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Effects of polymorphisms in alcohol metabolism and oxidative stress genes on survival from head and neck cancer

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ABSTRACT

Background: Heavy alcohol consumption increases risk of developing squamous cell carcinoma of the head and neck (SCCHN). Alcohol metabolism to cytotoxic and mutagenic intermediates acetaldehyde and reactive oxygen species is critical for alcohol-drinking-associated carcinogenesis. We hypothesized that polymorphisms in alcohol metabolism-related and antioxidant genes influence SCCHN survival.
Methods: Interview and genotyping data (64 polymorphisms in 12 genes) were obtained from 1227 white and African-American cases from the Carolina Head and Neck Cancer Epidemiology study, a population-based case–control study of SCCHN conducted in North Carolina from 2002 to 2006. Vital status, date and cause of death through 2009 were obtained from the National Death Index. Kaplan–Meier log-rank tests and adjusted hazard ratios were calculated to identify alleles associated with survival.
Results: Most tested SNPs were not associated with survival, with the exception of the minor alleles of rs3813865 and rs8192772 in *CYP2E1*. These were associated with poorer cancer-specific survival ($HR_{rs3813865}$, 95%CI = 2.00, 1.33–3.01; $HR_{rs8192772}$, 95%CI = 1.62, 1.17–2.23). Hazard ratios for 8 additional SNPs in *CYP2E1*, *GPx2*, *SOD1*, and *SOD2*, though not statistically significant, were suggestive of differences in allele hazards for all-cause and/or cancer death. No consistent associations with survival were found for SNPs in *ADH1B*, *ADH1C*, *ADH4*, *ADH7*, *ALDH2*, *GPx2*, *GPx4*, and *CAT*.
Conclusions: We identified some polymorphisms in alcohol and oxidative stress metabolism genes that influence survival in subjects with SCCHN. Previously unreported associations of SNPs in *CYP2E1* warrant further investigation.

1. Introduction

Head and neck cancers typically include cancers of the oral cavity, pharynx, and larynx. There were estimated to be 53,640 new cases and 11,520 deaths from oral cavity, pharyngeal, and laryngeal cancers in the U.S. in 2013 [1]. Globally in 2008, oral cavity tumors were among the top 10 incident cancers in men world-wide, and among the top 10 fatal cancers in men in developing countries [2]. Five-year relative survival for laryngeal, oral cavity, and pharyngeal cancer patients averages about 80% for localized cases, 50% for regional cases, and 33% for metastatic cases, with somewhat lower survival for laryngeal compared to oral cavity and pharyngeal cancers [3]. Age-adjusted mortality rates for African-American men with oral cavity and pharynx cancers are double those of men of other races, and disparities are especially pronounced for men with laryngeal cancer (4.7 deaths per 100,000 population in African-Americans compared to 2.1 in whites, 1.9 in Native Americans and Hispanics, and 0.7 in Asian/Pacific Islanders) [4].

It is estimated that 75% of new cases of SCCHN in the United States are caused by tobacco use, especially cigarette smoking,

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and/or drinking of alcoholic beverages [5]. In the past few decades the proportional incidence of oropharyngeal tumors associated with carcinogenic human papillomavirus sub-types has risen; such tumors appear to have better prognosis than non-HPV oropharyngeal tumors [6]. Multiple studies have reported associations between SCCHN *incidence* and polymorphisms in alcohol metabolism genes, especially *ADH1B*, *ADH1C*, *ADH4*, *ADH7*, *ALDH2*, and *CYP2E1* [7–16]. The primary biological mechanism responsible for this effect is hypothesized to be high levels of cytotoxic and mutagenic acetaldehyde, the metabolic intermediate between ethanol and acetate. Acetaldehyde, when associated with consumption of alcoholic beverages, is classified as a known human carcinogen by the International Agency for Research on Cancer [17]. Also, alcohol metabolism through CYP2E1, probably mediated by alcohol-induced stabilization of the CYP2E1 protein [18], is known to result in production of increased levels of reactive oxygen species (ROS) [19]. DNA damage by acetaldehyde or ROS has been suggested as one of the key mechanisms of alcohol drinking-associated carcinogenesis, and variation in associated pathway genes may modify progression and survival. Therefore, it is also of interest to know whether polymorphisms in genes encoding enzymes protective against oxidative stress (*SOD*, *GPx*, *CAT*) are associated with altered survival in SCCHN patients.

In addition to its role in alcohol metabolism, CYP2E1, as a member of the cytochrome P450 oxidative system, is involved in metabolism of xenobiotics and drugs [20], including the platinum-containing chemotherapeutic agents used to treat many head and neck cancers. Although CYP2E1 enzymatically inactivates some substrates, it has also been shown to bioactivate many compounds that are possibly carcinogenic; for example, the tobacco carcinogen N-nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [21].

While many studies have examined genetic associations with SCCHN *incidence*, there have been few reports of the effect of gene polymorphisms in alcohol metabolism and oxidative stress-related genes on *survival* of subjects with SCCHN. For example, only two studies investigated whether selected *ADH1C* and *CYP2E1* polymorphisms influence prognosis, and they reported evidence of an association with advanced clinical stages or higher recurrence [11,22]. Further, no studies have examined the influence on post-diagnosis survival of genetic variation in the oxidative stress pathways.

We examined the effect on survival of 64 SNPs in *ADH1B*, *ADH1C*, *ADH4*, *ADH7*, *ALDH2*, and *CYP2E1* alcohol metabolism genes, and in *CAT*, *GPx1*, *GPx2*, *GPx4*, *SOD1*, and *SOD2* antioxidant genes, using exposure, genetic, clinical, and outcome data from cases included in a large North Carolina (NC) population-based case–control study of SCCHN that included both white and African-American subjects.

2. Materials and methods

2.1. Study population

Cases for this analysis were obtained from the Carolina Head and Neck Cancer Epidemiology study (CHANCE), a population-based case–control study [23,24].

All cases of squamous cell carcinoma of the oral cavity, pharynx, and larynx diagnosed in 46 NC counties between January 1, 2002 and February 28, 2006 were eligible for enrollment. Rapid identification of cases was conducted by the NC Central Cancer Registry [24]. CHANCE cases included ICD-O-3 topography codes C0.00–C14.8, and C32.0–C32.9, excluding salivary gland tumors (C07.9, C08.0–C08.9), nasopharynx (C11.0–C11.9), nasal cavity (C30.0), and nasal sinuses (C31.0–C31.9). ICD-O-3 morphology codes included were 8010/3, 8051/3, 8083/3, 8071/3, 8072/3, 8073/3, 8074/3, and 8076/3. Benign tumors, carcinomas in situ, papillary carcinomas, and adenoid carcinomas were excluded. This analysis further excluded 21 lip cancers (C00.3–C00.9, C14.2), 26 cases of "other" race, and 115 without genotyping data, producing a study analysis group of 1227 cases, of which 922 were white/European-American and 305 were black/African-American.

Case tumors were classified into anatomic sub-site according to the following 5 ICD-O categories that are also used by the International Head and Neck Cancer Epidemiology (INHANCE) Consortium [25]: (1) oral cavity: C02.0–C02.3, C03.0, C03.1, C03.9, C04.0, C04.1, C04.8, C04.9, C05.0, C06.0–C06.2, C06.8, and C06.9; (2) oropharynx: C01.9, C02.4, C05.1, C05.2, C09.0, C09.1, C09.8, C09.9, C10.0–C10.4, C10.8, and C10.9; (3) oral cavity–oropharynx–hypopharynx NOS: C02.8, C02.9, C05.8, C05.9, C14.0, C14.2, and C14.8; (4) hypopharynx: C12.9, C13.0–C13.2, C13.8, and C13.9; and (5) larynx: C32.0–C32.3, and C32.8–C32.9.

Written informed consent was obtained from all subjects. The study was approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill.

2.2. Outcome assessment

We determined whether death had occurred in study participants by December 31, 2009, and, if so, the date and cause, through linkage with the United States National Death Index (NDI). The NDI is a national file of identified death record information compiled from computer files submitted by State vital statistics offices [26]; of all US sources of national death data, NDI has demonstrated the highest sensitivity and is the only national source with a coded cause of death field suitable for research purposes [27]. CHANCE collected multiple NDI matching data: social security number (SSN), date of birth (DOB), sex, race, state of residence, and name. Therefore there was a high proportion (>75%) of perfect/very close to perfect matches on SSN, DOB, and sex. The remaining near matches were further confirmed by examining the United States Social Security Death Index, which is created from Social Security Administration (SSA) records of persons with social security numbers whose deaths were reported to the SSA, usually by mortuaries, attorneys, or family members. A very small number were further confirmed by obituary search of newspaper websites. Partial matches on name, but having in common only a few SSN digits and parts of the DOB, that could not be confirmed by SSDI or obituary were excluded as non-matches. If the initial three digits of the first-listed cause of death code were C01–C06, C09, C10, C12–C14, or C32, the cause of death was classified as head and neck cancer.

2.3. SNP selection and genotyping

Blood samples were obtained at the time of questionnaire administration by nurse-interviewers trained in phlebotomy. If the subject was not willing or able to consent to the blood draw, they were asked to contribute a buccal cell sample via mouthrinse.

For this analysis, 75 SNPs (69 tag SNPs and 6 candidate SNPs found in prior studies to be associated with aerodigestive cancer incidence, breast cancer survival, alcohol dependence, or interaction with genes in the proposed carcinogenic pathway for vinyl chloride) were selected in 12 genes that are part of two metabolic pathways: *ADH1B*, *ADH1C*, *ADH4*, *ADH7*, *ALDH2*, and *CYP2E1* in the alcohol metabolism pathway in the upper aerodigestive tract; and *CAT*, *SOD1*, *SOD2*, *GPx1*, *GPx2*, and *GPx4* in the oxidative stress metabolic pathway. Tag SNPs, chosen to represent the genetic variation within each of the 12 candidate genes (gene and 2000 bp upstream and downstream) were selected using the Genome