Research Article

Cancer Epidemiology, Biomarkers & Prevention

Effects of *ERCC2* Lys751Gln (A35931C) and *CCND1* (G870A) Polymorphism on Outcome of Advanced-Stage Squamous Cell Carcinoma of the Head and Neck Are Treatment Dependent

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Abstract

Background: Germline variation in DNA damage response may explain variable treatment outcomes in squamous cell carcinoma of the head and neck (SCCHN). By grouping patients according to stage and radiation treatment, we compared SCCHN survival with regard to *ERCC2* A35931C (Lys751Gln, rs13181) and *CCND1* G870A (Pro241Pro, rs9344) genotypes.

Methods: In a hospital-based SCCHN case series (all white, 24.7% female, mean age 58.4 years), this treatment-outcome cohort study genotyped 275 stage III–IV cases that were initially treated with radiation (with or without chemotherapy) and 80 stage III–IV and 130 stage I–II cases that were initially treated without radiation or chemotherapy and used Kaplan–Meier and Cox regression analyses to compare genotype groups on the basis of overall, disease-specific, progression-free, and recurrence-free survival rates.

Results: *ERCC2* 35931 AA predicted worse survival in stage III–IV cases treated with radiation [multiply-adjusted HR = 1.66, 95% confidence interval (CI), 1.15–2.40; HR over the first 3 follow-up years = 1.92; 95% CI, 1.28–2.88] and better survival in stage III–IV cases not treated with radiation (HR = 0.26; 95% CI, 0.11–0.62). Although not associated with survival in stage III–IV cancers treated with radiation (HR = 1.00; 95% CI, 0.67–1.51), *CCND1*-870 GG predicted better survival in stage III–IV cancers not treated with radiation (HR = 0.14; 95% CI, 0.04–0.50). Survival in stage I–II did not depend on *ERCC2* A35931C or *CCND1* G870A genotype.

Conclusions: Although promoting tumor progression in untreated patients, germline differences in DNA-repair or cell-cycle control may improve treatment outcome in patients treated with DNA-damaging agents.

Impact: *ERCC2* A35931C may help distinguish advanced stage SCCHN with better outcomes from radiation treatment. *Cancer Epidemiol Biomarkers Prev; 20(11); 2429–37. ©2011 AACR.*

Introduction

The American Cancer Society expected nearly 50,000 oral cavity, pharynx, and larynx cancer cases in 2010, representing 3.2% of all cancer expected in the United States (1). During 2001–2007, 61% of oral cavity and pharynx cancer and 25% of larynx cancer presented at stages (regional or distant) that were associated with less than 60% 5-year relative survival rate (2). Primary treatment of these later stage cancers generally includes surgery and/or radiation, sometimes combined with chemotherapy (3). The less-than-desired results from these standard treatments motivate the search for predictors of treatment outcome. Based on the notion that tumor cells, as a result of germline genetic differences, may exhibit a different therapeutic response to the DNA damage caused by radiation and chemotherapy, a line of research aims to differentiate disease outcomes according to variation in DNA-repair and cell-cycle control genes.

In a hospital-based case series comprising 485 white patients with squamous cell carcinoma of the head and neck (SCCHN), we examined survival outcomes with regard to stage, treatment, and 2 polymorphisms, *ERCC2* A35931C (Lys751Gln, rs13181) and *CCND1* G870A (Pro241Pro, rs9344). *ERCC2* A35931C located on exon 23 of *ERCC2*, a gene that codes for a DNA helicase involved in nucleotide excision repair, produces an amino acid change (LysGln) associated with DNA–repair-phenotype differences (4–6), with *ERCC2* 35931C generally associated with lower *ERCC2* mRNA levels (6), lower

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DNA-repair efficiency (7), and higher DNA adduct levels (7). *CCND1*, the gene for a cyclin (cyclin D1) that controls the G_1 to S-phase cell-cycle transition, contains the exon 4 synonymous G870A (ProPro) polymorphism. The *CCND1* 870A variant promotes alternative splicing of the *CCND1* transcript, producing a truncated protein (cyclin D1b) with absent exon 5 (8–10). Cyclin D1b lacks 2 regulatory motifs, a PEST sequence and threonine 286, associated with protein degradation and nuclear export, respectively (10). In addition, cyclin D1b may possess intrinsic cancer-promoting properties (10). Current interest in the survival effects of these 2 polymorphisms includes not only cancer of the head and neck (11–17) but also cancers at various other sites (18–24).

Materials and Methods

Study population

We used a cohort study design to test genetic associations with SCCHN treatment outcome. Over a 7-year period (February 14, 2000, through February 14, 2008), the University Ear Nose and Throat Specialists practice at the University of Pittsburgh Medical Center recruited 686 white patients in the age range 20 to 81 years within 1 year of the diagnosis of a biopsy-verified primary SCC of the head and neck. A standardized interviewer-administered questionnaire was used to collect risk factor information (25) and blood samples were obtained for genotyping. Every case signed an informed consent (IRB #981041) form approved by the University of Pittsburgh Institutional Review Board. This case series constituted approximately one half of all 20- to 81-year-old white persons treated for a primary SCCHN by the University Ear Nose and Throat Specialists practice. Our analysis excluded 201 cases for the following 8 reasons: (i) DNA unavailable for analysis ($n = 24$); (ii) *ERCC2* and/or *CCND1* genotype assay failure ($n = 21$); (iii) outcome information unavailable ($n = 70$); (iv) enrollment after second or later primary head and neck cancer ($n = 24$); (v) visceral metastasis at diagnosis ($n = 3$); (vi) unknown stage at diagnosis ($n = 6$); (vii) chemotherapy as primary treatment without radiation therapy ($n = 6$); and (viii) radiation (with or without surgery) as primary treatment of stage I or II disease ($n = 47$). Compared with cases enrolled in the current study ($n = 485$), cases that were excluded ($n = 201$) were more frequently enrolled between 2006 and 2008 and less frequently enrolled between 2003 and 2005 ($P = 0.01$; Supplementary Table S1). In other regards, included and excluded cases were similar with respect to sex, age at diagnosis, education, cigarette smoking and alcohol-use history, and *ERCC2* A35931C and *CCND1* G870A genotypes ($P > 0.15$; Supplementary Table S1).

Genotyping

We used kits procured from Gentra Systems Inc. to isolate DNA from whole blood, a Thermo Scientific Nanodrop 1000 full-spectrum UV/visible spectrophotometer to evaluate DNA quantity and quality, and commercial TaqMan allele discrimination assays run on the ABI 7700 Sequence Detection System (Applied Biosystems) to determine *ERCC2* A35931C (rs13181) and *CCND1* G870A (rs9344) genotypes. Each amplification reaction included negative assay controls and positive control samples with known genotype confirmed by sequencing. Two laboratory technicians independently interpreted each assay. Assays of blind duplicate samples and repeated analysis of a 10% sample set produced completely concordant genotypic results.

Tumor and outcome information

Using inpatient and outpatient medical records, the Social Security Death Index, and a previously described computer system to control data quality (26), a dedicated and certified tumor registrar abstracted and recorded anatomic subsite, TNM stage, primary treatment, and clinical outcomes. Clinical outcome variables that were ascertained, classified, and dated included death, death due to head and neck cancer, treatments for head and neck cancer recurrence, progression, or metastasis, and treatments for new primary SCCHN. Recording disease activity [no evidence of disease (NED) vs. alive with disease (AOD)], the tumor registrar also maintained a catalog of outpatient visits to the University Ear Nose and Throat Specialists.

Study endpoints

Analyses considered 1 primary endpoint (overall survival rate) and 3 secondary endpoints (disease-specific, progression-free, and recurrence-free survival rates). The overall survival endpoint calculated survival as time between the treatment start date (date of definitive surgery or date chemotherapy or radiation therapy started for cases treated without surgery) and date of death. The disease-specific survival rate endpoint calculated survival as time between the treatment start date and date of death due to head and neck cancer. The progression-free and recurrence-free survival rate endpoints calculated survival as time between the treatment start date and the earliest (i) date of first AOD visit occurring after an NED visit; (ii) date of first treatment of head and neck cancer recurrence, progression, or metastasis; (iii) date of first treatment of new primary SCCHN; or (iv) date of death due to head and neck cancer. For cases not experiencing an endpoint, overall survival rate analyses censored follow-up on the date of last live contact. Disease-specific, progression-free, and recurrence-free survival rate analyses censored follow-up on either the date of death due to known causes unrelated to head and neck cancer or otherwise on the date of last alive contact. Analyses for recurrence-free survival included only cases with a posttreatment NED visit.

Other variables

Analyses used sex, age on treatment start date, primary head and neck tumor subsite, American Joint Committee on Cancer (AJCC) diagnostic stage group (27), and

treatment category (no radiation or chemotherapy, radiation therapy combined with platinum chemotherapy, and radiation therapy with or without nonplatinum chemotherapy) to define analytic cohorts and to adjust genotype associations with clinical outcome. Analyses grouped subsites into 4 categories: (i) lip and oral cavity; (ii) oropharynx (including soft palate, base of tongue, vallecula, and tonsil); (iii) larynx (ICD-O-3 C32.0 through C32.9); and (iv) other (including nasal cavity and sinus, nasopharynx, hypopharynx, and undetermined primary subsite). A previous publication defines cigarette smoking and alcohol-use history and describes procedures used to calculate lifetime cigarette and alcohol dose exposures (25).

Statistical analysis

We based inferences on 3 cohorts: 1 cohort of primary interest (a stage III–IV cohort exposed to radiation, with or without chemotherapy, as part of initial treatment) and 2 cohorts of secondary interest (stage I–II and stage III–IV cohorts, exposed neither to radiation nor to chemotherapy as part of initial treatment). We used the product-limit (Kaplan–Meier) method to estimate survival functions and the log-rank test with a Wilcoxon weight (the number at risk n_i at failure time t_i) to compare survival differences according to genotype. We evaluated overall survival differences under dominant and recessive genetic models, selected the model that produced the most statistically significant survival difference in our cohort of primary interest, and based statistical inferences on a Bonferroni-corrected P value $= 0.0125 = 0.05/4$ (for 2 polymorphisms evaluated under 2 models). Using SAS 9.2 (PROC PHREG), we fit proportional hazards (Cox) models to control genotype-outcome associations for potential confounders and applied a log-likelihood ratio test to genotype by cofactor interaction terms to screen for effect modification. To detect departures from the proportional hazards assumption, we examined Schoenfeld residual plots and tested the statistical significance of terms representing the time-dependent interaction between genotype and the natural logarithm of survival time. If results indicated nonproportional hazards, we examined Wald-type test statistics from single-variable and multiple-variable piecewise-constant time-varying coefficients (Gray's) models (28, 29) to confirm the statistical significance of outcome differences according to genotype.

Results

Description of study cohorts

Table 1 summarizes the 3 study cohorts, which represent SCCHN categorized according to stage at diagnosis and use of radiation as part of initial treatment. Among all cohorts, key characteristics include 24.7% female, mean age 58.4 years (SD = 10.7 years), 13% positive history of cancer, 76.7% history of cigarette smoking, mean 44.3 pack-years among ever smokers (SD = 30.0 pack-years), 86.2% history of alcohol use, and median 16,400 lifetime total drinks among ever drinkers [interquartile range (IQR) = 3,500–39,900], an amount corresponding to 1.5 drinks per day for 30 years. Male sex, younger age, and an oropharyngeal subsite primary tumor location characterized stage III–IV radiation-treated cases, the cohort of main interest.

Genetics of *ERCC2* A35931C (Lys751Gln) and *CCND1* G870A (Pro241Pro)

In an aggregate group of all 3 cohorts, *ERCC2* 35931 AA, AC, and CC genotypes appeared in 39.2%, 44.7%, and 16.1% (C minor allele frequency = 0.384, SE = 0.016) and *CCND1*-870 GG, GA, and AA genotypes in 27.8%, 49.3%, and 22.9% (A minor allele frequency = 0.475, SE = 0.016), respectively. Neither genotype distribution violated the Hardy–Weinberg equilibrium ($P > 0.2$). In a white control group described elsewhere (25), but not otherwise mentioned in this article, *ERCC2* 35931 AA, AC, and CC genotypes appeared in 36.4%, 46.5%, and 17% (C minor allele frequency = 0.403, SE = 0.012; $n = 834$) of cases and *CCND1*-870 GG, GA, and AA genotypes in 28.6%, 48.2%, and 23.2% (A minor allele frequency = 0.473; SE = 0.012; $n = 838$) cases, respectively. Whether evaluated under additive, dominant, or recessive genetic models, *ERCC2* A35931C and *CCND1* G870A case and control genotype differences were not statistically significant ($P > 0.3$).

Among all cases and among stage III–IV radiation-treated cases only, *ERCC2* A35931C and *CCND1* G870A genotypes were statistically independent of factors such as enrollment year, sex, age, prior history of cancer, smoking and alcohol histories, pack-years, and lifetime total drinks ($P > 0.05$; Supplementary Table S2). As shown in Table 1, *ERCC2* A35931C genotype distributions in the 3 study cohorts were statistically indistinguishable from each other. However, *CCND1* G870A genotype distributions differed ($P = 0.04$; Table 1), with relative paucity of 870A homozygotes among stage I–II cases. This latter finding corresponds to the lower proportion of stage I–II disease observed among all *CCND1* 870A homozygous cases (Supplementary Table S3).

Survival outcomes

Figure 1 shows overall survival, separately for each cohort, according to *ERCC2* A35931C and *CCND1* G870A genotypes. The radiation-treated stage III–IV cohort comprised 275 cases, including 155 censored before death at a median interval of 4.3 years (IQR = 3.2–6.0 years) after the start of treatment. The stage III–IV cohort untreated with radiation comprised 80 cases, including 46 censored before death at a median interval of 4.3 years (IQR = 2.6–6.8 years) after the start of treatment. The stage I–II cohort comprised 130 cases, including 103 censored before death at a median interval of 3.8 years (IQR = 2.1–5.6 years) after the start of treatment.

In stage III–IV cases, the effect of genotype depended on treatment. In cases treated with radiation, overall survival was worse in *ERCC2* 35931A homozygotes (AA) than in cases with at least 1 35931C allele (AC + CC; $P = 0.0013$)

Table 1. Characteristics of all study subjects and of study subjects subdivided into 3 cohorts defined by stage at diagnosis and use of radiation as part of initial treatment

		Stage III–IV			
Characteristic	**ALL ($n = 485$)**	**XRT ($n = 275$)**	**No XRT ($n = 80$)**	**Stage I–II no XRT ($n = 130$)**	**P^a**
Year enrolled					0.083
2000–2002	30.7	26.9	40.0	33.1	
2003–2005	30.5	29.5	30.0	33.1	
2006–2008	38.8	43.6	30.0	33.8	
Sex					0.028
Men	75.3	79.6	72.5	67.7	
Women	24.7	20.4	27.5	32.3	
Age, y					0.006
<50	19.6	21.8	20.0	14.6	
50–59	33.8	38.5	25.0	29.2	
60–69	30.5	28.4	36.3	31.5	
≥70	16.1	11.3	18.8	24.6	
History of cancer					0.896
Yes	13.0	12.4	13.8	13.8	
No	87.0	87.6	86.3	86.2	
Cigarette smoker					0.157
Ever	76.7	74.9	85.0	75.4	
Never	23.3	25.1	15.0	24.6	
Pack-years	(2)		(2)		0.283
Never smoker	23.4	25.1	15.4	24.6	
<15	11.8	10.5	12.8	13.8	
15–39	25.5	27.6	21.8	23.1	
≥40	39.3	36.7	50.0	38.5	
Alcohol drinker	(1)	(1)			0.826
Ever	86.2	85.8	85.0	87.7	
Never	13.8	14.2	15.0	12.3	
Lifetime total drinks	(4)	(3)	(1)		0.076
0–499	20.0	18.4	22.8	21.5	
500–4,999	19.3	15.8	25.3	23.1	
5,000–19,999	23.1	27.2	12.7	20.8	
≥20,000	37.6	38.6	39.2	34.6	
Subsite					<0.0001
Lip and oral cavity	49.5	36.7	56.3	72.3	
Oropharynx	26.4	41.8	8.8	4.6	
Larynx	17.9	13.5	28.8	20.8	
Other	6.2	8.0	6.3	2.3	
Stage group					
I	19.6			73.1	
II	7.2			26.9	
III	23.7	24.7	58.8		
IVa	46.8	71.3	38.8		
IVb	2.7	4.0	2.5		
Chemotherapy	(4)	(4)			
None	55.5	21.0	100.0	100.0	
Platinum	36.0	63.8			
Nonplatinum	8.5	15.1			

(Continued on the following page)

Table 1. Characteristics of all study subjects and of study subjects subdivided into 3 cohorts defined by stage at diagnosis and use of radiation as part of initial treatment (Cont'd)

		Stage III–IV			
Characteristic	**ALL ($n = 485$)**	**XRT ($n = 275$)**	**No XRT ($n = 80$)**	**Stage I–II no XRT ($n = 130$)**	***P*[a]**
ERCC2 A35931C (Lys751Gln)					0.566
AA	39.2	37.1	40.0	43.1	
AC	44.7	47.6	40.0	41.5	
CC	16.1	15.3	20.0	15.4	
CCND1 G870A (Pro241Pro)					0.040
GG	27.8	28.4	23.8	29.2	
GA	49.3	44.4	55.0	56.2	
AA	22.9	27.3	21.3	14.6	

NOTE: Values given are percentages. Parentheses contain the number of observations with missing data for the characteristic tabulated. Abbreviation: XRT, radiation treatment.
[a]χ^2 test.

and statistically indistinguishable between *CCND1* 870G homozygotes (GG) and cases with at least 1 870A allele (GA + AA; $P = 0.49$). In cases not treated with radiation, however, overall survival was better in *ERCC2* 35931A homozygotes than in cases with at least 1 35931C allele ($P = 0.0013$) as well as in *CCND1* 870G homozygotes than in cases with at least 1 870A allele ($P = 0.0089$). In stage I–II cases, the overall survival rate was independent of genotype. Although levels of statistical significance varied with the number of events, as shown in Table 2, analyses of genotype associations with disease-specific, progression-free, and recurrence-free survival were consistent in direction with the associations observed with overall survival.

Table 3 shows results from multiple variable models of overall survival. Controlling for sex, age, subsite, stage, platinum treatment, and *CCND1* G870A genotype, *ERCC2* 35931A homozygosity remained a statistically significant predictor of poor overall survival in radiation-treated stage III–IV SCCHN (HR = 1.66; 95% confidence interval (CI), 1.15–2.40). Controlling for sex, age, subsite, and stage, *ERCC2* 35931A and *CCND1* 870G homozygosity both remained statistically significant predictors of better overall survival in stage III–IV SCCHN not treated with radiation (*ERCC2* A35931C AA vs. AC + CC: HR = 0.26, 95% CI, 0.11–0.62; *CCND1* G870A GG vs. GA + AA: HR = 0.14; 95% CI, 0.04–0.50). Screening procedures suggested a nonproportional *ERCC2* A35931C hazard over time in relation to overall survival rate for radiation-treated stage III–IV SCCHN. However, the *ERCC2* A35931C genotype remained a statistically significant predictor in a multiple-variable piecewise-constant time-varying coefficients model (Table 3) and in a model that truncated follow-up 3 years following entry (HR = 1.92, 95% CI, 1.28–2.88; Supplementary Table S4). In stage III–IV cases treated with radiation, interaction tests

Table 2. Outcome summary, by cohort—number at risk, number of overall survival, disease-specific survival, and progression-free survival events, number with no evidence of disease activity posttreatment, number of recurrence-free survival events, and statistical significance (log-rank *P* values) of outcome differences according to *ERCC2* A35931C and *CCND1* G870A genotype

							***ERCC2* A35931C *P* values**				***CCND1* G870A *P* value**			
Cohort	***n***	**OS**	**DSS**	**PFS**	**NED**	**RFS**	**OS**	**DSS**	**PFS**	**RFS**	**OS**	**DSS**	**PFS**	**RFS**
Stage III–IV XRT	275	120	62	95	235	63	<0.01	0.02	0.03	0.16	0.49	0.21	0.80	0.83
Stage III–IV no XRT	80	34	18	32	71	26	<0.01	0.05	0.02	0.18	<0.01	0.17	0.02	0.01
Stage I–II no XRT	130	27	10	40	126	38	0.78	0.98	0.79	0.59	0.71	0.41	0.58	0.54

Abbreviations: DSS, disease-specific survival; *n*, number at risk; NED, number with no evidence of disease activity; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; XRT, radiation treatment.

Table 3. Results from multiple variable regression—multiply adjusted[a] risk factor associations with overall survival, estimated by proportional HRs, and 95% CIs

	Stage III–IV XRT ($n = 275$)				Stage III–IV no XRT ($n = 80$)			
Risk factor comparison	**HR**	**95% CI**	**PH**	**TVC**	**HR**	**95% CI**	**PH**	**TVC**
ERCC2[b]								
AA vs. AC + CC	1.66	1.15–2.40	<0.01	<0.01	0.26	0.11–0.62	<0.01	0.01
CCND1[c]								
GG vs. GA + AA	1.00	0.67–1.51	0.99	0.26	0.14	0.04–0.50	<0.01	0.03
Sex								
Female vs. male	1.12	0.72–1.75	0.61	0.99	0.60	0.25–1.44	0.25	0.24
Age								
Per year of age	1.03	1.01–1.05	<0.01	<0.01	1.04	1.00–1.07	0.06	0.08
Subsite								
Oropharynx vs. oral cavity	0.46	0.30–0.70	<0.01	<0.01	1.01	0.32–3.23	0.99	0.59
Larynx vs. oral cavity	0.51	0.28–0.92	0.03	0.06	0.49	0.21–1.15	0.10	0.39
Other vs. oral cavity	0.76	0.37–1.56	0.45	0.67	0.33	0.04–2.57	0.29	0.62
Stage								
IV vs. III	1.90	1.17–3.10	<0.01	<0.01	3.49	1.53–7.95	<0.01	0.03
Treatment								
Platinum vs. no platinum	0.91	0.63–1.32	0.62	0.70				

NOTE: Results from 2 models, one for stage III–IV tumors treated with radiation (XRT) and a second for stage III–IV not treated with radiation.

Abbreviations: PH, *P* value from proportional hazards model; TVC, *P* value from piecewise-constant time-varying coefficients model; XRT, radiation treatment.

[a]HRs mutually adjusted for *ERCC2* A35931C genotype, *CCND1* G870A genotype, sex, age, subsite, stage, and treatment.

[b]*ERCC2* A35931C genotype.

[c]*CCND1* G870A genotype.

did not identify statistically significant differences in the *ERCC2* A35931C polymorphism survival effect in relation to head and neck cancer subsite, stage, platinum chemotherapy, or *CCND1* G870A genotype.

Finally, in subgroup analyses restricted to persons without personal history of cancer, *ERCC2* 35931A homozygosity persisted as a statistically significant predictor of poor overall survival in stage III–IV SCCHN treated with radiation ($n = 241$, log-rank $P = 0.0006$) and better overall survival in stage III–IV SCCHN not treated with radiation ($n = 69$, log-rank $P = 0.0072$).

Discussion

In our cohort of main interest, comprising radiation-treated stage III–IV SCCHN cases, we observed worse overall survival rate in 102 patients who were homozygous for *ERCC2* 35931A than in 173 patients with at least 1 *ERCC2* 35931C allele ($P = 0.0013$; Fig. 1). *ERCC2* 35931A homozygosity predicted not only worse overall survival, but also worse disease-specific ($P = 0.02$) and progression-free survival ($P = 0.03$; Table 2). The *ERCC2* A35931C genotype overall survival effect appeared limited to the first 3 years of follow-up, after which time the product-limit estimated risks of death were 48% (SE = 5%) in *ERCC2* 35931A homozygotes and 30% (SE = 4%) in persons with at least 1 *ERCC2* 35931C allele, a 1.6-fold difference. Adjustment for *CCND1* genotype, sex, age, head and neck cancer subsite, stage, and platinum chemotherapy did not change this association (unadjusted HR = 1.65, 95% CI, 1.15–2.38; adjusted HR = 1.66, 95% CI, 1.15–2.40; Table 3). Finally, our statistical screens for effect modification in stage III–IV cases treated with radiation could not reject the null hypothesis of equivalent *ERCC2* A35931C genotype survival effects in *CCND1* 870G homozygotes versus persons with at least 1 *CCND1* 870A allele or in oropharyngeal versus nonoropharyngeal, in stage III versus stage IV, or in platinum-treated versus nonplatinum-treated SCCHN.

Two hypotheses can explain these results. The presence of at least 1 *ERCC2* 35931C allele could result in the development of tumors that respond more favorably to treatment. Alternatively, the presence of 2 *ERCC2* 35931A alleles could simply result in the development of tumors with greater malignant potential. However, relatively poor survival with *ERCC2* 35931 AA does not appear to represent a feature of SCCHN more generally. Most notably, for stage III–IV cancer not treated with radiation

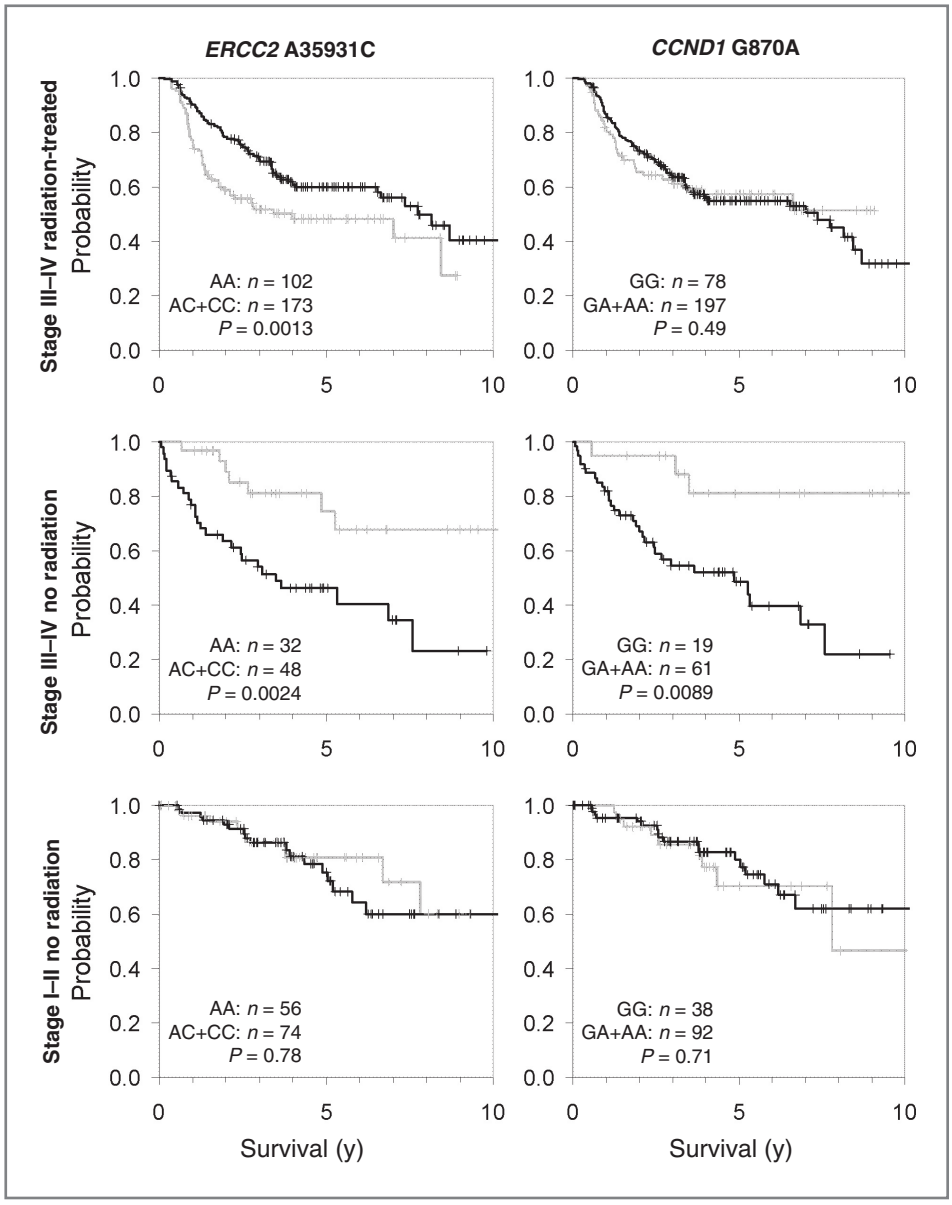

Figure 1. Overall survival (Kaplan–Meier plots) for each of 3 squamous cell carcinomas of head and neck cohorts: stage III–IV cases treated with radiation (with or without chemotherapy), stage III–IV cases not treated with radiation or chemotherapy, and stage I–II cases not treated with radiation or chemotherapy, according to *ERCC2* A35931C (AA in gray) and *CCND1* G870A (GG in gray). Plus ("+") symbols signify censored observations.

or chemotherapy, overall survival was better, not worse, in 32 patients with *ERCC2* 35931 AA than in 48 patients with *ERCC2* 35931 AC or CC ($P = 0.0024$; Fig. 1). These results (the *ERCC2* 35931C allele associating with better survival in patients exposed, and worse survival in those unexposed, to DNA-damaging treatment) concur with observations in esophageal cancer either treated or not treated with cisplatin (18). A simple explanation for a treatment's influence follows. Ineffective DNA damage response due to germline polymorphisms in DNA-repair or cell-cycle control genes may promote the death of tumor cells that are unable to repair the DNA damage caused by radiation and, thereby, render these tumor cells more vulnerable to radiation therapy. Absent radiation, however, ineffective DNA damage response may actually promote tumor cell genetic instability and, consequently, tumor progression. The general notion that *ERCC2* 35931C signifies reduced DNA repair (6, 7) is consistent with the better survival rate we observed in radiation-treated stage III–IV cases with at least one *ERCC2* 35931C allele.

A *CCND1*-870 GG survival benefit also appeared to depend on treatment, with survival difference only observed in stage III–IV disease not treated with radiation (Fig. 1). Stage I–II disease occurred less often in cases with *CCND1*-870 AA than in cases with *CCND1*-870 GG or GA (Supplementary Table S3), an occurrence that explains the relatively low frequency of the *CCND1*-870 AA genotype in our stage I–II cohort (Table 1). To the extent that the *CCND1* 870A allele may contribute to malignant potential, the association observed between *CCND1*-870 AA and advanced

stage among all cases complements the association observed between *CCND1*-870 GG and better survival in stage III–IV patients with cancers not treated with radiation.

A literature search located only 2 published studies, both included in a 2008 review (12), of *ERCC2* A35931C and head and neck cancer outcome. Reporting in 2005, Gal and colleagues (11) determined the *ERCC2* A35931C genotype for 276 18- to 65-year-old western Washington residents (71% male, mean age: 54 years, 93% white) with oral SCC (25% oropharynx, 60% treated with radiation, stage distribution not specified) incident between 1988 and 1995. Although the *ERCC2* 35931 CC genotype tended to predict lower risk of subsequent second primary SCCHN (adjusted HR = 0.17, 95% CI, 0.02–1.29), the *ERCC2* A35931C genotype did not predict overall or disease-specific survival rates, with survival results said to be similar for persons who received radiotherapy as part of initial treatment. Reporting in 2006, Quintela-Fandino and colleagues (16) determined the *ERCC2* A35931C genotype for 103 Spanish patients (94% male, median age: 60 years) treated between 1988 and 1995 for primary or relapsed stage IV SCCHN (48% oropharynx, 18% oral cavity, 16% larynx, and 18% other). Chemotherapy-naive patients received platinum-based induction chemotherapy, with radiation in 25% of cases. Patients carrying the 35931C allele experienced better survival than patients homozygous for the 35931A allele ($P = 0.0012$), a result that is in agreement with our observations in stage III–IV cases treated with radiation (with or without chemotherapy).

Four other publications mention *CCND1* G870A-dependent outcomes in SCCHN, with reports from (i) Germany ($n = 194$; ref. 14); (ii) Portugal ($n = 66$; ref. 15); (iii) India ($n = 165$; ref. 17); and (iv) the United States ($n = 698$; ref. 13). Each study observed worse overall and/or disease-free survival rates in relation to *CCND1* 870G. These studies did not examine variation in effect with regard to both stage and treatment. In contrast, we observed better, not worse, overall, progression-free, and recurrence-free survival rates in relation to *CCND1*-870 GG, but only within a small subgroup, stage III–IV not treated with radiation.

Study limitations include noncomparable stage III–IV cohorts, one treated with radiation and another not treated. Radiation treatment cannot be regarded as a random exposure. In our study population, for example, more recent enrollment period, very late stage (stage IV), and involvement of oropharyngeal subsite characterized the radiation-treated cohort (Table 1). Although we can observe significantly better survival in radiation-treated than in nontreated patients with at least 1 *ERCC2* 35931C allele and significantly worse survival in radiation-treated than in nontreated patients homozygous for *ERCC2* 35931A (Supplementary Figure), the noncomparability of radiation-treated and nontreated cohorts precludes isolation and measurement of the specific effects of radiation treatment. Nevertheless, our results suggest that radiation, if effective, is either more effective or only effective in patients with at least 1 *ERCC2* 35931C allele.

Other study limitations arose as a consequence of (i) case loss secondary to missing data; (ii) uncertainty regarding disease-specific, progression-free, and recurrence-free survival outcomes; (iii) incomplete knowledge of the extent or amount of radiation or chemotherapy delivered; (iv) unknown human papilloma virus (HPV) tumor status; (v) sample size; and (vi) polymorphism selection. Many factors contributed to case loss. However, case loss as a whole appeared to be random with regard to genotype and other prognostic variables (Supplementary Table S1). Moreover, our inclusion and exclusion criteria defined 3 distinct and relatively homogeneous cohorts that, together, encompass most of head and neck cancer clinically. Our primary analysis used a hard endpoint: death due to any cause. However, one would expect genetic factors interacting with treatment to affect, in particular, the cancer-relevant outcomes including disease-specific, progression-free, and recurrence-free survival rates. Results with these latter outcomes corroborated overall survival results (Table 2). However, determination of causes of death and ascertainment of cancer-progression events from medical records entail subjectivity and introduce variability. Our reliance on one research-dedicated and certified tumor registrar may have produced more reliable information about these difficult-to-measure secondary endpoints. More importantly, outcome determinations occurred in a manner blind to genotype. We documented radiation and type of chemotherapy started as part of primary treatment, but not the amount of radiation or doses of chemotherapy actually completed. This limitation prohibited study of genetic effect according to treatment intensity. We lacked information about HPV tumor status, a favorable prognostic factor usually observed in oropharyngeal cancer (30). However, the genotypic effects we observed on overall SCCHN survival were not only statistically equivalent in oropharyngeal and nonoropharyngeal radiation-treated stage III–IV SCCHN but also were observed in analyses restricted to oral cavity cancer (data not shown). Although our study included many cases ($n = 485$), sample size limited our ability to compare genotype effects between important subgroups, in particular radiation-treated stage III–IV cases subgrouped according to platinum chemotherapy. Finally, we studied only 2 polymorphisms in 2 genes. However, these genetic variants were chosen because of their known functional significance. Fully useful approaches to outcome prediction or treatment selection may require more complete genetic characterization of DNA-repair and cell-cycle control pathways (12, 16, 31).

Although genetic determinants of head and neck cancer outcome and treatment response remain poorly understood, we find that *ERCC2* A35931C and, perhaps,

CCND1 G870A when combined with information about stage and treatment may predict outcomes in advanced-stage SCCHN and help clinicians tailor treatment selection.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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