow cytometry (Epics Profile II Flow Cytometer, Beckman Coulter, Inc., Fullerton, CA) according to the manufacturer's instructions. Specific apoptosis (the apoptotic index) was determined by the formula $[100 \times (\text{experimental apoptosis} - \text{spontaneous apoptosis}) / 100 - \text{spontaneous apoptosis}]$ (26). However, the phenotype assays were performed 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 χ^2 tests to compare the distributions of demographic variables and selected risk factors between the patients and controls, while we used the Student *t* test, the analysis of one-way variance analysis, and the Pearson correlation for the apoptosis index. The Hardy-Weinberg equilibrium (HWE; $p^2 + 2pq + q^2 = 1$, where *p* is the frequency of the variant allele and *q* = 1 – *p*) was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies with expected genotype frequencies under the HWE in the controls. The association between case-control status and each individual SNP, measured by odds ratios (ORs) and 95% confidence intervals (CIs), was 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. Because Logistic regression analysis was also used for both stratification by covariates and trend test. We also calculated the linkage disequilibrium (LD) for the *CASP8* -652 6N ins/del and codon D302H G>C SNPs. We reconstructed haplotypes for each subject on the basis of the observed *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 performed using Pearson's chi-square test. All analyses were performed using Statistical Analysis System software (version 9.1, SAS Institute Inc., Cary, NC). 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 minority (each group <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 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 HWE 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 -652 6N del allele appeared 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 LD between the -652 6N del and 302H (C) variant alleles was incomplete in all control subjects ($D' = 0.415$, $r^2 = 0.027$), 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.

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: young than the median age of 56 years of the controls (adjusted OR = 0.74, 95% CI = 0.56–0.99), old 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 may be the result of fewer observations in some strata, or the differences may 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 interactions in risk associated with the *CASP8* -652 6N variant and by selected stratification factors nor by tumor sub-site (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 the CPT-induced apoptosis assay 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 vs. female), smoking status (ever vs. never) and alcohol use (ever vs. never) (data not shown). However, there appeared to be a weak statistical correlation ($r = 0.250$, $P = 0.001$) between increasing apoptotic 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) ($P = 0.951$) (Figure 1B). However, the apoptotic index appeared 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, respectively) (Figure 1C), but this trend was not statistically significant ($P = 0.109$).

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, compared with the ins/ins genotype. This lower risk was not observed for any of the D302H genotypes. However, haplotype analysis suggested that the del allele, in the presence 302D (Del-D haplotype of -652 6N/D302H), but not the 302H, allele (i.e., Del-H, which had fewer observations), was also associated with a significantly lower

SCCHN risk, compared with the ins-D haplotype, regardless tumor sites or tumor-related clinical characteristics. Furthermore, the apoptotic index appeared 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 between the *CASP8* -652 6N polymorphism and risk of cancers other than SCCHN. The earliest Chinese study, comparing patients with several types of cancer and cancer-free controls, found that *CASP8* -652 6N del variant genotypes were associated with significantly reduced risks of lung, breast, esophageal, gastric, colorectal, and cervical cancers (14). However, a later, larger multi-center study of 7,753 breast cancer patients and 7,921 controls indicated that the *CASP8* -652 6N del variant had no significant effect on breast cancer risk in Europeans (17). An Italian study of 580 familial breast cancer patients 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 diagnosis (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 colorectal cancer and 6,266 controls from different ancestries found that -652 6N del variant genotypes were not associated with the risk of breast, colorectal, or prostate cancer in populations with Asian, European, or African origin (16). 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 a 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 physiological 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 a 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 US Caucasian populations 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 United Kingdom study of 3,191 breast cancer patients and 3,258 cancer-free controls found that *CASP8* 302H variant genotypes were associated with a significantly reduced breast cancer risk (15). Later, a small US study of 216 colon cancer patients and 255 controls 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 familial breast cancer patients 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 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 CPT-induced and p53-mediated apoptosis, because the inhibition of topoisomerase I by CTP leads to increased levels of DNA strand breaks, a strong apoptotic signal (22). Interestingly, we found that the apoptotic index appeared 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 leads to a reduced probability of carcinogenesis. Although our experiments were performed 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 in order to better understand what region of the gene may 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 performed to validate our observed association and identify the true disease-causing variants and their functionality.

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Abbreviations used

SNP	single nucleotide polymorphism
OR	odds ratio
CI	confidence interval
SCCHN	squamous cell carcinoma of head and neck
CPT	Camptothecin

References

1. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. *J Dent Res* 2007;86:104–114. [PubMed: 17251508]
2. Jemal A, Siegel R, Ward E, et al. Cancer statistics 2008. *CA Cancer J Clin* 2008;58:71–96. [PubMed: 18287387]
3. Franceschi S, Talamini R, Barra S, et al. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. *Cancer Res* 1990;50:6502–6507. [PubMed: 2208109]
4. Ho T, Wei Q, Sturgis EM. Epidemiology of carcinogen metabolism genes and risk of squamous cell carcinoma of the head and neck. *Head Neck* 2007;29:682–699. [PubMed: 17274053]
5. Gattas GJ, de Carvalho MB, Siraque MS, et al. Genetic polymorphisms of CYP1A1, CYP2E1, GSTM1, and GSTT1 associated with head and neck cancer. *Head Neck* 2006;28:819–826. [PubMed: 16721740]
6. Li G, Liu Z, Sturgis EM, et al. Genetic polymorphisms of p21 are associated with risk of squamous cell carcinoma of the head and neck. *Carcinogenesis* 2005;26:1596–1602. [PubMed: 15878916]
7. Li G, Sturgis EM, Wang LE, et al. Association of a p73 exon 2 G4C14-to-A4T14 polymorphism with risk of squamous cell carcinoma of the head and neck. *Carcinogenesis* 2004;25:1911–1916. [PubMed: 15180941]
8. Lowe SW, Lin AW. Apoptosis in cancer. *Carcinogenesis* 2000;21:485–495. [PubMed: 10688869]
9. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. *Nature* 2001;411:342–348. [PubMed: 11357141]
10. Grenet J, Teitz T, Wei T, Valentine V, Kidd VJ. Structure and chromosome localization of the human CASP8 gene. *Gene* 1999;226:225–232. [PubMed: 9931493]
11. Bethke L, Sullivan K, Webb E, et al. The Common D302H Variant of CASP8 Is Associated with Risk of Glioma. *Cancer Epidemiol Biomarkers Prev* 2008;17:987–989. [PubMed: 18398042]
12. Rajaraman P, Wang SS, Rothman N, et al. Polymorphisms in apoptosis and cell cycle control genes and risk of brain tumors in adults. *Cancer Epidemiol Biomarkers Prev* 2007;16:1655–1661. [PubMed: 17684142]
13. Lan Q, Zheng T, Chanock S, et al. Genetic variants in caspase genes and susceptibility to non-Hodgkin lymphoma. *Carcinogenesis* 2007;28:823–827. [PubMed: 17071630]
14. Sun T, Gao Y, Tan W, et al. A six-nucleotide insertion-deletion polymorphism in the CASP8 promoter is associated with susceptibility to multiple cancers. *Nat Genet* 2007;39:605–613. [PubMed: 17450141]
15. MacPherson G, Healey CS, Teare MD, et al. Association of a common variant of the CASP8 gene with reduced risk of breast cancer. *J Natl Cancer Inst* 2004;96:1866–1869. [PubMed: 15601643]

16. Haiman CA, Garcia RR, Kolonel LN, Henderson BE, Wu AH, Le Marchand L. A promoter polymorphism in the CASP8 gene is not associated with cancer risk. *Nat Genet* 2008;40:259–260. author reply 60–1. [PubMed: 18305469]
17. Frank B, Rigas SH, Bermejo JL, et al. The CASP8 -652 6N del promoter polymorphism and breast cancer risk: a multicenter study. *Breast Cancer Res Treat.* 2007
18. De Vecchi G, Verderio P, Pizzamiglio S, et al. Evidences for association of the CASP8 -652 6N del promoter polymorphism with age at diagnosis in familial breast cancer cases. *Breast Cancer Res Treat.* 2008
19. Cox A, Dunning AM, Garcia-Closas M, et al. A common coding variant in CASP8 is associated with breast cancer risk. *Nat Genet* 2007;39:352–358. [PubMed: 17293864]
20. Chen K, Hu Z, Wang LE, et al. Single-nucleotide polymorphisms at the TP53-binding or responsive promoter regions of BAX and BCL2 genes and risk of squamous cell carcinoma of the head and neck. *Carcinogenesis* 2007;28:2008–2012. [PubMed: 17693666]
21. Li C, Zhao H, Hu Z, et al. Genetic variants and haplotypes of the caspase-8 and caspase-10 genes contribute to susceptibility to cutaneous melanoma. *Human mutation.* 2008
22. Fusaro G, Dasgupta P, Rastogi S, Joshi B, Chellappan S. Prohibitin induces the transcriptional activity of p53 and is exported from the nucleus upon apoptotic signaling. *J Biol Chem* 2003;278:47853–47861. [PubMed: 14500729]
23. Pantazis P, Early JA, Mendoza JT, DeJesus AR, Giovanella BC. Cytotoxic efficacy of 9-nitrocamptothecin in the treatment of human malignant melanoma cells in vitro. *Cancer Res* 1994;54:771–776. [PubMed: 8306340]
24. Yazici YD, Kim S, Jasser SA, et al. Antivascular therapy of oral tongue squamous cell carcinoma with PTK787. *Laryngoscope* 2005;115:2249–2255. [PubMed: 16369175]
25. Cotter TG. Induction of apoptosis in cells of the immune system by cytotoxic stimuli. *Semin Immunol* 1992;4:399–405. [PubMed: 1286166]
26. Seker H, Butkiewicz D, Bowman ED, et al. Functional significance of XPD polymorphic variants: attenuated apoptosis in human lymphoblastoid cells with the XPD 312 Asp/Asp genotype. *Cancer Res* 2001;61:7430–7434. [PubMed: 11606376]
27. Danial NN, Korsmeyer SJ. Cell death: critical control points. *Cell* 2004;116:205–219. [PubMed: 14744432]
28. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770–776. [PubMed: 11048727]
29. Medema JP, Scaffidi C, Kischkel FC, et al. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *Embo J* 1997;16:2794–2804. [PubMed: 9184224]
30. Scully C, Field JK, Tanzawa H. Genetic aberrations in oral or head and neck squamous cell carcinoma (SCCHN): 1. Carcinogen metabolism, DNA repair and cell cycle control. *Oral Oncol* 2000;36:256–263. [PubMed: 10793327]
32. Liu H, Chang DW, Yang X. Interdimer processing and linearity of procaspase-3 activation. A unifying mechanism for the activation of initiator and effector caspases. *J Biol Chem* 2005;280:11578–11582. [PubMed: 15664982]
32. Goodman JE, Mechanic LE, Luke BT, Ambs S, Chanock S, Harris CC. Exploring SNP-SNP interactions and colon cancer risk using polymorphism interaction analysis. *Int J Cancer* 2006;118:1790–1797. [PubMed: 16217767]
33. Frank B, Hemminki K, Wappenschmidt B, et al. Association of the CASP10 V410I variant with reduced familial breast cancer risk and interaction with the CASP8 D302H variant. *Carcinogenesis* 2006;27:606–609. [PubMed: 16251207]
34. Andersen MH, Becker JC, Straten P. Regulators of apoptosis: suitable targets for immune therapy of cancer. *Nat Rev Drug Discov* 2005;4:399–409. [PubMed: 15864269]

Figure 1. Graphic presentation of apoptotic index by age and *CASP8* genotypes

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* H302D genotypes: there is no trend of 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) allele increased, which is consistent with its observed inverse association, assuming the lymphocytes serve as the surrogates for target cells.

Table 1

Baseline clinical characteristics of cases and controls

Variables	Cases n = 1023 (%)	Controls n = 1052 (%)	P*
Age (y)			0.785
≤ 56	505 49.4	513 48.8	
> 56	518 50.6	539 51.2	
Sex			
Male	790 77.2	807 76.7	0.782
Female	233 22.8	245 23.3	
Smoking status			
Current	361 35.3	160 15.2	<0.001
Former	390 38.1	386 36.7	
Never	272 26.6	506 48.1	
Drinking status			
Current	524 51.2	428 40.7	<0.001
Former	225 22.0	169 16.1	
Never	274 26.8	455 43.2	
Histological type			
Oral cavity	295 28.8		
Pharynx [‡]	564 55.1		
Larynx	164 16.1		
Primary tumor (T) [‡]			
T ₁	260 25.4		
T ₂	373 36.5		
T ₃	200 19.5		
T ₄	190 18.6		
Lymph node metastasis (N) [§]			
N ₀	375 36.6		
N ₁	149 14.6		
N ₂	455 44.5		

Variables	Cases <i>n</i> = 1023 (%)	Controls <i>n</i> = 1052 (%)	<i>P</i> [*]
N ₃	44	4.3	

^{*} *P* values for a two-sided χ^2 test.

[†] Included both oropharyngeal and hypopharyngeal cancer cases.

[‡] T: The extent of the primary T₁: tumor ≤ 2 cm at the greatest dimension; T₂ to T₄: increasing greatest dimensions.

[§] N: regional lymph node involvement. N₀: no regional lymph nodes involved; N₁ to N₃: increasing involvement of regional lymph nodes.

Table 2

Allele, genotype, and haplotype frequencies of *CASP8* among cases and controls and their associations with squamous cell carcinoma of the head and neck

Variables	Cases No. (%)	Controls* No. (%)	P	Adjusted OR (95% CI) †
Genotypes				
Total no. subjects	1023	1052		
Total no. alleles	2046	2104		
<i>CASP8</i> -652 6N (AGTAAG /-)			0.002‡	
ins/ins	311 (30.4)	257 (24.4)		1.00 (ref.)
ins/del	456 (44.6)	542 (51.5)		0.65 (0.52–0.81)
del/del	256 (25.0)	253 (24.1)		0.80 (0.62–1.03)
Trend test				P = 0.060
ins/del + del/del	712 (69.6)	795 (75.6)		0.70 (0.57–0.85)
del allele	0.473	0.498	0.107 §	
<i>CASP8</i> D302H			0.706 †	
DD	745 (72.8)	783 (74.4)		1.00 (ref.)
DH	261 (25.5)	252 (24.0)		1.07 (0.87–1.31)
HH	17 (1.7)	17 (1.6)		1.00 (0.50–2.00)
Trend test				P = 0.608
DH+HH	278 (27.2)	269 (25.6)		1.06 (0.87–1.30)
H allele	0.144	0.136	0.444 §	
Haplotypes^{//}				
Total no. haplotypes	2046	2104		
ins - D	998 (48.8)	972 (46.2)	0.121 **	1.00 (ref.)
del - D	751 (36.7)	846 (40.2)		0.73 (0.55–0.97)
del - H	217 (10.6)	202 (9.6)		1.00 (0.64–1.58)
ins - H	80 (3.9)	84 (4.0)		0.84 (0.37–1.93)

* The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$) ($\chi^2 = 0.97$, $P = 0.324$ for *CASP8* -652 6N ins/del; $\chi^2 = 0.41$, $P = 0.522$ for *CASP8* D302H).

† OR (odds ratio) and CI (confidence interval) 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.

// Haplotypes constructed in the order of variant alleles of *CASP8* -652 ins/del and D302H.

** Globe tests for differences in haplotype frequencies between cases and controls.

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 3

Variables	Cases (n = 1023)			Controls (n = 1052)			Adjusted OR 95% CI [*]
	ins/ins No. (%)	ins/del No. (%)	ins/del+del/del No. (%)	ins/ins No. (%)	ins/del No. (%)	ins/del+del/del No. (%)	
Age (years)							
< 56	151 (29.9)	354 (70.1)	128 (25.0)	385 (75.0)			0.74 (0.56–0.99)
≥ 56	160 (30.9)	358 (69.1)	129 (23.9)	410 (76.1)			0.66 (0.50–0.88)
Sex							
Male	231 (29.2)	559 (70.8)	193 (23.9)	614 (76.1)			0.73 (0.58–0.92)
Female	80 (34.3)	153 (65.7)	64 (26.1)	181 (73.9)			0.61 (0.40–0.93)
Smoking status							
Never	89 (32.7)	183 (67.3)	140 (27.7)	366 (72.3)			0.78 (0.57–1.07)
Ever	222 (29.6)	529 (70.4)	117 (21.4)	429 (78.6)			0.66 (0.50–0.85)
Drinking status							
Never	89 (32.5)	185 (67.5)	119 (26.2)	336 (73.8)			0.72 (0.51–1.01)
Ever	222 (29.6)	527 (70.4)	138 (23.1)	459 (76.9)			0.69 (0.54–0.89)
Tumor site							
Oral cavity	84 (28.5)	211 (71.5)	257 (24.4)	795 (75.6)			0.77 (0.57–1.04)
Pharynx [†]	174 (30.9)	390 (69.1)	257 (24.4)	795 (75.6)			0.69 (0.54–0.87)
Larynx	53 (32.3)	111 (67.7)	257 (24.4)	795 (75.6)			0.59 (0.41–0.87)

^{*} OR (odds ratio) and CI (confidence interval) were adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.

[†] Included both oropharyngeal and hypopharyngeal cancer cases.