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CTLA4 Variants, UV-Induced Tolerance, and Risk of Non-Melanoma Skin Cancer

Marleen M. Welsh[1], **Katie M. Applebaum**[1,2], **Steven K. Spencer**[3], **Ann E. Perry**[4], **Margaret R. Karagas**[5], and **Heather H. Nelson**[6,7]

[1]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts

[2]Department of Epidemiology, Boston University School of Public Health, Boston, Massachusetts

[3]Section of Dermatology, Department of Medicine, Dartmouth Medical School, Lebanon, New Hampshire

[4]Department of Pathology, Dartmouth Hitchcock Medical Center, Dartmouth Medical School, Lebanon, New Hampshire

[5]Section of Biostatistics and Epidemiology, Department of Community and Family Medicine, and the Norris Cotton Cancer Center, Dartmouth Medical School, Lebanon, New Hampshire

[6]Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota

[7]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota

Abstract

Although skin tumors are highly immunogenic, exposure to UV radiation is known to suppress immune responses via regulatory T cells. Specifically, the activity of cytotoxic lymphocyte-associated antigen-4 (CTLA-4) is integral in regulating the development of UV-induced tolerance and, concomitantly, skin cancers. Due to the inverse relationship between tumor surveillance and autoimmunity, we hypothesize that the same genetic variant in the *CTLA4* locus that increases risk for autoimmune diseases is associated with decreased risk of non-melanoma skin cancer (NMSC). We analyzed whether the polymorphism CT60 or haplotypes of *CTLA4* influence odds of developing the major types of NMSC, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), in a population-based case-control study of Caucasians in New Hampshire (849 controls, 930 BCC, and 713 SCC). The *CTLA4* CT60 GG genotype was associated with decreased odds for BCC and SCC, controlling for age, sex, lifetime number of severe sunburns, and skin type [BCC: odds ratio (OR), 0.7; 95% confidence interval (95% CI), 0.5–0.9; SCC: OR, 0.7; 95% CI, 0.5–1.0]. For BCC, this decrease was apparent largely among those with a higher lifetime number of severe sunburns ($P_{interaction}$ = 0.0074). There were significantly decreased odds of disease associated with two haplotypes, which possess the CT60 G allele. Additionally, lifetime number of severe sunburns modestly altered the effects of the *CTLA4* haplotypes in BCC, and the association seemed driven by the CT60 single nucleotide polymorphism. In conclusion, genetic variation at the *CTLA4* locus may be etiologically important in NMSC, the most prevalent malignancy in the United States.

Requests for reprints: Heather H. Nelson, University of Minnesota Cancer Center, 554A Cancer Center Research Building, 420 Delaware Street Southeast, MMC 806, Minneapolis, MN 55455. Phone: 612-626-9887; Fax: 612-626-4842; hhnelson@umn.edu.

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No potential conflicts of interest were disclosed.

Introduction

Immunity, specifically the ability of the immune system to prevent outgrowth of abnormal cells, is a key component of cancer prevention. This involves a delicate balance between activation of tumor surveillance mechanisms while concomitantly avoiding development of autoimmunity. Central to this process of recognizing malignant ”nonself” from normal ”self” are T-regulatory (Treg) cells. Treg cells exist in the tumor environment where they can suppress CTL activity, inhibiting the normal antitumor response of the body (1). Expressed on Treg cells, cytotoxic lymphocyte-associated antigen-4 (CTLA-4) interacts with antigen-presenting cells to inhibit T-cell activation (2,3). CTLA-4 is important to the proper functioning of Tregs as antibody neutralization of CTLA-4 on Tregs ablates their suppressive activity (4).

Non-melanoma skin cancers (NMSC) are particularly immunogenic. In humans, organ transplant recipients, who are highly immunosuppressed to prevent organ rejection, have >65-fold increased risk of developing squamous cell carcinoma (SCC) and 10-fold increased risk of developing basal cell carcinoma (BCC) of the skin (5). In mice, transfer of skin tumor cells from syngeneic donors leads to tumor rejection, unless the mice are first immunosuppressed either by drugs or UV (6–8). Experiments in mice have shown that UV-induced suppression of tumor surveillance can be adoptively transferred via spleen cells from a UV-exposed mouse to a nonexposed mouse (9). Transfer of either serum or nonviable splenic cells was not able to make the mice tumor susceptible, implying an active regulatory T lymphocyte, not a virus or soluble factor, was generated in response to UV.

CTLA-4 plays an important role in UV-induced immune suppression as well as in the development of skin cancers. Neutralizing CTLA-4 antibodies block the development of hapten-specific tolerance due to UV exposure in normal mice (10). In addition, depletion of CTLA-4^+ cells from spleen and lymph node cells blocks the ability of UV-exposed mice to transfer hapten-specific tolerance to unexposed mice, whereas administering a small number of just the CTLA-4^+ cells allows adoptive transfer to occur (10). Aside from being a marker of Tregs developed as part of UV-induced immune suppression, CTLA-4 seems to have a vital role in Treg functioning. Additionally, transgenic mice that express a skin-specific CTLA-4 antagonist, CTLA-4Ig, developed fewer skin tumors after chronic exposure to UV (11). They were more resistant to UV-induced immunosuppression of delayed-type hypersensitivity reactions than wild-type counterparts, potentially due to an innate Th1 bias in these animals (11). Further, in mice experiments, activity specific to CTLA-4 seems to be integral in regulating the development of UV-induced tolerance and, concomitantly, skin cancers.

In humans, the genetic region that includes *CTLA4* is highly variable, and several variants in this gene have been associated with autoimmune diseases (reviewed in ref. 12). Recently, one study reported a new single nucleotide polymorphism (SNP), CT60, in the 3′-untranslated region of *CTLA4* in which the G allele was associated with increased risk of type 1 diabetes, autoimmune thyroid disease, and Graves’ disease (13). Further functional examination of the SNP determined that it altered basal Treg frequency, such that the G allele was associated with lower Treg levels (14). Due to the inverse relationship between tumor surveillance and autoimmunity, we hypothesize that the same genetic variant in the *CTLA4* locus that increases risk for autoimmune diseases is associated with decreased risk of NMSC. In this study, we examine the association of both the purported functional SNP CT60 and *CTLA4* haplotypes with NMSC and for the presence of effect modification by UV exposure or pigmentation.

Materials and Methods

Study population

Newly diagnosed cases of histologically confirmed BCC and SCC in New Hampshire were identified using an incident survey established through the collaboration of dermatologists, dermatopathologists, and pathology laboratories throughout the state and bordering regions from July 1, 1993 to June 30, 1995 (series 1) and July 1, 1997 to March 30, 2000 (series 2; ref. 15). The study design for The New Hampshire Health Study has been described previously by Karagas and colleagues (15). Briefly, eligibility criteria for cases were as follows: (*a*) between the ages of 25 and 74 y, (*b*) had a listed telephone number, and (*c*) spoke English. Eligible SCC cases and a ratio of approximately two to one BCC cases in series 1 and one to one ratio in series 2 were selected to take part in the study. The BCC cases were randomly sampled to ensure representativeness of age, sex, and anatomic site for all incident BCCs within New Hampshire. Controls ages 25 to 64 y were identified from the New Hampshire State Department of Transportation files and those ages 65 to 74 y were obtained from enrollment lists from the Center for Medicaid and Medicare Services. Potential controls were frequency matched on age and gender to the combined distribution of case groups.

A personal interview was conducted with consenting cases and controls, with about 80% of cases and 72% of controls agreeing to participate. The interviews, usually conducted in the participant's home, covered demographic factors, pigmentation characteristics, sun exposure and sensitivity, and other factors (15). Blood draws and/or buccal samples were obtained from cases and controls from both phases of the study for DNA analysis. Approximately 85% of subjects consented to providing a DNA sample; 90% of which were blood derived. All study protocol and materials were approved by the Dartmouth College Committee for the Protection of Human Subjects, and all participants provided informed consent.

SNP selection and genotyping

Gene sequence of chromosome section 2q33 (nucleotides 204556102-204571101) was obtained from Genbank.[8] Tagging SNPs were chosen using the Haploview program, with a minor allele frequency ≥0.05 and r^2 threshold = 0.8. Eight haplotype-tagging SNPs were found using this method (rs16840252, rs231731, rs11571317, rs733618, rs5742909, rs3087243, rs231775, and rs231777). Additionally, SNPs rs11571302 and rs11571297 were reported (13) to be associated with several autoimmune diseases but are not listed in HapMap. Therefore, these SNPs were also genotyped. Genotyping for all SNPs was performed by the University of Minnesota Genotyping Facility in the Biomedical Genomics Center using the Sequenom platform. For rs231775, there was a high level of missing genotype data (>10%) using the Sequenom method, so it was regenotyped using a Taqman allelic discrimination platform (Applied Biosystems). Taqman primers, probes, and conditions are available on request. Among the samples successfully genotyped by both methods (n = 2,341), the genotype concordance was 97.7%. Therefore, we combined the genotype data collected by both methods for our final data set.

Statistical analyses

Adjusted odds ratios (ORs) and 95% confidence intervals (95% CI) for models examining the CT60 polymorphism as a risk factor for NMSC were obtained using unconditional logistic regression. All models were adjusted for age at diagnosis (continuous), gender, skin type, and lifetime number of severe sunburns. Self-reported sunburn history was described

[8] http://www.ncbi.nlm.nih.gov/Genbank/index.html

as the lifetime number of painful sunburns that last 2 or more days. For all analyses, cutoffs were determined based on the median within controls, creating groups of low (0–1) and high (≥2) burns. The measure of skin type, skin reaction to acute sun exposure, was also dichotomized. Those who responded that they burned then tanned or only tanned were placed in the ”tanner” category, whereas those who responded that they burned and peeled or burned and freckled were placed in the ”burner” category. For all CT60 genotype associations, a univariate Cochran-Armitage test for trend was used.

To test for statistical interaction between CT60 genotype and sunburns, models were generated that included separate main effect terms for each of the variables (i.e., genotype and burns) as well as additional cross-product interaction terms. The log likelihood of the full model was then compared with the log likelihood from a similar model that did not contain the cross-product terms, and a two–degree of freedom test was performed. A similar test was performed for haplotype-sunburns interaction, including six cross-product terms and using a six–degree of freedom test. All tests were two sided, and a *P* value of ≤0.05 was considered significant.

Logistic regression was also used to analyze the effects of CT60 by anatomic site, controlling for age, sex, sunburns, and skin type. BCCs or SCCs that developed on the head or neck were grouped together as ”sun-exposed” sites, whereas NMSCs that developed on any other site of the body were generally termed ”sun-protected” sites. The distribution of these sites has been previously described (15). In this study, 529 BCCs were on the head and neck and 300 were at other sites; for SCCs, 303 were on the head and neck and 183 were at other sites. For each histologic type, separate models were used to predict odds of disease at each anatomic site compared with controls. To generate the *P* values for differences by anatomic site, a case-case comparison was used for sun-exposed and sun-protected sites within each histology in logistic regression models adjusted for age, sex, sunburns, and skin type.

Haplotypes were estimated using the HAPPY macro in SAS v9.1.[9] Unconditional logistic regression was performed using the most frequent haplotype as the referent group, and all models were controlled for age, gender, lifetime history of sunburns, and skin type. A global χ^2 test was performed to assess association of haplotypes with BCC and SCC.

Results

Genotype data at the *CTLA4* locus were available for 2,476 participants (849 controls, 930 BCC cases, and 713 SCC cases). The frequency distributions for age, sex, and other known risk factors for NMSC, such as skin reaction to acute sun exposure and lifetime number of sunburns, are shown in Table 1. The average age of participants was 61.3 years (SD, 10.5) for controls, 58.7 years (SD, 11.1) for BCC, and 64.1 years (SD, 8.7) for SCC. In all groups, there were more men than women. As expected, BCC and SCC cases were more likely to burn and more likely to have experienced severe sunburns than controls.

In our population, the prevalence of the G allele of the CT60 polymorphism was 54.8%, similar to the prevalence of 54.2% reported in the CEU population of HapMap. After controlling for age, gender, skin type, and lifetime number of severe sunburns, the GG genotype was associated with a decrease in odds of both BCC and SCC (BCC: OR, 0.7; 95% CI, 0.5–0.9; SCC: OR, 0.7; 95% CI, 0.5–1.0). No association was observed between the heterozygote and either disease (Table 2).

[9] http://www.hsph.harvard.edu/faculty/kraft/soft.htm

The association between CT60 genotype and BCC, but not SCC, was strongest among those with a history of two or more severe sunburns (Table 3). In this stratum, the heterozygote and the GG homozygote had decreased risk of BCC (AG: OR, 0.6; 95% CI, 0.4–0.9; GG: OR, 0.5; 95% CI, 0.3–0.7) compared with the AA genotype. In contrast, we did not observe evidence of effect modification by skin type in either histologic type of NMSC (BCC: $P_{interaction} = 0.98$; SCC: $P_{interaction} = 0.64$; data not shown).

When examining the effects by anatomic site, the GG genotype was associated with a statistically significantly decreased risk of BCCs of the head and neck (OR, 0.7; 95% CI, 0.5–1.0) and other sites (OR, 0.6; 95% CI, 0.4–0.9). However, there was no difference in effects between these sites ($P = 0.42$). Examining SCC, GG genotype was significantly associated with decreased risk of tumors of the head and neck (OR, 0.7; 95% CI, 0.5–1.0). A nonsignificant risk estimate was observed for other anatomic sites (OR, 0.8; 95% CI, 0.5–1.3), and the difference in *CTLA4* CT60 risk by anatomic site was not statistically significant ($P = 0.61$).

For the haplotype analysis, 10 SNPs were genotyped. Two SNPs not in HapMap but identified in the literature, J031 (rs11571302) and J027_1 (rs11571297), were highly correlated with rs3087243 (97.3% and 96.0%, respectively) in our population and therefore were not included in the haplotype analysis. The final list of SNPs used for haplotype analysis is presented in Table 4. The results of univariate and adjusted analysis for each of these SNPs are shown in Table 5, and the results of the haplotype estimation are shown in Table 6. Six haplotypes occurred with >0.05 frequency in the total population. The last category, H7, corresponds to the pool of all other haplotypes that did not reach this prevalence cutoff. The most prevalent haplotype H1 (0-0-0-0-0-1-0-0) was used as the referent group in all logistic regression models. Haplotype H2 (0-0-0-0-0-0-1-0), which contains the G allele of CT60, was associated with decreased odds of both BCC and SCC (BCC: OR, 0.8; 95% CI, 0.7–0.9; SCC: OR, 0.8; 95% CI, 0.7–1.0) when controlling for age, sex, lifetime number of severe sunburns, and skin type. Additionally, haplotype H5 (1-1-0-0-0-0-0-1), which also included the CT60 G allele, was associated with decreased risk of SCC only (OR, 0.6; 95% CI, 0.4–0.9). The global test on haplotypes suggested that the haplotypes were not strongly predictive for either disease (BCC: $P = 0.14$; SCC: $P = 0.12$).

We further tested for effect modification between sunburn history and *CTLA4* haplotypes (Table 7). Although interaction terms were not statistically significant (BCC: $P = 0.086$; SCC: $P = 0.79$), among those with a high number of lifetime sunburns, haplotypes H2, H3 (1-1-0-0-1-0-0-1), and H5 were associated with a decreased odds of BCC (H2: OR, 0.6; 95% CI, 0.5–0.8; H3: OR, 0.7; 95% CI, 0.5–0.9; H5: OR, 0.6; 95% CI, 0.4–0.9); for SCC, H5 was associated with decreased odds of disease (OR, 0.5; 95% CI, 0.3–0.8). Finally, pigmentation did not seem to modify the haplotypic relationships for either type of cancer (BCC: $P_{interaction} = 0.34$; SCC: $P_{interaction} = 0.79$; data not shown).

When examining these effects by anatomic site, H2 was significantly associated with BCCs that developed at sites of low sun exposure (sites other than the head and neck: OR, 0.8; 95% CI, 0.6–0.9) and with BCCs that developed on the head or neck (OR, 0.8; 95% CI, 0.7–1.0); this difference between anatomic sites was not significant ($P = 0.51$). For SCCs, H2 and H5 were significantly associated with cancers of the head or neck (H2: OR, 0.8; 95% CI, 0.6–1.0; H5: OR, 0.6; 95% CI, 0.4–1.0); however, the difference by anatomic site was not significant for either haplotype (H2: $P = 0.24$; H5: $P = 0.75$).

Discussion

We have examined the role of genetic variation in the *CTLA4* locus in NMSC, the most common cancer in the United States. We found the GG genotype of SNP CT60 to be associated with decreased odds of both BCC and SCC compared with the AA genotype after controlling for age, sex, skin type, and lifetime number of severe sunburns. Additionally, there were no significant effects of genotype by anatomic site. One *CTLA4* haplotype (H2) was associated with decreased risk of BCC, whereas two haplotypes (H2 and H5) were associated with decreased risk of SCC. Further, there was evidence of effect modification of haplotype risk by sunburns (BCC) but no differential effects by anatomic site.

To our knowledge, no previous studies have analyzed the association between variation at the *CTLA4* locus and risk of NMSC. However, Cozar and colleagues (16) found an increased risk of renal cancer associated with the AA genotype of CT60 (OR, 2.12; 95% CI, 1.28–3.50) compared with the AG and GG genotypes pooled, which is consistent with our data. A study of breast cancer and *CTLA4* promoter SNPs found no association between any of the three SNPs [−1722T/C (rs733618), −1661A/G (rs4553808), and −318C/T (rs5742909)] and case status (17). In our population, we also found that haplotypes containing the variant alleles of these SNPs were not significantly associated with altered risk of cancer (rs4553808 and tagging SNP rs16840252 are in complete linkage disequilibrium). Finally, one small study examined the effect of CT60 on risk of gastric mucosa-associated lymphoid tissue lymphoma, a rare tumor of distinct etiology, and found no association (18). Low power and differential effects of the SNP on solid tumors versus lymphomas could contribute to the null results in this study.

In our study, several haplotypes (H2, H3, and H5) were significantly associated with decreased odds of BCC among those who experienced a higher number of severe sunburns. The common allele these haplotypes share that differs from the referent group H1 is CT60G. Only one common haplotype, H6, that contained CT60G was not significantly associated with decreased odds of developing BCC, and it occurs at a relatively low frequency in our population. It is possible that lack of statistical power in the H6 haplotype group may have led to an imprecise estimate of association or, alternatively, that the H6 haplotype has a different biology than the other haplotypes containing CT60. Specifically, the variation captured in the H6 haplotype might affect the CTLA-4 response to sunburns. In addition, effects of similar magnitude and direction for haplotypes H2 and H5 were observed for the main effects of the haplotypes and the anatomic site effects. We believe that the CT60G allele may be driving this association and that the large number of degrees of freedom used by the haplotype analysis likely resulted in nonstatistically significant *P* values on the *CTLA4* global haplotype test and for interaction terms.

Exposure to UV light results in the development of Tregs that induce tolerance to tumor antigens (1). High doses of UV exposure in combination with the *CTLA4* CT60A allele that already has higher basal levels of Tregs could alter risk of NMSC. In this analysis, we used a high lifetime number of severe sunburns as a metric of UV exposure. Our results fit this model, as decreasing the number of A alleles is associated with decreased risk of BCC in those with a high lifetime number of sunburns. Similar gene-burns interactions were observed for BCC and SCC in this population with the UV immune suppression gene *histidine ammonia lyase* (*HAL*), which synthesizes a photoreceptor for the pathway (19).

Although skin type is a major risk factor for NMSC, we found no interaction between CT60 genotype and self-reported skin type. This result is consistent with past evidence that the prevalence of UV immunosuppression susceptibility is invariant across Caucasian, African-American, and Indian-American populations (20).

Interestingly, there were no differential effects of either CT60 or *CTLA4* haplotypes by anatomic site. This may be related to the nature of UV-induced immunosuppression, which can be both local and systemic. Hapten applied to UV-irradiated skin immediately after acute, low-dose exposure results in only local suppression within the first 24 hours, but 3 days after application, systemic suppression is observed (21). Our data support the concept of systemic UV-induced immunosuppression, as the effects of genotypes and haplotypes were as strong on sun-protected sites as they were on sun-exposed sites.

One of the advantages of this study is the use of both a candidate functional SNP approach as well as a haplotype-tagging SNP approach. In testing association between a purported functional SNP and risk of disease, we can have greater confidence that it is the causal SNP as there is prior evidence of mechanism. Additionally, the use of haplotype-tagging SNPs helps capture most of the variation in a gene. The results of both single SNP analysis and haplotype analysis indicate that CT60 is the relevant SNP within the *CTLA4* locus that is associated with risk of NMSC.

In this study, we have elucidated the important role of *CTLA4* variation, specifically the purported functional SNP CT60, in skin cancer risk. Our data suggest that the CT60G allele, and the resulting lower Treg frequency, is associated with significantly decreased risk of NMSC, most likely due to decreased tumor surveillance. Although our study only examines its effects on NMSCs, these cancer types are thought of as excellent model systems for studying the role of the immune system in the etiology of solid tumors.

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Table 1

Population demographics and exposures

	Controls (*n* = 849)	BCC (*n* = 930)	SCC (*n* = 713)
Age (SD)	61.3 (10.5)	58.7 (11.1)	64.1 (8.7)
Gender			
Male	509 (60.0%)	522 (56.1%)	454 (63.7%)
Female	340 (40.1%)	408 (43.9%)	259 (36.3%)
Skin reaction to first strong summer sun for 1 h			
Tan	584 (69.0%)	529 (56.9%)	395 (55.5%)
Burn	262 (31.0%)	400 (43.1%)	317 (44.5%)
Missing	3	1	1
Lifetime severe sunburns*			
Low (1-0)	415 (51.7%)	349 (38.8%)	275 (40.2%)
High (≥2)	388 (48.3%)	551 (61.2%)	410 (59.9%)
Missing	46	30	28

* Cutoff based on median in controls.

Table 2

Association of *CTLA4* SNP CT60 with SCC and BCC

CT60 genotype	Controls (*n* = 821)	BCC (*n* = 901)	OR (95% CI)*	SCC (*n* = 690)	OR (95% CI)*
AA	156 (19.0%)	207 (23.0%)	Reference	143 (20.7%)	Reference
AG	385 (46.9%)	442 (49.1%)	0.9 (0.7–1.1)	349 (50.6%)	1.0 (0.7–1.3)
GG	280 (34.1%)	252 (28.0%)	0.7 (0.5–0.9)	198 (28.7%)	0.7 (0.5–1.0)
		Trend test †: *P* = 0.0034		Trend test †: *P* = 0.051	

*ORs adjusted for gender, age at tumor diagnosis, skin reaction to acute sun exposure, and lifetime number of severe sunburns.

†Cochran-Armitage test for trend.

Table 3

Association of lifetime severe sunburns, CT60 genotype, and NMSC

Burns	Controls (*n* = 776)	BCC (*n* = 872)	Stratified OR (95% CI)*	Joint effects OR (95% CI)*	SCC (*n* = 663)	Stratified OR (95% CI)*	Joint effects OR (95% CI)*
0–1	*n* = 400	*n* = 336			*n* = 265		
AA	89	65	Reference	Reference	65	Reference	Reference
AG	182	168	1.3 (0.9–1.9)	1.3 (0.9–1.9)	131	1.0 (0.7–1.5)	1.0 (0.7–1.5)
GG	129	103	1.1 (0.7–1.6)	1.1 (0.7–1.6)	69	0.7 (0.5–1.1)	0.7 (0.5–1.1)
			P trend = 0.76 †			*P* trend = 0.15 †	
2+	*n* = 376	*n* = 536			*n* = 398		
AA	58	133	Reference	2.7 (1.7–4.3)	74	Reference	1.7 (1.0–2.7)
AG	179	259	0.6 (0.4–0.9)	1.7 (1.2–2.5)	203	0.9 (0.6–1.4)	1.5 (1.0–2.3)
GG	139	144	0.5 (0.3–0.7)	1.2 (0.8–1.8)	121	0.7 (0.5–1.1)	1.2 (0.8–1.8)
			P trend < 0.001 †			*P* trend = 0.051 †	
				$P_{interaction}$ = 0.0074			$P_{interaction}$ = 0.86

*ORs adjusted for gender, age at tumor removal, and skin reaction to acute sun exposure.

†Cochran-Armitage test for trend.

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Table 4

Haplotype-tagging SNPs for the 15-kb region including the *CTLA4* locus

SNP number	dbSNP ID (common name)	Polymorphism (0/1)
1	rs16840252	C/T
2	rs231731	T/C
3	rs11571317	C/T
4	rs733618	A/G
5	rs5742909	C/T
6	rs3087243 (CT60)	G/A
7	rs231775	A/G
8	rs231777	C/T

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Table 5

Univariate and adjusted analysis of *CTLA4* tagging SNPs

	Controls, *n* (%)	BCC, *n* (%)	Univariate OR (95% CI)	Adjusted OR (95% CI)*	SCC, *n* (%)	Univariate OR (95% CI)	Adjusted OR (95% CI)*
rs16840252							
CC	565 (68.3)	631 (69.8)	Reference	Reference	483 (70.7)	Reference	Reference
CT	236 (28.5)	249 (27.5)	0.9 (0.8–1.2)	1.0 (0.8–1.2)	183 (26.8)	0.9 (0.7–1.1)	0.9 (0.7–1.1)
TT	26 (3.1)	24 (2.7)	0.8 (0.5–1.5)	0.8 (0.4–1.3)	17 (2.5)	0.8 (0.4–1.4)	0.6 (0.3–1.1)
rs231731							
TT	520 (62.9)	580 (63.8)	Reference	Reference	453 (65.1)	Reference	Reference
CT	272 (32.9)	292 (32.1)	1.0 (0.8–1.2)	1.0 (0.8–1.2)	212 (30.5)	0.9 (0.7–1.1)	0.9 (0.7–1.1)
CC	35 (4.2)	37 (4.1)	0.9 (0.6–1.5)	0.9 (0.6–1.5)	31 (4.5)	1.0 (0.6–1.7)	0.9 (0.6–1.6)
rs11571317							
CC	679 (86.4)	743 (85.4)	Reference	Reference	561 (86.3)	Reference	Reference
CT	99 (12.6)	114 (13.1)	1.1 (0.8–1.4)	1.0 (0.7–1.3)	83 (12.8)	1.0 (0.7–1.4)	0.9 (0.7–1.3)
TT	8 (1.0)	13 (1.5)	1.5 (0.6–3.6)	1.5 (0.6–3.7)	6 (0.9)	0.9 (0.3–2.6)	1.0 (0.3–3.1)
rs733618							
AA	706 (84.9)	782 (86.1)	Reference	Reference	592 (85.1)	Reference	Reference
GA	122 (14.7)	121 (13.3)	0.9 (0.7–1.2)	0.9 (0.7–1.2)	99 (14.2)	1.0 (0.7–1.3)	1.0 (0.8–1.4)
GG	4 (0.5)	5 (0.6)	1.1 (0.3–4.2)	1.4 (0.3–5.9)	5 (0.7)	1.5 (0.4–5.6)	2.0 (0.5–8.5)
rs5742909							
CC	682 (83.1)	745 (82.4)	Reference	Reference	561 (81.7)	Reference	Reference
CT	132 (16.1)	152 (16.8)	1.1 (0.8–1.4)	1.1 (0.8–1.4)	120 (17.5)	1.1 (0.8–1.5)	1.0 (0.8–1.4)
TT	7 (0.9)	7 (0.8)	0.9 (0.3–2.6)	1.0 (0.3–2.8)	6 (0.9)	1.0 (0.3–3.1)	1.0 (0.3–3.1)
rs3087243 (CT60)							
AA	156 (19.0)	207 (23.0)	Reference	Reference	143 (20.7)	Reference	Reference
AG	385 (46.9)	442 (49.1)	0.9 (0.7–1.1)	0.9 (0.7–1.1)	349 (50.6)	1.0 (0.8–1.3)	1.0 (0.7–1.3)
GG	280 (34.1)	252 (28.0)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	198 (28.7)	0.8 (0.6–1.0)	0.7 (0.5–1.0)
rs231775							
AA	318 (38.8)	388 (43.3)	Reference	Reference	272 (39.8)	Reference	Reference
AG	353 (43.1)	388 (43.3)	0.9 (0.7–1.1)	0.9 (0.7–1.2)	319 (46.6)	1.1 (0.8–1.3)	1.1 (0.9–1.4)
GG	148 (18.1)	121 (13.5)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	93 (13.6)	0.7 (0.5–1.0)	0.7 (0.5–1.0)

	Controls, *n* (%)	BCC, *n* (%)	Univariate OR (95% CI)	Adjusted OR (95% CI)*	SCC, *n* (%)	Univariate OR (95% CI)	Adjusted OR (95% CI)*
rs231777							
CC	590 (72.7)	663 (74.2)	Reference	Reference	506 (74.2)	Reference	Reference
CT	205 (25.3)	211 (23.6)	0.9 (0.7–1.1)	0.9 (0.7–1.2)	159 (23.3)	0.9 (0.7–1.1)	0.9 (0.7–1.1)
TT	17 (2.1)	20 (2.2)	1.0 (0.5–2.0)	1.0 (0.5–1.9)	17 (2.5)	1.2 (0.6–2.3)	0.9 (0.5–1.9)

*ORs adjusted for gender, age at tumor diagnosis, skin reaction to acute sun exposure, and lifetime number of severe sunburns.

Table 6

Estimated haplotypes of *CTLA4* and association with SCC and BCC

Haplotypes	Tagging SNPs	Frequency (%)*		OR (95% CI) †,‡	Frequency (%)*	OR (95% CI) †,‡
	1-2-3-4-5-6-7-8	Controls	BCC		SCC	
H1	0-0-0-0-0-1-0-0	0.353	0.393	Reference	0.386	Reference
H2	0-0-0-0-0-0-1-0	0.316	0.283	0.8 (0.7–0.9)	0.297	0.8 (0.7–1.0)
H3	1-1-0-0-1-0-0-1	0.090	0.092	0.9 (0.7–1.2)	0.097	0.9 (0.7–1.2)
H4	0-0-1-0-0-1-0-0	0.074	0.081	0.9 (0.7–1.2)	0.075	0.9 (0.6–1.2)
H5	1-1-0-0-0-0-0-1	0.059	0.052	0.8 (0.6–1.1)	0.044	0.6 (0.4–0.9)
H6	0-1-0-1-0-0-1-0	0.051	0.052	0.9 (0.7–1.2)	0.052	1.0 (0.7–1.4)
H7	<0.05§	0.056	0.048	0.7 (0.5–1.0)	0.050	0.9 (0.6–1.2)

*Based on 801 controls, 900 BCC, and 685 SCC.

†ORs controlled for age at diagnosis, sex, skin reaction to first summer sun exposure for 1 h, and lifetime number of severe sunburns.

‡Global test on haplotypes: BCC, $P = 0.14$; SCC, $P = 0.12$.

§This category combines other haplotypes generated from these eight tagging SNPs with a frequency below 5%.

Table 7

Modification of effects of estimated haplotypes of *CTLA4* by lifetime history of severe sunburns in association with SCC and BCC

Sunburns	Frequency		OR (95% CI)*	Frequency	OR (95% CI)*
	Controls	BCC		SCC	
Low (0–1)	*n* = 413	*n* = 349		*n* = 275	
H1	0.384	0.387	Reference	0.421	Reference
H2	0.309	0.309	1.0 (0.8–1.3)	0.279	0.8 (0.6–1.1)
H3	0.081	0.100	1.2 (0.9–1.8)	0.090	1.0 (0.7–1.5)
H4	0.070	0.070	1.0 (0.7–1.5)	0.075	1.0 (0.6–1.6)
H5	0.046	0.049	1.1 (0.6–1.8)	0.035	0.8 (0.4–1.4)
H6	0.051	0.046	0.9 (0.6–1.5)	0.045	0.8 (0.5–1.4)
H7	0.057	0.047	0.8 (0.5–1.3)	0.054	0.9 (0.6–1.6)
High (≥2)	*n* = 388	*n* = 551		*n* = 410	
H1	0.317	0.402	Reference	0.367	Reference
H2	0.326	0.265	0.6 (0.5–0.8)	0.306	0.8 (0.6–1.0)
H3	0.101	0.089	0.7 (0.5–0.9)	0.100	0.8 (0.6–1.2)
H4	0.077	0.085	0.8 (0.6–1.2)	0.072	0.7 (0.5–1.1)
H5	0.071	0.055	0.6 (0.4–0.9)	0.049	0.5 (0.3–0.8)
H6	0.052	0.056	0.9 (0.6–1.3)	0.058	1.1 (0.7–1.7)
H7	0.056	0.048	0.7 (0.4–1.0)	0.048	0.8 (0.5–1.3)

*ORs controlled for age at diagnosis, sex, and skin reaction to first summer sun exposure for 1 h.

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