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Case-Control Study of Sunlight Exposure and Cutaneous Human Papillomavirus Seroreactivity in Basal Cell and Squamous Cell Carcinomas of the Skin

Michelle R. Iannacone
University of South Florida, Chelsea92902@aol.com

Case-Control Study of Sunlight Exposure and Cutaneous Human Papillomavirus Seroreactivity in Basal Cell and Squamous Cell Carcinomas of the Skin

by

Michelle Rose Iannacone Allen

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Co-Major Professor: Heather Stockwell, Sc.D.
Co-Major Professor: Dana E. Rollison, Ph.D.
Wei Wang, Ph.D.
Kathleen O'Rourke, Ph.D.

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DEDICATION

I would like to thank my grandmother, Rose Iannacone, for planting the seeds to know God and for sharing her untimely love for Jesus. It is the most precious gift ever received.

My dissertation work is dedicated to my parents, Ferdinand and Christine Iannacone. Their love and support has been endless, allowing me to dream big, reach for the stars, and never give up. A child could not ask for better parents than mine and I hope to make them proud each day of my life.

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ABSTRACT

Non-melanoma skin cancer (NMSC), comprised of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is the most common cancer in Caucasians. Ultraviolet radiation (UVR) exposure is the most important environmental risk factor for both BCC and SCC development. However, the precise relationship between UVR and the risk of NMSC is complex, and the relationship may differ by skin cancer type. It has been hypothesized that intermittent patterns and childhood sunlight exposure are important for BCC while continuous (chronic) and lifelong (i.e. childhood and adulthood) sunlight exposure is important for SCC. Epidemiologic studies have demonstrated that cutaneous human papillomavirus (HPV) infection may also be a risk factor for developing NMSC. However, the pathway by which cutaneous HPV is associated with NMSC remains unclear. It is hypothesized that UVR exposure may interact synergistically with cutaneous HPV in NMSC development.

The goal of the research study was to evaluate the relationship between levels of sunlight exposure and BCC and SCC and to investigate differences in sunlight-associated BCC and SCC risk by genus-specific cutaneous HPV serostatus. To address these goals, we conducted a clinic based case-control study of histologically confirmed BCC and SCC cases recruited from a university dermatology clinic and controls with no history of cancer and screened negative for current skin cancer. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the associations between measures of sunlight exposure and BCC and SCC.

Multiplicative interactions were tested by placing an interaction term for the product of genus-specific HPV seroreactivity and sunlight related factors in the logistic regression models.

Measures of both intermittent and continuous patterns of sunlight exposure were associated with both types of skin cancer (i.e. BCC and SCC). Specifically, history of blistering sunburn (a marker of intermittent sunlight exposure) and occupational sunlight exposure (i.e. having a job in the sun for ≥3 months for >10 years) were both associated with BCC and SCC. The major differences in patterns of sunlight exposure between BCC and SCC were observed for sunlight exposure in one's thirties. Additionally, sunlight exposure in one's twenties was associated with SCC, regardless of pattern of exposure; similar associations were not observed for BCC. Measures of timing of sunlight exposure consistently demonstrated that childhood/adolescent sunlight exposure was more important for SCC than BCC. These included number of moles on the forearms and entire body (measure of increased childhood sunlight exposure), and younger age at first and tanning bed use. Younger age at first blistering sunburn was statistically significantly associated with both BCC and SCC.

NMSC cases were more likely to be seropositive for cutaneous HPV antibodies compared to controls. Compared to tanning, having a propensity to sun burn ($p=0.006$), or poor tanning ability ($p=0.003$) were significantly associated with a higher seroprevalence to genus beta HPV types within SCC cases. Statistically significant interactions were observed between poor tanning ability and genus-specific seropositivity with NMSC. Specifically, the associations between poor tanning ability and BCC ($p_{interaction}=0.02$) and SCC ($p_{interaction}=0.01$) were significantly stronger among individuals that were seropositive for antibodies to genus alpha HPV types. Similarly, the association between poor tanning ability and SCC was stronger among those

seropositive for genus beta HPV types ($p_{interaction}$=0.001). No additional significant interactions were observed for BCC or SCC between cutaneous sensitivity, history of blistering sunburn, or cumulative sunlight exposure and genus-specific seroreactivity.

In conclusion, associations with patterns of sunlight exposure appeared to be similar between BCC and SCC cases. With the exception of age at first blistering sunburn, factors measuring timing of sunlight exposure demonstrated stronger and statistically significant relationships with SCC. Additionally, of the sunlight related factors measured, only the associations between poor tanning ability and BCC and SCC were significantly modified by HPV seropositivity to types in genera alpha or beta.

CHAPTER 1:

INTRODUCTION AND THEORETICAL FRAMEWORK

Descriptive epidemiology of non-melanoma skin cancer

Non-melanoma skin cancer (NMSC), comprised of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is the most common cancer in Caucasians, with more than one million new cases diagnosed annually in the United States alone(1). While the mortality associated with NMSC is low(2), patients with multiple NMSC's may experience substantial morbidity, and treatment costs for NMSC are high at the national level. Furthermore, a history of NMSC has been consistently associated with increased risk of subsequent primary cancers of other sites in studies from both the U.S. and Europe(3-11).

Risk factors for non-melanoma skin cancer

Identified risk factors for BCC and SCC include older age, male sex, light eye (blue, green, or hazel), hair (red or blonde), and skin (fair) color, and immunosuppression(12). Lifestyle factors such as smoking have also been proposed as risk factors for NMSC, mainly SCC, although findings are inconsistent across studies(13-28). Ultraviolet radiation (UVR) exposure has been implicated in the etiology of skin cancer and is considered the most important environmental risk for both BCC and SCC development. However, the precise relationship between UVR and the risk of NMSC is

complex, and the relationship may differ by skin cancer type. In addition to sunlight exposure, epidemiologic studies have demonstrated that cutaneous human papillomavirus (HPV) infection may be a risk factor for developing NMSC(29-38). However, the pathway by which cutaneous HPV is associated with NMSC remains unclear. It is hypothesized that UVR exposure may interact synergistically with cutaneous HPV in NMSC development(34, 39-46).

Patterns and Timing of sun exposure in basal cell and squamous cell carcinoma

Beginning in the late 1950s, researchers began to conduct case-control studies to identify risk factors for NMSC, including total (cumulative) outdoor sunlight exposure hours and sunlight exposure on working and non-working days(19, 47-49). Observations from these studies helped recognize that BCC and SCC may have different exposure-response relationships with sunlight exposure. However, few epidemiologic studies have formally evaluated the relationship between patterns and timing of sunlight exposure in BCC and SCC. Patterns of exposure refer to whether sunlight exposure was experienced continuously (chronic exposure) or sporadically (intermittent exposure). For example, persons working outdoors, such as farmers, or living in geographic regions with a high annual UV index, such as Florida, are classified as having had chronic sunlight exposure. Alternatively, intermittent sunlight exposure refers to persons working indoors and experiencing most of their sunlight exposure on the weekends or persons living in northern latitudes with a low UV index being exposed to high doses of sunlight exposure while on vacation to regions with high UV index. Continuous or chronic sunlight exposure has been postulated to be associated with the development of SCC, whereas intermittent sunlight exposure has been observed to be associated with BCC. Timing of sunlight exposure refers to what period in life the majority of a person's exposure was received, in early childhood, adulthood or both.

Others have speculated that a high level of sunlight exposure in childhood is more strongly associated with BCC while exposure in adulthood is more strongly associated with SCC.

Cutaneous human papillomavirus and UV radiation in non-melanoma skin cancer

Human papillomaviruses belong to a large family of more than 100 genotypes, with genus alpha comprising types that infect predominantly mucosal epithelia (including "high-risk" types associated with cervical cancer and "low-risk" types inducing benign mucosal lesions), and types that infect cutaneous epithelia(50). HPV types that infect cutaneous epithelia have also been identified from genera beta, gamma, mu, and nu(50). Epidemiologic studies have demonstrated a potential role for cutaneous HPV infections in NMSC development. Furthermore, it has been hypothesized that cutaneous HPV may interact synergistically with UV radiation exposure in NMSC development. Several lines of evidence suggest that UV radiation exposure is associated with cutaneous HPV infection, and that these two factors may play a synergistic role in the development of cutaneous SCC. UV radiation produces distinct mutations in DNA, and tandem mutations, specifically CC→TT transitions in the TP53 gene (thymine dimers), are a hallmark of UV-induced DNA damage in SCC(42). UV-B radiation can also stimulate the promoter activity of HPV 5 and 8(39). In turn, the E6 proteins of genus beta HPV types have been shown to inhibit UV radiation-induced apoptosis through p53-independent pathways(45, 46), and cells expressing the E6 protein of HPV type 5 have reduced capacity to repair UV radiation-induced thymine dimers(43). In addition, HPV 38 E6 and E7 can alter the regulation of cell cycle checkpoints activated by UV radiation(41).

Limitations in literature

There are several limitations in the literature that should be addressed. Studies investigating the associations between the amount, patterns, and timing of sunlight exposure and NMSC are few in number and have been limited to populations outside of the United States(51-53), with the exception of the study conducted by Vitasa et al among watermen from Maryland. However, Vitasa et al measured cumulative exposure to UVB while the other studies(51-53) conducted among residents from Southern Europe and Australia used indirect measurements of sunlight exposure such as hours spent outdoors. Measuring lifetime sunlight exposure is difficult and measurement methods have varied across studies making it difficult to compare results.

Evidence in the published literature investigating the association between cutaneous HPV infection and NMSC is limited, and more epidemiologic studies are needed to better understand the association between UV radiation exposure and cutaneous HPV infection as they relate to NMSC development. A majority of the studies investigating the association between cutaneous HPV seropositivity and NMSC only included cutaneous HPV types from genus beta and did not present stratified analyses by factors, such as sunlight exposure, that may explain the variability observed across study populations.

Public health significance

Non-melanoma skin cancer (NMSC), comprised of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), is the most frequently occurring cancer among U.S. men and women. Exposure to Ultraviolet (UV) radiation is an established risk factor for NMSC, but despite the current knowledge about the harm of sunlight exposure, and increased use of sunscreen, NMSC incidence rates continue to increase,

emphasizing the critical need to better understand the role of sunscreen use in preventing NMSC and differences in sunlight exposure response relationships for BCC and SCC. Furthermore, it's important to identify additional risk factors for NMSC that may better characterize individuals at high risk and aid in the development of novel prevention strategies.

Many epidemiologic studies provide evidence for the role of UV radiation exposure in the etiology of all types of skin cancer. However, few studies have formally evaluated the association between patterns and timing of sunlight exposure as they relate to BCC and SCC. Understanding how sunlight exposure response differs for BCC and SCC is important for better educating the public in sun safe behaviors. Simply advising a reduction in sunlight exposure will not help reduce the incidence of NMSC if changes in sunlight exposure patterns are related to skin cancer development. For example, reducing continuous sunlight exposure (i.e. high doses of daily sunlight exposure) may decrease the incidence of SCC but not BCC if intermittent sun exposure, as experienced on holidays and vacations, is still received in high doses. Epidemiologic studies conducted in several countries have demonstrated an association between cutaneous HPV infection and NMSC, particularly SCC, and there is limited evidence to support the interaction between sunlight exposure and cutaneous HPV seropositivity as they relate to SCC. There is growing interest in utilizing a vaccine approach to preventing cancers caused by HPV, such as NMSC. However, much remains to be understood regarding the epidemiology of cutaneous HPV infections and their relationship with UV radiation exposure and NMSC development before such an approach can be incorporated into public health practice.

Specific Aims

Non-melanoma skin cancer (NMSC), comprised of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), is the most frequently occurring cancer among U.S. men and women, with an estimated annual case burden of more than one million cases. NMSC, though not as fatal as other cancers, is associated with high treatment costs at the national level and an increased risk of developing other cancers. Exposure to ultraviolet (UV) radiation has been established as a risk factor for NMSC. Evidence from previous studies suggest that intermittent sunlight exposure is important for the pathogenesis of BCC, whereas cumulative sunlight exposure is important for both BCC and SCC, but the exact relationship between patterns and timing of sunlight exposure and risk of BCC and SCC still remain unclear.

With UV radiation exposure being the most important environmental risk factor for NMSC and increasing annual incidence of NMSC despite the increased use of sunscreen products, there is a need to identify cofactors that may interact with UV radiation exposure to increase the risk of NMSC so novel prevention strategies can be developed. Epidemiologic studies have demonstrated that cutaneous human papillomavirus (HPV) infection may be a risk factor for developing NMSC. DNA from cutaneous HPV types, especially genus beta types, have been detected in NMSC tissues, and antibodies against genus beta HPV types have been associated with a 50-400% increased risk of NMSC in several epidemiologic studies. However, the pathway by which cutaneous HPV is associated with NMSC remains unclear. It is hypothesized that UV radiation exposure may interact synergistically with cutaneous HPV in NMSC development.

Identifying how differences in sunlight exposure and cutaneous HPV infections influence the development of BCC and SCC may help characterize individuals at high risk and aid in the development of novel prevention strategies. Utilizing data collected from a previous case control study of NMSC funded by a James and Esther King Biomedical Research Grant (30-14953-99-01), we conducted a case-control analysis of sunlight exposure and cutaneous HPV seropositivity in NMSC among control patients recruited from Moffitt's Lifetime Cancer Screening and Prevention Clinic and the University of South Florida (USF) Family Medicine Clinic and among NMSC patients recruited from the USF Dermatology Clinic. The **goal** of the research study was to evaluate the relationship between levels of sunlight exposure and BCC and SCC and to investigate differences in sunlight-associated BCC and SCC risk by genus-specific cutaneous HPV serostatus. The specific aims for the current study were:

1) To evaluate the association between self-reported patterns (continuous vs. intermittent) of sunlight exposure and BCC and SCC of the skin.
2) To evaluate differences in the associations between self-reported timing (childhood vs. adulthood) of sunlight exposures and BCC and SCC of the skin.
3) To investigate the interaction effects of genus-specific cutaneous HPV seroreactivity and measures of sunlight exposure as they relate to BCC and SCC of the skin.

We **hypothesized** that intermittent and childhood sunlight exposure will be associated with BCC and that chronic, life-long sunlight exposure will be associated with SCC. Finally, we hypothesize that sunlight exposure will be associated with BCC and SCC more strongly among those who are seropositive for antibodies to one or more cutaneous HPV types.

The current study is innovative in that it will be the first study to formally evaluate the relationship between measures of patterns and timing of sunlight exposure in a high risk U.S population. It will also be the first to estimate interaction and joint effects between measures of sunlight exposure (i.e. patterns and timing) and cutaneous HPV seropositivity among a U.S. population. Findings from the proposed study will be of potential public health significance by identifying how differences in patterns and timing of sunlight exposure relate to BCC and SCC. Furthermore, results from the current study may potentially provide evidence to support the interaction between sunlight exposure and cutaneous HPV as they are related to BCC and SCC. This information may help identify high-risk individuals and aid in the development of novel prevention strategies with the intent of reducing the burden of NMSC in populations experiencing high UVR exposure.

CHAPTER 2:

CASE-CONTROL STUDY OF PATTERNS AND TIMING OF SUNLIGHT EXPOSURE IN BASAL CELL AND SQUAMOUS CELL CARCINOMAS OF THE SKIN

Abstract

A case-control study was conducted among Florida residents in the United States to investigate identical measures of patterns (intermittent vs. continuous) and timing (childhood vs. adulthood) of sunlight exposure in basal (BCC) and squamous (SCC) cell carcinomas of the skin. Participants included 218 BCC and 169 SCC cases recruited from a university dermatology clinic and 316 controls with no history of skin or other cancers. A history of blistering sunburn (a measure of intermittent sunlight exposure) was associated with both BCC and SCC. Additionally, having a job in the sun for ≥3 months for 10 years or longer (a measure of continuous sunlight exposure) was also associated with both BCC and SCC in our study population. Measures of timing of sunlight exposure included the presence of moles on one's forearms and entire body (a marker of increased childhood sunlight exposure), age at first blistering sunburn and age at first tanning bed use. With the exception of younger age at first blistering sunburn, measures of younger age at sunlight exposure tended to be associated with SCC, but not BCC risk. Results from the current study provided evidence that both intermittent and continuous patterns of sunlight exposure may be important in both BCC and SCC risk. Additionally, it appeared as though sunlight exposure at younger age was

important for SCC but not BCC in our study population. Further studies are required to identify potential differences or similarities in exposure-response relationships in different types of non-melanoma skin cancer.

Introduction

Non-melanoma skin cancer (NMSC), comprised of basal cell (BCC) and squamous cell (SCC) carcinomas, is the most common cancer in Caucasians, with more than one million new cases diagnosed annually in the United States (U.S.) alone(1). While the mortality associated with NMSC is low(2, 54), patients with multiple NMSC's may experience substantial morbidity, and treatment costs for NMSC are high at the national level(55). Furthermore, a history of NMSC has been consistently associated with increased risk of subsequent primary cancers of other sites in studies from both the U.S. and Europe(3-11).

Ultraviolet radiation (UVR) exposure is considered the most important environmental risk for both BCC and SCC. However, the precise relationship between UVR and the risk of NMSC is complex, and the relationship may differ by skin cancer type. Starting in the late 1950s, researchers began to identify total (cumulative) outdoor sunlight exposure hours and sunlight exposure on working and non-working days(19, 47-49) as risk factors for NMSC. Results from these studies suggested that BCC and SCC may have different exposure-response relationships with sunlight.

Patterns of sunlight exposure are continuous (i.e. persons working outdoors or living in a geographic region with a high annual UV index) or intermittent (i.e. persons working indoors and experiencing most of their sunlight exposure on the weekends or while vacationing to regions with a higher UV index than their place of residence). Timing of sunlight exposure refers to the period of life during which the majority of a

person's sunlight exposure was experienced: childhood/adolescence, adulthood or both. Evidence from previous studies suggests that intermittent and childhood sunlight exposure may be important for the pathogenesis of BCC, whereas continuous, lifelong sunlight exposure may be important for SCC(52, 53, 56-58).

A major limitation of previously published studies is that they do not present direct comparisons between BCC and SCC from the same study population for associations with measures of patterns and timing of sunlight exposure. Therefore, differences in the observed associations may be explained by methodological inconsistencies in exposure measurement between study populations that investigate BCC or SCC alone. This is the first case-control study to simultaneously evaluate identical measures of patterns and timing of sunlight exposure as they are related to both BCC and SCC in the same U.S. population with high annual UVR exposure. The goal of the current study was to identify potential differences or similarities in sunlight exposure responses for BCC and SCC risk.

Materials and methods

Study design and population

A clinic-based case-control study was conducted to evaluate the relationship between patterns and timing of sunlight exposure and risk of BCC and SCC. Complete study procedures have been described in detail elsewhere(59). The University of South Florida (USF) Dermatology (D) clinic served as the primary location for recruitment of NMSC cases, comprised of patients with histologically-confirmed BCC or SCC. Control participants were recruited from the USF Family Medicine (FM) clinic and Moffitt's Lifetime Cancer Screening (LCS). Controls were individuals who self-reported no history of skin or other types of cancer and underwent a skin cancer screening exam at the time

of study enrollment and screened negative for skin cancer. Additionally, any patient that screened positive for a suspicious lesion, underwent a biopsy and were determined to be negative for skin cancer were also included as controls. All participants were recruited between October 30, 2006 and December 24, 2008. All participants provided written informed consent, and all study procedures were approved by the institutional review board at the University of South Florida.

Participation rates for the USF-D, the USF-FM, and LCS clinics were 80%, 47%, and 65%, respectively. There were no statistically significant differences in age or gender between those NMSC patients who agreed to participate and those that refused. The current study population was restricted to White individuals and includes 218 BCC and 169 SCC cases and 316 controls, between the ages of 18 and 80.

Exposure assessment

Self-administered questionnaires were used to obtain information on sunlight exposures and potential confounding factors, including age, gender, ethnicity, education, eye and hair color, ever smoking, skin sensitivity to sunlight exposure (measured by skin reaction to one hour of sunlight exposure for the first time without sunscreen), and tanning ability (measured by change in skin color to repeated exposure to the summer sun). Patterns of sunlight exposure were measured using questions on history of blistering sunburn (yes/no), ever having a job in the sunlight for ≥3 months (yes/no), the number of years with a job in the sunlight for ≥3 months (<1, 1-5, 6-10, or >10 years), lifetime frequency of tanning bed use (≤10, 11-50, 51-100, >100 times), frequency of sunscreen application with a sunlight protection factor (SPF) of ≥15 when outside for more than 15 minutes during the summer (always, often, sometimes, rarely, never), and the number of hours of mid-day sunlight exposure on a typical weekday (<1, 1-2, 3-4, 5-6 hours) and weekend day (<1, 1-2, 3-4, 5-6 hours) in the summer during one's teen

years, twenties, thirties, and the past ten years prior to study enrollment. Experiencing blistering sunburn is considered a marker of intermittent sunlight exposure. Additionally, using sunscreen always/often or rarely/never is considered experiencing continuous sunlight exposure and using sunscreen some of time is considered intermittent sunlight exposure.

Timing of sunlight exposure was measured using questions on the age at which a blistering sunburn was experienced (≤5, 6-10, 11-15, 16-20, >20 years), the number of moles larger than one quarter of an inch in diameter on the forearms (none, <10, 10-25, >25 moles) and on the entire body (none, <10, 10-25, >25 moles), the age at first tanning bed use (≤15, 16-20, >20 years), and the number of hours of mid-day sunlight exposure on a typical weekday (<1, 1-2, 3-4, 5-6 hours) and weekend day (<1, 1-2, 3-4, 5-6 hours) in the summer during one's teen years, twenties, thirties, and in the past ten years prior to study enrollment. The presence of moles is considered an indicator of increased sunlight exposure in childhood or adolescence(60-65).

Statistical analysis

Demographic and skin cancer risk factors were compared between cases and controls using the chi-square test. To test whether measures of patterns or timing of sunlight exposure were associated with BCC or SCC, separate odds ratios (OR) and corresponding 95% confidence intervals (CI) for each skin cancer type were calculated using unconditional logistic regression. Backward stepwise elimination was used to identify confounders from those factors previously shown to be associated with sunlight exposure and NMSC, including age (as a continuous variable), gender, ethnicity, education, eye, hair, and un-tanned skin color, cutaneous sensitivity and tanning ability to sunlight exposure, history of ever smoking, and alcohol consumption in the past year. Each factor retained in the model at $p<.10$ was included in the final regression models;

these factors include age, gender, ethnicity, education, eye and hair color, cutaneous sensitivity, tanning ability, and history of ever smoking. Variance inflation factors and Pearson correlation coefficients were estimated to identify multicollinear relationships between independent risk factors. No collinearity between co-factors and measures of patterns and timing of sunlight exposure was observed.

Factors associated with skin susceptibility factors to sunlight exposure have the potential to be factors on the causal pathway between UVR exposure and skin cancer. Therefore, to demonstrate the impact of these factors on the associations of interest, we present results from two different multivariate analyses. The first multivariate analysis adjusted for demographic and lifestyle factors only (i.e. age, gender, education, and history of ever smoking) and the second adjusted for demographic and lifestyle factors, as well as measures of skin susceptibility to sunlight exposure (i.e. ethnicity, eye and hair color, cutaneous sensitivity and tanning ability to sunlight exposure).

To compare the effects sizes between BCC and SCC for each sun-related factor measured a case-only analysis was conducted. OR and 95% CI were estimated using logistic regression where the dependent variable included NMSC cases only (1=BCC; 0=SCC). A p-value <0.05 for the beta coefficient for each sunlight related factor was considered statistically significant for differences in the magnitudes of associations observed for each independent factor.

Utilizing data collected on the number of hours of sunlight exposure experienced on a typical weekday and weekend day during the summer in different time periods, summary scores were calculated. To measure cumulative sunlight exposure in *early life* (i.e. teens, twenties, and thirties), a median value was applied to each category of hours of sunlight exposure (<1 hour=0.5; 1-2 hours=1.5; 3-4 hours=3.5; 5-6 hours=5.5) on a

weekday and weekend day. The median values for weekday and weekend sunlight exposure were summed for each age group and then summed across the age groups (i.e. teens, twenties, and thirties) and divided into three categories: low, medium, and high. For intermittent sunlight exposure in *early life*, median values were once again applied to each category of hours of sunlight exposure. The ratio of median hours on a weekend day relative to that on a weekday was estimated separately for one's teen years, twenties, and thirties, summed across the three decades, and divided into three groups: low (representing continuous sunlight exposure), medium, and high. Analyses including summary scores measuring sunlight exposure in *early life* were restricted to participants who were ≥40 years of age.

For patterns of sunlight exposure by age at exposure (i.e. one's teens, twenties, thirties, and the 10 years prior to study enrollment), the participant was considered as having had *continuous sunlight exposure* if the reported number of hours of weekday sunlight exposure (1-2 or 3-6 hours) equaled that of weekend sunlight exposure (1-2 or 3-6 hours). However, if the reported number of hours of weekday sunlight exposure was less than that of weekend sunlight exposure, then the participant was considered as having *intermittent* sunlight exposure. Participants classified as having *continuous* or *intermittent* sunlight exposure were compared to participants with <1 hour of sunlight exposure on a typical weekday and weekend day. Daily sunlight exposure by age at exposure was measured by summing the median values of weekday and weekend hours of sunlight exposure and then dividing the values into three categories: low, medium, and high, independently for each time period.

All analyses were performed using the SAS statistical software package (version 9.1.3; SAS Institute).

Results

Demographic, lifestyle, and skin susceptibility factors are presented for cases and controls in Table 1. Compared to controls, cases were significantly more likely to be male (BCC: p=<.0001; SCC: p=<.0001), older in age (BCC: p=<.0001; SCC: p=<.0001), less educated (BCC: p=0.0004; SCC: p=0.001), and ever smokers (BCC: p=0.002; SCC: p=<.0001). Additionally, NMSC cases were more likely to have light eye and hair color, a greater tendency to burn and a lesser tendency to tan from sunlight exposure, compared to controls.

Associations between patterns of sunlight exposure and NMSC are presented in Table 2. When adjusting for demographic and lifestyle factors only, a history of blistering sunburn was positively associated with both BCC (OR=1.96, 95% CI=1.27-3.03) and SCC (OR=2.02, 95% CI=1.22-3.33). Ever having a job in the sunlight for ≥3 months was significantly associated with SCC (OR=1.73, 95% CI=1.06-2.83) but not BCC (OR=1.38, 95% CI=0.89-2.14). However, having a job in the sunlight for ≥3 months for >10 years was significantly associated with both BCC (OR=2.14, 95% CI=1.12-4.11) and SCC (OR=2.54, 95% CI=1.23-5.28). With the exception of having a job in the sunlight for >10 years, the associations described above were no longer statistically significant when adding skin susceptibility co-factors to the multivariate models. When adjusting for demographic and lifestyle factors only, no associations were observed between levels of cumulative sunlight exposure or patterns of exposure in one's twenties or thirties and either BCC or SCC. However, after additional adjustment for measures of skin susceptibility, medium levels of cumulative sunlight exposure were associated with BCC (OR=1.88, 95% CI=1.07-3.31) and medium (OR=2.36, 95% CI=1.22-4.57) and high (OR=1.25-4.91) levels of cumulative sunlight exposure were significantly associated with SCC, compared to low levels in early life. Additionally, sunlight exposure in one's

twenties was associated with SCC regardless of the pattern of exposure; specifically, an OR of 2.99 (95% CI=1.19-7.48) was associated with continuous hours and an OR of 3.15 (95% CI=1.27-7.83) was associated with intermittent hours of exposure compared to <1 hour of sunlight exposure. Finally, in one's thirties, statistically significant associations were observed between intermittent hours of sunlight exposure and BCC (OR=2.09, 95% CI=1.11-3.93) while continuous hours of sunlight exposure were associated with SCC (OR=2.25, 95% CI=1.02-4.94), compared to <1 hour of exposure, when adjusting for skin susceptibility co-factors. Regardless of the covariates included in the multivariate models, no statistically significant associations in BCC or SCC were observed with tanning bed use, sunscreen use, levels of intermittent sunlight exposure in early life, and patterns of sunlight exposure in one's teens and the past ten years prior to study enrollment.

Table 3 presents the associations between measures of timing of sunlight exposure and BCC and SCC. When adjusting for demographic and lifestyle factors only, associations with SCC were observed for the presence of >10 moles on the forearms (OR=3.27, 95% CI=1.12-9.58) and entire body (OR=2.12, 95% CI=1.11-4.06), compared to no moles. Similar associations were not observed with BCC. Experiencing a blistering sunburn in young childhood or adolescence was significantly associated with both BCC (<10 years: OR=1.97, 95% CI=1.14-3.42; 10-20 years: OR=2.15, 95% CI=1.32-3.52) and SCC (<10 years: OR=2.25, 95% CI=1.22-4.13; 10-20 years: OR=2.37, 95% CI=1.34-4.21), compared to never experiencing blistering sunburn. SCC cases were more likely to begin using a tanning bed prior to age 20 (OR=1.97, 95% CI=1.01-3.85), compared to never users. No significant associations with BCC were observed for age at first tanning bed use. Elevated OR estimates were observed for high levels of daily sunlight exposure during the summer with BCC and SCC across all

time periods, however, none of these associations achieved statistical significance. When including measures of skin susceptibility to sunlight exposure to the multivariate models, little differences were observed in the magnitudes of associations between measures of timing of sunlight exposure and BCC/SCC.

In summary, measures of both intermittent and continuous patterns of sunlight exposure were associated with both types of skin cancer (i.e. BCC and SCC). Specifically, history of blistering sunburn (a marker of intermittent sunlight exposure) and occupational sunlight exposure (i.e. having a job in the sun for ≥3 months for >10 years) were both associated with BCC and SCC. The major differences in patterns of sunlight exposure between BCC and SCC were observed for sunlight exposure in one's thirties when adjusting for skin susceptibility factors. Additionally, sunlight exposure in one's twenties was associated with SCC, regardless of pattern of exposure; similar associations were not observed for BCC. Measures of timing of sunlight exposure consistently demonstrated that childhood/adolescent sunlight exposure was statistically significantly more important for SCC. However, despite differences in statistical significance in sun-related factors between BCC and SCC, case-only analyses demonstrated that the observed ORs were not significantly different in magnitude between BCC and SCC for measures of patterns and timing of sunlight exposure (data not shown).

Discussion

A clinic based case-control study was conducted to identify associations between patterns and timing of sunlight exposure and two types NMSC, BCC and SCC. It has been suggested that BCC and SCC risk may differ by the patterns and timing in which sunlight exposure was received. Unlike previously published studies, we investigated

multiple measures of sunlight exposure in BCC and SCC simultaneously and many similarities were observed in measures of intermittent and continuous patterns of sunlight exposure between the two types of skin cancer. For example, history of blistering sunburn, having a job in sun for >10 years, and cumulative sunlight exposure in early life were associated with both BCC and SCC. With the exception of experiencing blistering sunburn at a younger age, measures of timing of sunlight exposure tended to be more important for SCC than BCC risk. For example, the presence of moles on one's forearms or entire body (a marker of childhood/adolescent sun exposure) was associated with SCC, but not BCC. Additionally, using a tanning bad for the first time at a younger age was positively associated with SCC, but not BCC.

Previous studies that aimed to quantify the association between the amount of sunlight exposure and NMSC have provided evidence to support the hypothesis that intermittent sunlight exposure is associated with BCC(52, 66) while chronic sunlight exposure is associated with SCC(13, 24, 49, 51, 53, 67) . Utilizing similar information as previous studies (i.e. number of hours of sunlight exposure to define intermittent and continuous exposure), findings from the current study provide evidence that BCC and SCC risk do not differ by patterns of exposure, but in fact that intermittent and continuous patterns of sunlight exposure are important for both BCC and SCC. Additionally, information from previous studies investigating measures of sunlight exposure, such as blistering sunburn, has been used to potentially support the current hypotheses regarding patterns of sunlight exposure and NMSC. A history of blistering sunburn (an indicator of intermittent sunlight exposure) was positively associated with both BCC and SCC in our study population. This agrees with two case-control studies of SCC(53, 67) , but contradicts observations from four studies of BCC(14, 52, 67, 68) and one of SCC(68). Blistering sunburn is believed to result from high doses of intense UVR

exposure in short increments of time. Therefore, it's considered a measure of intermittency. However, blistering sunburn is also a measure of cutaneous sensitivity to sunlight exposure and may explain the observed associations in our study population for both BCC and SCC when co-factors measuring skin susceptibility to sunlight exposure were excluded from the multivariate models.

It has been estimated that approximately 25% of lifetime sunlight exposure occurs before 18 years of age(69). Young childhood and adolescence is considered a time period when individuals have greater vulnerability to toxic exposure, such as UVR(69). Associations with first occurrence of blistering sunburn during childhood or adolescence (age periods prior to skin cancer diagnosis) were similar for BCC and SCC risk in our study population. However, among residents of Western Australia, blistering sunburn between 10 to 14 years of age was associated with BCC(52) while sunburn between 35 to 39 years of age was associated with SCC(53). Many epidemiologic studies have investigated the association between sunlight exposure in early childhood and nevus development and provide evidence that increasing sunlight exposure in early years of life is associated with melanocytic nevus development(60-65). Since most nevi develop by the age of 10, their presence in adulthood may be considered an indicator of high UV exposure in childhood. Self-reported presence of >10 moles on the entire body were significantly and positively associated with SCC in our study population. Similar results were not observed for BCC. A limited number of studies have reported findings for the association between the presence of moles and NMSC, of which, one case-control study from Western Australia(70) and one prospective cohort study of U.S. male health professionals(27) observed a positive dose-response relationship between an increasing number of moles and BCC. In contrast, among adults from the U.S.(68), the presence of moles was not associated with either BCC or SCC risk.

The current study has some limitations. Clinic based study populations are not necessarily representative samples of the general population. Case-control studies are often subject to recall bias because cases tend to think about their exposures more carefully as they might relate to their current cancer diagnosis. The sample size was small, limiting the ability to detect statistically significant associations, especially when adjusting for multiple co-factors. Few differences were observed in the magnitudes of the estimated effects when adjusting for skin susceptibility factors. However, when including these factors in the multivariate model, precision decreased and in some instances statistical significance was no longer observed, mostly likely due to a decrease in the sample size.

Unlike previous studies(51-53), we measured intermittency of sunlight exposure in the current study by assuming that weekend hours were “non-working” hours for our study population and we were unable to estimate “lifetime” sunlight exposure or consider the amount of ambient solar irradiance received by study participants. Additionally, sunlight exposure was not assessed at the site of BCC or SCC diagnosis, as done in previous studies(52, 53). Depending on the site of skin cancer diagnosis, this may result in participants underestimating the amount of sunlight exposure to the site of skin cancer diagnosis which, in turn would result in small effect differences being observed. Approximately 61% of skin cancers in our study population occurred on the face. Since the face is chronically exposed to sunlight exposure regardless of the outdoor activity or type of clothing being worn (even hats do not block or filter 100% of UV radiation), this could result in participants under-reporting their sunlight exposure. We also did not collect information on sunlight exposure during holidays or recreational activities. It is difficult to compare results across studies for the relationship between sunlight exposure

and skin cancer due mainly to inconsistencies and variations in the methods used to measure sunlight exposure.

The current study is the first case-control study to formally evaluate measures of patterns and timing of sunlight exposure in NMSC in a high risk U.S. population as well as present findings simultaneously for both skin cancer types, BCC and SCC. This presentation allowed for direct comparisons of patterns and timing of sunlight by skin cancer type. The controls were screened for current signs of BCC and SCC by a nurse practitioner to avoid misclassification of case-control status that may result from self-reported data. This is an important strength of our study as a portion of the screened patients were included as cases in the current study population.

The current study does not support clear differences in the exposure response relationships between patterns of sunlight exposure for BCC and SCC. We conducted a case-only analysis to identify statistically significant differences in the observed ORs between BCC and SCC. Results of this analysis provided evidence to support that the associations between patterns of sunlight exposure were in fact more similar, than different for each type of NMSC. Based on the evidence provided by the current study we conclude that both intermittent and continuous patterns of sunlight exposure are important for both BCC and SCC risk. Additionally, despite statistically significant ORs observed between measures of timing of sunlight exposure and SCC, the case-only analysis revealed no strong differences in timing of sunlight exposure between BCC and SCC.

Understanding how sunlight exposure responses may potentially differ by NMSC type is important for better educating the public in sun safe behaviors. Simply advising a reduction in sunlight exposure will not help reduce the incidence of NMSC if changes in

sunlight exposure patterns are related to skin cancer development. For example, applying sunscreen while on vacation may decrease BCC risk associated with intermittent sunlight exposure, but may not impact the risk of SCC, which may be more strongly related with continuous sunlight exposure. Further studies are needed to highlight similarities and differences in the exposure-response relationship of patterns and timing of sunlight exposure with BCC and SCC. Furthermore, standardized methods for measuring sunlight exposure should be established to enable comparisons across different study populations.

Acknowledgements

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Table 2.1. Demographic, life-style, and skin cancer risk factors in basal cell and squamous cell carcinoma cases and controls

Variable	Controls (n = 316) n (%)	BCC (n = 218) n (%)	p[1]	SCC (n = 169) n (%)	p[1]
Age mean(S.D.)	55.6 (11.8)	62.8 (11.9)	<.0001	64.8 (9.6)	<.0001
Age (years)					
18-29	9 (2.9)	1 (0.5)		1 (0.6)	
30-39	21 (6.7)	6 (2.8)		2 (1.2)	
40-49	55 (17.4)	24 (11.0)		10 (5.9)	
50-59	109 (34.5)	46 (21.1)		30 (17.8)	
60-69	88 (27.9)	64 (29.4)		68 (40.2)	
70-80	34 (10.8)	77 (35.3)	<.0001	58 (34.3)	<.0001
Gender					
Male	117 (37.0)	133 (61.0)		108 (63.9)	
Female	199 (63.0)	85 (39.0)	<.0001	61 (36.1)	<.0001
Ethnicity					
Non-Hispanic	280 (88.6)	208 (95.4)		161 (95.3)	
Hispanic	32 (10.1)	7 (3.2)	0.003	2 (1.2)	0.0003
Education					
≤ 12 years	32 (10.1)	46 (21.1)		36 (21.3)	
> 12 years	280 (88.6)	168 (77.1)	0.0004	129 (76.3)	0.0006
Smoked 100 cigarettes					
Never	161 (50.9)	81 (37.2)		51 (30.2)	
Ever	154 (48.7)	134 (61.5)	0.002	114 (67.5)	<.0001
Alcohol consumption					
≥ 1 drink in past year	274 (86.7)	177 (81.2)		130 (76.9)	
No drinks in past year	40 (12.7)	39 (17.9)	0.09	35 (20.7)	0.02
Eye color					
Blue	94 (29.7)	87 (40.0)		69 (40.8)	
Green	50 (16.1)	24 (11.0)		25 (14.8)	
Hazel	52 (16.5)	48 (22.0)		31 (18.3)	
Light brown	36 (11.4)	22 (10.1)		18 (10.7)	
Dark brown	80 (25.3)	35 (16.1)	0.009	22 (13.0)	0.02
Hair Color					
Black/Brown	245 (77.5)	152 (69.7)		113 (66.9)	
Blonde/Red	70 (22.2)	65 (29.8)	0.04	53 (31.4)	0.02
Color of un-tanned skin					
White	299 (94.9)	209 (96.3)		161 (95.3)	
Brown	15 (4.8)	7 (3.2)	0.38	6 (3.6)	0.55
Cutaneous sensitivity to sunlight exposure					
Sunburn with blisters	29 (9.2)	33 (15.1)		22 (13.0)	
Sunburn w/o blisters	96 (30.4)	95 (43.6)		71 (42.0)	
Mild sunburn/tan	144 (45.6)	65 (29.8)		50 (29.6)	
Tan/no change color	44 (13.9)	21 (9.6)	0.0001	22 (13.0)	0.005
Tanning ability to sunlight exposure					
It is unable to tan	22 (7.0)	15 (6.9)		26 (15.4)	
Tan if you work at it	103 (32.6)	93 (42.7)		77 (45.6)	
It tans easily	186 (58.9)	104 (47.7)	0.04	62 (37.6)	<.0001

[1]p-value for chi-square test

Table 2.2. Associations between measures of patterns of sunlight exposure and basal cell and squamous cell carcinoma cases and controls

	Controls (n=316)	Basal cell carcinoma (n=218)			Squamous cell carcinoma (n=169)		
Variable	n (%)	n (%)	OR (95% CI)[1]	OR (95% CI)[2]	n (%)	OR (95% CI)[1]	OR (95% CI)[2]
Blistering Sunburn							
No	101 (32.3)	54 (25.0)	1.00 (reference)	1.00 (reference)	38 (23.0)	1.00 (reference)	1.00 (reference)
Yes	212 (67.7)	162 (75.0)	1.96 (1.27-3.03)	1.56 (0.96-2.54)	127 (77.0)	2.02 (1.22-3.33)	1.24 (0.71-2.18)
Job in sun ≥3 months							
No	227 (72.8)	120 (55.3)	1.00 (reference)	1.00 (reference)	86 (51.8)	1.00 (reference)	1.00 (reference)
Yes	85 (27.2)	97 (44.7)	1.38 (0.89-2.14)	1.31 (0.81-2.12)	80 (48.2)	1.73 (1.06-2.83)	1.72 (0.99-2.97)
# years with job							
≤10	57 (18.7)	47 (22.3)	1.17 (0.70-1.94)	1.07 (0.61-1.86)	44 (27.0)	1.64 (0.94-2.86)	1.64 (0.88-3.07)
>10	21 (6.9)	44 (20.9)	2.14 (1.12-4.11)	2.12 (1.05-4.27)	33 (20.2)	2.54 (1.23-5.28)	2.36 (1.07-5.20)
Lifetime tanning bed use							
Never used	209 (71.3)	175 (82.5)	1.00 (reference)	1.00 (reference)	127 (80.9)	1.00 (reference)	1.00 (reference)
1-10 times	44 (15.0)	25 (11.8)	0.99 (0.56-1.76)	0.99 (0.53-1.82)	18 (11.5)	1.01 (0.52-1.98)	0.80 (0.38-1.71)
>10 times	40 (13.7)	12 (5.7)	0.64 (0.30-1.36)	0.64 (0.30-1.36)	12 (7.6)	1.67 (0.75-3.73)	1.85 (0.74-4.62)
Apply SPF[3] ≥15							
Always/often	124 (39.6)	82 (37.8)	1.00 (reference)	1.00 (reference)	53 (32.5)	1.00 (reference)	1.00 (reference)
Sometimes	98 (31.3)	59 (27.2)	0.79 (0.50-1.26)	0.87 (0.52-1.45)	56 (34.4)	0.83 (0.49-1.42)	0.86 (0.47-1.59)
Rarely/never	91 (29.1)	76 (35.0)	0.83 (0.52-1.32)	0.93 (0.56-1.54)	54 (33.1)	0.79 (0.46-1.36)	0.87 (0.48-1.60)
Cumulative sunlight exposure							
Low	87 (32.3)	52 (27.4)	1.00 (reference)	1.00 (reference)	35 (22.7)	1.00 (reference)	1.00 (reference)
Medium	92 (34.2)	54 (28.4)	1.02 (0.61-1.69)	1.42 (0.81-2.50)	57 (37.0)	1.49 (0.84-2.64)	2.36 (1.22-4.57)
High	90 (33.5)	84 (44.2)	1.37 (0.83-2.27)	1.88 (1.07-3.31)	62 (40.3)	1.59 (0.88-2.87)	2.47 (1.25-4.91)
Intermittent sunlight exposure							
Low	91 (33.8)	80 (42.1)	1.00 (reference)	1.00 (reference)	59 (38.3)	1.00 (reference)	1.00 (reference)
Medium	84 (31.2)	52 (27.4)	0.99 (0.60-1.64)	1.26 (0.72-2.22)	46 (29.9)	1.25 (0.71-2.20)	1.58 (0.83-3.00)
High	94 (34.9)	58 (30.5)	1.15 (0.70-1.88)	1.23 (0.72-2.10)	49 (31.8)	1.48 (0.85-2.58)	1.57 (0.83-2.94)

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Table 2.2 continued. Associations between measures of patterns of sunlight exposure and basal cell and squamous cell carcinoma cases and controls

	Controls (n=316)	Basal cell carcinoma (n=218)			Squamous cell carcinoma (n=169)		
Variable	n (%)	n (%)	OR (95% CI)[1]	OR (95% CI)[2]	n (%)	OR (95% CI)[1]	OR (95% CI)[2]
Patterns by age at exposure							
Teens							
<1 hour	18 (6.0)	12 (6.0)	1.00 (reference)	1.00 (reference)	4 (2.5)	1.00 (reference)	1.00 (reference)
Continuous hours	151 (50.0)	113 (56.8)	1.04 (0.45-2.41)	0.97 (0.38-2.48)	96 (60.4)	2.33 (0.69-7.90)	1.76 (0.48-6.47)
Intermittent hours	133 (44.0)	74 (37.2)	1.08 (0.46-2.54)	1.10 (0.42-2.83)	59 (37.1)	2.26 (0.66-7.78)	1.86 (0.50-6.94)
Twenties							
<1 hour	34 (11.3)	18 (9.0)	1.00 (reference)	1.00 (reference)	11 (6.9)	1.00 (reference)	1.00 (reference)
Continuous hours	102 (33.9)	91 (45.5)	1.36 (0.68-2.71)	1.58 (0.75-3.36)	74 (46.5)	2.01 (0.86-4.67)	2.99 (1.19-7.48)
Intermittent hours	165 (54.8)	91 (45.5)	1.30 (0.66-2.56)	1.56 (0.74-3.26)	74 (46.5)	2.11 (0.92-4.88)	3.15 (1.27-7.83)
Thirties							
<1 hour	60 (20.5)	27 (13.6)	1.00 (reference)	1.00 (reference)	18 (11.3)	1.00 (reference)	1.00 (reference)
Continuous hours	85 (29.0)	79 (39.9)	1.31 (0.72-2.40)	1.77 (0.90-3.49)	69 (43.4)	1.55 (0.77-3.10)	2.25 (1.02-4.94)
Intermittent hours	148 (50.5)	92 (46.5)	1.38 (0.79-2.41)	2.09 (1.11-3.93)	72 (45.3)	1.47 (0.76-2.85)	1.95 (0.92-4.12)
Past 10 years							
<1 hour	63 (28.6)	52 (30.2)	1.00 (reference)	1.00 (reference)	49 (33.1)	1.00 (reference)	1.00 (reference)
Continuous hours	74 (33.6)	83 (48.3)	0.88 (0.51-1.52)	1.14 (0.62-2.12)	64 (43.2)	0.81 (0.46-1.42)	1.35 (0.69-2.64)
Intermittent hours	83 (37.7)	37 (21.5)	0.57 (0.32-1.03)	0.67 (0.35-1.28)	35 (23.6)	0.60 (0.33-1.10)	0.93 (0.46-1.89)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, gender, education, and history of ever smoking

[2]OR and 95% CI adjusted for age, gender, education, history of ever smoking, ethnicity, eye and hair color, cutaneous sensitivity and tanning ability to sunlight exposure

[3]SPF=sun protection factor

Table 2.3. Associations between measures of timing of sunlight exposure and basal cell and squamous cell carcinoma cases and controls

	Controls (n=316)	Basal cell carcinoma (n=218)			Squamous cell carcinoma (n=169)		
Variable	n (%)	n (%)	OR (95% CI)[1]	OR (95% CI)[2]	n (%)	OR (95% CI)[1]	OR (95% CI)[2]
# of moles on forearms							
None	220 (71.4)	155 (71.8)	1.00 (reference)	1.00 (reference)	115 (69.7)	1.00 (reference)	1.00 (reference)
<10	80 (26.0)	53 (24.5)	0.84 (0.54-1.31)	0.65 (0.40-1.06)	39 (23.6)	0.92 (0.56-1.52)	0.94 (0.54-1.64)
≥10	8 (2.6)	8 (3.7)	1.65 (0.57-4.77)	1.75 (0.55-5.61)	11 (6.7)	3.27 (1.12-9.58)	2.69 (0.75-9.59)
# of moles on entire body							
None	118 (39.1)	79 (36.9)	1.00 (reference)	1.00 (reference)	56 (34.1)	1.00 (reference)	1.00 (reference)
<10	147 (48.7)	107 (50.0)	1.11 (0.73-1.67)	1.03 (0.66-1.60)	76 (46.3)	1.15 (0.72-1.86)	1.22 (0.71-2.09)
≥10	37 (12.3)	28 (13.1)	1.18 (0.64-2.19)	1.06 (0.55-2.04)	32 (19.5)	2.12 (1.11-4.06)	2.16 (1.03-4.52)
Age at 1st sunburn							
None	101 (32.9)	54 (25.2)	1.00 (reference)	1.00 (reference)	38 (23.5)	1.00 (reference)	1.00 (reference)
<10 years	62 (20.2)	47 (22.0)	1.97 (1.14-3.42)	1.35 (0.73-2.49)	46 (28.4)	2.25 (1.22-4.13)	1.07 (0.53-2.15)
10-20 years	108 (35.2)	84 (39.3)	2.15 (1.32-3.52)	1.73 (1.00-2.99)	65 (40.1)	2.37 (1.34-4.21)	1.62 (0.86-3.04)
>20 years	36 (11.7)	29 (13.6)	1.71 (0.89-3.28)	1.67 (0.83-3.37)	13 (8.0)	0.96 (0.42-2.20)	0.81 (0.33-2.01)
Age at 1st tanning bed use							
Never used	209 (67.0)	175 (80.3)	1.00 (reference)	1.00 (reference)	127 (76.5)	1.00 (reference)	1.00 (reference)
≤20 years	38 (12.2)	20 (9.2)	1.09 (0.58-2.06)	1.10 (0.55-2.18)	23 (13.9)	1.97 (1.01-3.85)	1.97 (0.91-4.27)
>20 years	65 (20.8)	23 (10.6)	0.64 (0.64-1.12)	0.56 (0.30-1.05)	16 (9.6)	0.77 (0.40-1.50)	0.78 (0.37-1.65)
Daily sunlight exposure by age							
Teens							
Low	63 (20.9)	27 (13.6)	1.00 (reference)	1.00 (reference)	21 (13.2)	1.00 (reference)	1.00 (reference)
Medium	104 (34.4)	68 (34.2)	1.28 (0.71-2.30)	1.18 (0.62-2.25)	55 (34.6)	1.24 (0.64-2.42)	0.94 (0.45-1.97)
High	135 (44.7)	104 (52.3)	1.38 (0.78-2.43)	1.47 (0.78-2.77)	83 (52.2)	1.43 (0.75-2.73)	1.40 (0.68-2.89)
Twenties							
Low	121 (40.2)	62 (31.0)	1.00 (reference)	1.00 (reference)	53 (33.3)	1.00 (reference)	1.00 (reference)
Medium	114 (37.9)	79 (39.5)	1.20 (0.77-1.88)	1.35 (0.82-2.21)	51 (32.1)	0.82 (0.49-1.37)	0.97 (0.54-1.73)
High	66 (21.9)	59 (29.5)	1.22 (0.73-2.31)	1.31 (0.75-2.31)	55 (34.6)	1.40 (0.80-2.44)	1.56 (0.83-2.91)
Thirties							
Low	152 (51.9)	87 (43.9)	1.00 (reference)	1.00 (reference)	64 (40.3)	1.00 (reference)	1.00 (reference)
Medium	103 (35.2)	63 (31.8)	0.90 (0.58-1.39)	0.98 (0.60-1.58)	62 (39.0)	1.08 (0.66-1.75)	1.36 (0.78-2.37)
High	38 (13.0)	48 (24.2)	1.20 (0.68-2.10)	1.28 (0.69-2.36)	33 (20.8)	1.15 (0.61-2.18)	1.30 (0.63-2.68)
Past 10 years							
Low	126 (57.3)	76 (44.2)	1.00 (reference)	1.00 (reference)	80 (54.1)	1.00 (reference)	1.00 (reference)
Medium	65 (29.5)	59 (34.3)	1.05 (0.64-1.74)	1.15 (0.66-2.01)	40 (27.0)	0.77 (0.45-1.31)	1.13 (0.61-2.10)
High	29 (13.2)	37 (21.5)	1.41 (0.74-2.68)	1.62 (0.79-3.30)	28 (18.9)	1.16 (0.59-2.25)	1.57 (0.73-3.36)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, gender, education, and history of ever smoking

[2]OR and 95% CI adjusted for age, gender, education, history of ever smoking, cutaneous sensitivity and tanning ability to sunlight exposure

CHAPTER 3:

SUNLIGHT EXPOSURE AND CUTANEOUS HUMAN PAPILLOMAVIRUS IN NON-MELANOMA SKIN CANCER

Abstract

Non-melanoma skin cancer (NMSC), comprised of basal cell (BCC) and squamous cell carcinoma (SCC), is the most common cancer in Caucasians. Ultraviolet radiation (UVR) exposure is the most important environmental risk factor for both BCC and SCC development. Recently, epidemiologic studies have demonstrated that cutaneous human papillomavirus (HPV) infection may also be a risk factor for developing NMSC. However, the pathway by which cutaneous HPV is associated with NMSC remains unclear. It is hypothesized that UVR exposure may interact synergistically with cutaneous HPV in NMSC development. To investigate differences in sunlight-associated BCC and SCC risk by genus-specific cutaneous HPV serostatus, a clinic based case-control study was conducted. NMSC cases included patients with histologically confirmed BCC (n=204) and SCC (n=156) diagnoses recruited from a university dermatology clinic and controls were participants with no history of cancer and screened negative for current skin cancer (n=297). NMSC cases were more likely to be seropositive for cutaneous HPV antibodies compared to controls. Compared to tanning, having a propensity to sun burn (p=0.006), or poor tanning ability (p=0.003) were significantly associated with a higher seroprevalence to genus beta HPV types within

SCC cases. Statistically significant interactions were observed between poor tanning ability and genus-specific seropositivity with NMSC. Specifically, the associations between poor tanning ability and BCC ($p_{interaction}$=0.02) and SCC ($p_{interaction}$=0.01) were significantly stronger among individuals that were seropositive for antibodies to genus alpha HPV types. Similarly, the association between poor tanning ability and SCC was stronger among those seropositive for genus beta HPV types ($p_{interaction}$=0.001). In conclusion, evidence from the current study supports the hypothesis that cutaneous HPV infection may play a potential role in the association between UVR and NMSC.

Introduction

Non-melanoma skin cancer (NMSC), comprised of basal (BCC) and squamous (SCC) cell carcinomas, is the most common cancer in Caucasians, with more than one million new cases diagnosed annually in the United States alone(1). Constitutional factors, including light eye, hair, and skin color, as well as older age, male sex, and immunosuppression(12) have been identified as risk factors for BCC and SCC. Ultraviolet radiation (UVR) exposure has been implicated in the etiology of skin cancer and is considered the most important environmental risk for both BCC and SCC development.

Several lines of evidence suggest that UVR exposure may play a synergistic role along with cutaneous human papillomavirus (HPV) infection in the development of cutaneous NMSC. HPVs belong to a large family of more than 100 genotypes, including types that infect cutaneous epithelia identified from genera alpha, beta, gamma, mu, and nu(50). Presence of antibodies against cutaneous HPV types has been associated with SCC in several epidemiologic studies(29, 30, 34, 71-73); however, results from epidemiologic studies of cutaneous HPV and BCC are less consistent(29, 30, 34, 71, 72)

and fewer in number. UV-B radiation has been shown to stimulate the promoter activity of HPV 5 and 8(39). In turn, the E6 and/or E7 proteins of genus beta HPV types have been shown to inhibit UVR-induced apoptosis through p53-independent pathways(45, 46), reduced capacity to repair UVR-induced mutations(43), and alter the regulation of UVR-activated cell cycle checkpoints.

The goal of the current study was to investigate the potential modifying effects of cutaneous HPV seroreactivity on the associations between sunlight related factors and BCC and SCC.

Materials and Methods

Study design and population

To investigate differences in sunlight-associated BCC and SCC risk by cutaneous HPV seroreactivity, a clinic-based case-control analysis was conducted. Study procedures have been described previously(59). Participants were recruited from the Dermatology (D) and Family Medicine (FM) clinics at the University of South Florida (USF), as well as Moffitt's Lifetime Cancer Screening and Prevention (LCS) clinic. Eligible cases were patients, ages 18-80 years, diagnosed with a histologically-confirmed BCC or SCC. Controls were patients who reported no history of any type of skin cancer at the time of study recruitment and screened negative for skin cancer as determined by a full body skin cancer screening exam conducted by a nurse practitioner. Participation rates for the USF-D, the USF-FM, and LCS clinics were 80%, 47%, and 65%, respectively. No significant differences in age or gender were observed between study participants and non-participants from the USF-D clinic. Significant differences in age, but not gender, were observed between study participants and non-participants from the USF-FM and LCS clinics.

All study participants were asked to complete a self-administered questionnaire including questions on demographic, constitutional characteristics, life-style factors, and measures of sunlight exposure, and to provide a blood sample for cutaneous HPV antibody measurement. A total of 204 BCC, 156 SCC, and 297 controls had available questionnaire data and cutaneous HPV antibody results. Participants that reported a race other than white or had missing data on race were excluded from the current study analysis. All participants provided written informed consent. All study procedures were approved by the institutional review board at the University of South Florida.

Measurement of antibodies to cutaneous human papillomavirus types

At the time of study enrollment, blood was drawn using a sterile needle into serum separator tubes with clot activators. Following centrifugation, serum was aliquoted into cryovials and stored at -80^{o}C until being shipped on dry ice to Dr. Pawlita's laboratory at the German Cancer Research Center (Deutsches Krebsforschungzentrum, (DKFZ)), for analysis. Samples were analyzed for antibodies to the major capsid protein L1 for 7 types in genus alpha (2, 3, 7, 10, 27, 57, 77), 17 types in genus beta (5, 8, 9, 15, 17, 20, 23, 24, 25, 36, 38, 49, 75, 76, 92, 96, 107), 8 types in genus gamma (4, 48, 50, 65, 88, 95, 101, 103), and 1 type in both genus mu (1) and genus nu (41), using a detection method based on Glutathione-S-Transferase (GST) capture ELISA as described in Sehr et al.(74, 75) in combination with fluorescent bead technology (Luminex) as recently described(76). Briefly, full-length viral proteins were expressed in bacteria in fusion with an N-terminal glutathione S-transferase (GST) domain. Glutathione-crosslinked to casein was coupled to fluorescence-labeled polystyrol beads and GST fusion proteins were affinity-purified on the beads directly in a one-step procedure. Bead types of different color and carrying different antigens were mixed and incubated with human sera. Antibody bound to the beads via the viral antigens was

stained by biotinylated anti-human-Ig and streptavidin-phycoerythrin. Beads were analyzed in a luminex analyzer that identifies the bead color - and thus the antigen carried by the bead – and quantified the antibody bound to viral antigen via the median phycoerythrin fluorescence intensity of at least 100 beads of the same internal color. Cutoff points to define seropositivity were applied as described elsewhere(29, 35).

Statistical analysis

Differences in the distributions of demographic and skin cancer risk factors, as well as genus-specific HPV seroreactivity between NMSC cases and controls were tested using the chi-square test. The sunlight exposure factors included cutaneous sensitivity to one hour of sunlight exposure for the first time without sunscreen (experience a sunburn with or without blistering, a mild sunburn that turns to a tan, tanning or no change in skin color); tanning ability from repeated sunlight exposure (it is unable to tan, it can tan if you work at it, it tans easily); history of blistering sunburn (yes/no); and cumulative sunlight exposure in early life (low, high). To measure cumulative sunlight exposure in early life (i.e. <30 years of age), a median value was applied to each category of hours of sunlight exposure (<1 hour=0.5; 1-2 hours=1.5; 3-4 hours=3.5; 5-6 hours=5.5) experienced on a weekday and weekend day during the summer in different time periods (i.e. one's teens, twenties, and thirties). The median values applied to weekday and weekend day hours of exposure were first summed for each individual age period and then summed across the three age periods and divided into two categories: low and high. Analyses involving cumulative sunlight exposure in early life were restricted to participants who were ≥40 years of age.

Logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the associations between sun-related factors and BCC and SCC. Confounding by constitutional, demographic, lifestyle and skin cancer risk factors

was assessed, and with the exception of age and gender, inclusion of additional co-factors did not alter the calculated estimates by more than 10%, thus final models included only age and gender as covariates.

Participants were classified as HPV-seropositive or HPV-seronegative for antibodies to each individual cutaneous HPV type measured based on HPV type-specific cut points assigned. Cutaneous HPV types were then grouped by genus. Overall genus-specific seropositivity was calculated as the proportion of participants who tested positive for antibodies to at least one of the types in that genus. Genus-specific seropositive participants were compared to participants that tested negative for all types in that genus. The chi-square test was used to describe differences in the distributions of genus-specific HPV seropositivity across sun-related factors among cases and controls. The associations between sun-related factors and BCC and SCC were stratified by genus-specific HPV serostatus (seropositive, seronegative), and stratum-specific OR and corresponding 95% CIs were estimated. Statistical significance of multiplicative interactions between genus-specific seroreactivity and sun-related factors as they related to BCC and SCC was tested by placing an interaction term for the product of genus-specific seroreactivity and the sun-related factors in the logistic regression models. A p-value of <0.05 for the beta coefficient corresponding to the interaction term was considered statistically significant. All analyses were conducted using the SAS statistical software package (version 9.2; SAS Institute).

Results

Compared to controls, NMSC cases were significantly more likely to be male, older in age, more likely to burn from sunlight exposure, and exhibit diminished ability to tan (Table 1). Additionally, SCC cases reported higher levels of cumulative sunlight exposure ($p=0.03$) compared to controls (Table 1). Seroprevalence was highest for

cutaneous HPV types in genus beta for SCC cases (73.1%) and controls (60.3%), followed by genus gamma (62.8% and 52.2% in SCC cases and controls, respectively) (Table 1). BCC cases were equally likely as controls to be seropositive for HPV types in genus beta (68.1%) and genus gamma (68.6%). Statistically significant case-control differences in HPV seropositivity were observed for HPV types in genus alpha and BCC (p=0.01), in genus beta and SCC (p=0.01), and in genus gamma and BCC (p=0.0002) as well as SCC (p=0.03).

Associations between sunlight related factors with BCC and SCC with adjustment for age and sex are presented in Table 2. Cutaneous sensitivity, specifically experiencing a sunburn when exposed to at least one hour of sunlight, poor tanning ability, and history of blistering sunburn were statistically significantly associated with both BCC and SCC. Cumulative sunlight exposure was associated with SCC; similar associations were not observed for BCC.

Differences in genus-specific HPV seropositivity by sun-related factors within BCC/SCC case groups and the control group are presented in Table 3. Among SCC cases, seroprevalence for HPV types in genus beta was significantly associated with a propensity to burn when exposed to sunlight (p=0.006) and inability to tan after repeated sunlight exposure (p=0.003) (Table 3). Additionally, among controls, seroprevalence for the single HPV type in genus mu was significantly associated with a propensity to burn when exposed to sunlight (p=0.02) (Table 3).

Given that cutaneous sensitivity to sunlight exposure and tanning ability were associated with HPV seropositivity, associations between these two sun-related factors and BCC/SCC were stratified by genus-specific HPV serostatus to investigate potential effect modification. Associations between propensity to sunburn and BCC/SCC were

relatively similar across categories of cutaneous HPV serostatus, with none of the interaction terms being statistically significant (Table 4). Poor tanning ability was associated with statistically significant increased risks of BCC (OR=4.71, 95% CI=2.29-9.66) and SCC (OR=15.60, 95% CI=5.40-45.1) among those who were seropositive to genus alpha HPV types, whereas more modest risks of BCC (OR=1.48, 95% CI=0.88-2.48) and SCC (OR=2.53, 95% CI=1.43-4.46) were observed among those who were seronegative to HPV types in genus alpha. Both interactions were statistically significant (p=0.02 for BCC, p=0.01 for SCC). Additionally, the association between poor tanning ability and SCC was significantly greater among genus beta HPV-seropositive individuals (OR=6.86, 95% CI=3.68-12.80) than seronegative individuals (OR=1.39, 95% CI=0.59-3.31) (p for interaction=0.001) (Table 4). No significant interactions were observed between sun-related factors and seropositivity for HPV types in genera gamma, mu or nu in relation to either BCC or SCC.

Discussion

A case-control study was conducted to investigate the potential modifying effects of cutaneous HPV seroreactivity on the associations between skin cancer risk factors and basal cell (BCC) and squamous cell (SCC) carcinomas of the skin. With the exception of cumulative sunlight exposure and BCC, all sun-related measures were associated with BCC and SCC in the current study population. The associations between poor tanning ability and BCC/SCC were significantly greater among those who were seropositive for HPV types in genus alpha, and the association between poor tanning ability and SCC was significantly greater among those who were seropositive to HPV types in genus beta.

It is hypothesized that UVR exposure may interact synergistically with cutaneous HPV in NMSC development, in which case one would expect to observe interactions

between cutaneous HPV seropositivity and sun-related factors in relation to BCC and SCC. Tanning ability, specifically poor tanning ability, was the only sun-related factor measured that demonstrated statistically significant multiplicative interactions with cutaneous HPV seropositivity in BCC and SCC in the current study population. Two population based case-control studies from New Hampshire(34, 72) investigated modifying effects of genus beta HPV seroreactivity on the associations between SCC and cutaneous sensitivity to sunlight exposure and the number of lifetime painful sunburns, but no statistically significant interactions were observed. A multi-center study(77) observed a statistically significant interaction between lighter skin photo-type and genus beta seropositivity in SCC among residents of the Netherlands. Similar observations were not observed among residents of Italy and Australia(77). Interactions between genus beta seroreactivity and the number of sunburns before age 20 and the average daily sun exposure in SCC were also investigated; no statistically significant interactions were observed among residents from any site(77). Differences across the study populations may be explained by varying levels of UVR exposure by geographic regions as well as differences in the underlying characteristics of the study populations, including age and sex. Additionally, direct comparisons between reported observations across study populations have been based on different numbers of beta-HPV types analyzed. This may explain differences in the observed interactions between sunlight related factors and genus beta HPV seroreactivity in SCC.

The proposed study has some limitations. Case-control studies are often subject to recall bias because cases tend to think about their exposures more carefully as they might relate to their current cancer diagnosis. However, the participants did not know their HPV serostatus at the time of questionnaire completion, and therefore, the interaction results should not be subject to recall bias. Sample sizes were small limiting

stratified analyses and the ability to detect statistically significant interactions. Despite the limitations of the proposed study, several strengths should also be noted. The current study presents cutaneous HPV genus-specific associations outside of genus alpha and beta in a U.S. population. It is the first study to investigate interaction effects between genus-specific HPV seropositivity and multiple measures of sunlight exposure as they relate to both BCC and SCC in a U.S. population. The use of a multiplexed assay to assess seropositivity to multiple cutaneous HPV types is a great strength of the proposed study. Dr. Pawlita's laboratory has been used in most of the sero-epidemiologic studies of cutaneous HPV published to date(29, 34-37, 78), including the two studies published from the U.S. in New Hampshire(34, 72). This allowed us to directly compare our results to those observed in New Hampshire where levels of UV radiation exposure are significantly lower compared to Florida.

UV radiation exposure is the most important environmental risk factor for NMSC, and given that the incidence of NMSC is increasing despite the increased use of sunscreen products, there is a need to identify cofactors that may interact with UV radiation exposure to increase the risk of NMSC so novel prevention strategies can be developed. Epidemiologic studies have demonstrated a potential role for cutaneous HPV infections in NMSC development(29, 30, 34, 71-73), and accumulating evidence suggests that cutaneous HPV may interact synergistically with UV radiation exposure in NMSC development. However, additional studies are needed, including those that measure infection with HPV types in multiple genera. Identifying how cutaneous HPV infections may influence sunlight-associated risks of NMSC may lead to improved characterization of high-risk individuals and aid in the development of novel prevention strategies.

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Table 3.1. Demographic and skin cancer risk factors in basal cell and squamous cell carcinoma cases and controls

Variable	Controls (n=297) n (%)	BCC (n=204) n (%)	p[1]	SCC (n=156) n (%)	p[1]
Age (years) mean (S.D.)	55.2 (11.7)	62.6 (12.0)	<.0001	64.7 (9.8)	<.0001
Age (years)					
18-39	28 (9.4)	7 (3.4)		3 (1.9)	
40-49	54 (18.2)	24 (11.8)		10 (6.4)	
50-59	104 (35.0)	42 (20.6)		28 (18.0)	
60-69	83 (28.0)	60 (29.4)		63 (40.4)	
70-80	28 (9.4)	71 (34.8)	<.0001	52 (33.3)	<.0001
Gender					
Male	111 (37.4)	123 (60.3)		100 (64.1)	
Female	186 (62.6)	81 (39.7)	<.0001	56 (35.9)	<.0001
Cutaneous sensitivity					
Mild sunburn turn to tan/tan	177 (60.0)	80 (40.0)		65 (42.8)	
Sunburn/blistering	118 (40.0)	120 (60.0)	<.0001	87 (57.2)	0.001
Tanning ability					
Tans easily	173 (59.3)	96 (48.5)		56 (36.8)	
Tan if work at it/ unable to tan	119 (40.8)	102 (51.5)	0.02	96 (63.2)	<.0001
History of blistering sunburn					
No	92 (31.3)	52 (25.7)		35 (23.0)	
Yes	202 (68.7)	150 (74.3)	0.18	117 (77.0)	0.07
Cumulative sunlight exposure					
Low	82 (32.5)	51 (28.7)		31 (22.0)	
High	170 (67.5)	127 (71.4)	0.39	110 (78.0)	0.03
Genus Alpha					
Negative	193 (65.0)	109 (53.4)		96 (61.5)	
Positive	104 (35.0)	95 (46.6)	0.01	60 (38.5)	0.47
Genus Beta					
Negative	118 (39.7)	65 (31.9)		42 (26.9)	
Positive	179 (60.3)	139 (68.1)	0.07	114 (73.1)	0.01
Genus Gamma					
Negative	142 (47.8)	64 (31.4)		58 (37.2)	
Positive	155 (52.2)	140 (68.6)	0.0002	98 (62.8)	0.03
Genus Mu					
Negative	202 (68.0)	126 (61.8)		94 (60.3)	
Positive	95 (32.0)	78 (38.2)	0.15	62 (39.7)	0.10
Genus Nu					
Negative	263 (88.6)	180 (88.2)		136 (87.2)	
Positive	34 (11.4)	24 (11.8)	0.91	20 (12.8)	0.67

[1]p-value for chi-square test

Table 3.2. Associations between sunlight related factors and basal cell and squamous cell carcinoma cases and controls

	Controls (n=297)	Basal cell carcinoma (n=204)		Squamous cell carcinoma (n=156)	
Sunlight related factor	n (%)	n (%)	OR (95% CI)[1]	n (%)	OR (95% CI)[1]
Cutaneous sensitivity					
Mild sunburn turn to tan/tan	177 (60.0)	80 (40.0)	1.00 (reference)	65 (42.8)	1.00 (reference)
Sunburn/blistering	118 (40.0)	120 (60.0)	2.75 (1.84-4.11)	87 (57.2)	2.39 (1.53-3.74)
Tanning ability					
Tans easily	173 (59.3)	96 (48.5)	1.00 (reference)	56 (36.8)	1.00 (reference)
Tan if work at it/unable to tan	119 (40.8)	102 (51.5)	2.23 (1.48-3.34)	96 (63.2)	4.09 (2.52-6.64)
History of blistering sunburn					
No	92 (31.3)	52 (25.7)	1.00 (reference)	35 (23.0)	1.00 (reference)
Yes	202 (68.7)	150 (74.3)	1.59 (1.04-2.46)	117 (77.0)	1.79 (1.08-2.96)
Cumulative sunlight exposure					
Low	82 (32.5)	51 (28.7)	1.00 (reference)	31 (22.0)	1.00 (reference)
High	170 (67.5)	127 (71.4)	1.21 (0.77-1.89)	110 (78.0)	1.85 (1.08-3.15)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and gender

Table 3.3a. Distribution of cutaneous sensitivity to sunlight exposure by genus-specific HPV seropositivity in basal cell and squamous cell carcinoma cases and controls

	% Genus-specific HPV seropositive					
	Controls (n=297)		BCC (n=204)		SCC (n=156)	
Cutaneous sensitivity	n (%[1])	p[2]	n (%[1])	p[2]	n (%[1])	p[2]
	Alpha		**Alpha**		**Alpha**	
Mild sunburn to tan/tan	60 (33.9)		37 (46.3)		24 (36.9)	
Sunburn/blistering	43 (36.4)	0.65	57 (47.5)	0.86	35 (40.2)	0.68
	Beta		**Beta**		**Beta**	
Mild sunburn to tan/tan	103 (58.2)		52 (65.0)		40 (61.5)	
Sunburn/blistering	75 (63.6)	0.36	83 (69.2)	0.54	71 (81.6)	**0.006**
	Gamma		**Gamma**		**Gamma**	
Mild sunburn to tan/tan	89 (50.3)		58 (72.5)		41 (63.1)	
Sunburn/blistering	66 (55.9)	0.34	80 (66.7)	0.38	54 (62.1)	0.90
	Mu		**Mu**		**Mu**	
Mild sunburn to tan/tan	48 (27.1)		28 (35.0)		24 (36.9)	
Sunburn/blistering	47 (39.8)	**0.02**	49 (40.8)	0.41	36 (41.4)	0.58
	Nu		**Nu**		**Nu**	
Mild sunburn to tan/tan	16 (9.0)		10 (12.5)		10 (15.4)	
Sunburn/blistering	18 (15.3)	0.10	14 (11.7)	0.86	10 (11.5)	0.48

[1]Percent includes the proportion of individuals that are HPV seropositive for types within a given genus
[2]chi-square p-value

Table 3.3b. Distribution of tanning ability to sunlight exposure by genus-specific HPV seropositivity in basal cell and squamous cell carcinoma cases and controls

	% Genus-specific HPV seropositive					
	Controls (n=297)		BCC (n=204)		SCC (n=156)	
Tanning ability	n (%[1])	p[2]	n (%[1])	p[2]	n (%[1])	p[2]
	Alpha		**Alpha**		**Alpha**	
Tans easily	67 (38.7)		41 (42.7)		19 (33.9)	
If work at it/unable to tan	35 (29.4)	0.10	52 (51.0)	0.24	40 (41.7)	0.35
	Beta		**Beta**		**Beta**	
Tans easily	107 (61.9)		64 (66.7)		33 (58.9)	
If work at it/unable to tan	69 (58.0)	0.51	71 (69.6)	0.66	78 (81.3)	**0.003**
	Gamma		**Gamma**		**Gamma**	
Tans easily	90 (52.0)		61 (63.5)		32 (57.1)	
If work at it/unable to tan	64 (53.8)	0.77	76 (74.5)	0.09	62 (64.6)	0.36
	Mu		**Mu**		**Mu**	
Tans easily	56 (32.4)		34 (35.4)		17 (30.4)	
If work at it/unable to tan	38 (31.9)	0.94	42 (41.2)	0.41	43 (44.8)	0.08
	Nu		**Nu**		**Nu**	
Tans easily	19 (11.0)		12 (12.5)		5 (8.9)	
If work at it/unable to tan	14 (11.8)	0.84	12 (11.8)	0.87	15 (15.6)	0.24

[1]Percent includes the proportion of individuals that are HPV seropositive for types within a given genus
[2]chi-square p-value

Table 3.3c. Distribution of history of blistering sunburn by genus-specific HPV seropositivity in basal cell and squamous cell carcinoma cases and controls

	% Genus-specific HPV seropositive					
	Controls (n=297)		BCC (n=204)		SCC (n=156)	
Blistering sunburn	n (%[1])	p[2]	n (%[1])	p[2]	n (%[1])	p[2]
	Alpha		**Alpha**		**Alpha**	
No	33 (35.9)		24 (46.2)		11 (31.4)	
Yes	70 (34.7)	0.84	71 (47.3)	0.88	47 (40.2)	0.35
	Beta		**Beta**		**Beta**	
No	50 (54.4)		37 (71.2)		25 (71.4)	
Yes	128 (63.4)	0.14	101 (67.3)	0.61	85 (72.7)	0.89
	Gamma		**Gamma**		**Gamma**	
No	42 (45.7)		36 (69.2)		23 (65.7)	
Yes	112 (55.5)	0.12	103 (68.7)	0.94	71 (60.7)	0.59
	Mu		**Mu**		**Mu**	
No	24 (26.1)		14 (26.9)		12 (34.3)	
Yes	69 (34.2)	0.17	64 (42.7)	0.05	49 (41.9)	0.42
	Nu		**Nu**		**Nu**	
No	10 (29.4)		4 (7.7)		4 (11.4)	
Yes	24 (11.9)	0.80	20 (13.3)	0.28	16 (13.7)	0.73

[1]Percent includes the proportion of individuals that are HPV seropositive for types within a given genus
[2]chi-square p-value

Table 3.3d. Distribution of levels of cumulative sunlight exposure by genus-specific HPV seropositivity in basal cell and squamous cell carcinoma cases and controls

	% Genus-specific HPV seropositive					
	Controls (n=297)		BCC (n=204)		SCC (n=156)	
Cumulative sun exposure	n (%[1])	p[2]	n (%[1])	p[2]	n (%[1])	p[2]
	Alpha		**Alpha**		**Alpha**	
Low	27 (32.9)		19 (37.3)		16 (51.6)	
High	50 (29.4)	0.57	63 (49.6)	0.14	37 (33.6)	0.07
	Beta		**Beta**		**Beta**	
Low	46 (56.1)		34 (28.3)		22 (71.0)	
High	101 (59.4)	0.62	86 (67.7)	0.89	84 (76.4)	0.54
	Gamma		**Gamma**		**Gamma**	
Low	37 (45.1)		35 (68.6)		22 (71.0)	
High	91 (53.5)	0.21	88 (69.3)	0.93	67 (60.9)	0.31
	Mu		**Mu**		**Mu**	
Low	32 (39.0)		21 (41.2)		11 (35.5)	
High	50 (29.4)	0.13	50 (39.8)	0.82	48 (43.6)	0.42
	Nu		**Nu**		**Nu**	
Low	11 (13.4)		8 (15.7)		2 (6.5)	
High	16 (9.4)	0.34	14 (11.0)	0.39	17 (15.5)	0.19

[1]Percent includes the proportion of individuals that are HPV seropositive for types within a given genus
[2]chi-square p-value

Table 3.4. Associations between sunlight related factors and basal cell and squamous cell carcinoma cases by genus-specific human papillomavirus serostatus

	Basal cell carcinoma (n=204)			Squamous cell carcinoma (n=156)		
	HPV serostatus			HPV serostatus		
	Positive	Negative		Positive	Negative	
Sunlight factor	OR (95% CI)[1]	OR (95% CI)[1]	p[2]	OR (95% CI)[1]	OR (95% CI)[1]	p[2]
Cutaneous sensitivity						
	Alpha			**Alpha**		
Mild sunburn turn to tan/tan	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Sunburn/blistering	2.39 (1.28-4.47)	2.94 (1.74-4.99)	0.55	2.52 (1.17-5.42)	2.32 (1.33-4.02)	0.88
	Beta			**Beta**		
Mild sunburn turn to tan/tan	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Sunburn/blistering	2.63 (1.60-4.35)	2.90 (1.48-5.69)	0.82	2.75 (1.60-4.75)	1.38 (0.60-3.18)	0.14
	Gamma			**Gamma**		
Mild sunburn turn to tan/tan	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Sunburn/blistering	2.05 (1.24-3.37)	4.68 (2.30-9.52)	0.08	1.95 (1.09-3.50)	3.02 (1.50-6.12)	0.35
	Mu			**Mu**		
Mild sunburn turn to tan/tan	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Sunburn/blistering	2.02 (1.05-3.87)	3.19 (1.90-5.36)	0.51	1.75 (0.83-3.68)	2.88 (1.62-5.13)	0.32
	Nu			**Nu**		
Mild sunburn turn to tan/tan	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Sunburn/blistering	1.23 (0.40-3.76)	3.17 (2.05-4.90)	0.12	0.90 (0.26-3.11)	2.77 (1.71-4.49)	0.10

[1]Odds ratios (OR) and 95% confidence (CI) intervals adjusted for age and gender
[2]p-value for interaction between genus specific HPV seroreactivity and sunlight related factor

Table 3.4 continued. Associations between sunlight related factors and basal cell and squamous cell carcinoma cases by genus-specific human papillomavirus serostatus

	Basal cell carcinoma (n=204)			Squamous cell carcinoma (n=156)		
	HPV serostatus			HPV serostatus		
	Positive	Negative		Positive	Negative	
Sunlight factor	OR (95% CI)[1]	OR (95% CI)[1]	p[2]	OR (95% CI)[1]	OR (95% CI)[1]	p[2]
Tanning Ability						
	Alpha			**Alpha**		
Tans easily	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Tan if work at it/unable to tan	4.71 (2.29-9.66)	1.48 (0.88-2.48)	**0.02**	15.6 (5.40-45.1)	2.53 (1.43-4.46)	**0.01**
	Beta			**Beta**		
Tans easily	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Tan if work at it/unable to tan	2.94 (1.73-4.98)	1.44 (0.75-2.78)	0.13	6.86 (3.68-12.8)	1.39 (0.59-3.31)	**0.001**
	Gamma			**Gamma**		
Tans easily	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Tan if work at it/unable to tan	2.50 (1.49-4.20)	1.67 (0.85-3.29)	0.30	4.42 (2.33-8.38)	3.65 (1.72-7.76)	0.61
	Mu			**Mu**		
Tans easily	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Tan if work at it/unable to tan	2.52 (1.28-4.95)	2.10 (1.25-3.54)	0.37	6.08 (2.59-14.3)	3.29 (1.80-5.98)	0.19
	Nu			**Nu**		
Tans easily	1.00 (reference)	1.00 (reference)		1.00 (reference)	1.00 (reference)	
Tan if work at it/unable to tan	2.16 (0.65-7.21)	2.22 (1.44-3.42)	0.84	8.58 (1.83-40.3)	3.76 (2.25-6.29)	0.33

[1]Odds ratios (OR) and 95% confidence (CI) intervals adjusted for age and gender

[2]p-value for interaction between genus specific HPV seroreactivity and sunlight related factor

CHAPTER 4:

CONCLUSIONS AND FUTURE DIRECTIONS

Non-melanoma skin cancer (NMSC), comprised of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), is the most frequently occurring cancer among U.S. men and women. Exposure to ultraviolet (UV) radiation is an established risk factor for NMSC, but despite the current knowledge about the harm of sunlight exposure and increased use of sunscreen, NMSC incidence rates continue to increase, emphasizing the critical need to better understand the role of sunscreen use in preventing NMSC and differences in sunlight exposure response relationships for BCC and SCC. Furthermore, it's important to identify additional risk factors for NMSC that may better characterize individuals at high risk and aid in the development of novel prevention strategies. A case-control study was conducted to investigate sunscreen use and to identify differences in the exposure response relationship between measures of patterns and timing of sunlight exposure and BCC and SCC. We also investigated the potential modifying effects of cutaneous HPV seroreactivity on the associations between sunlight exposure and NMSC.

Unlike previously published studies, we investigated multiple measures of sunlight exposure in BCC and SCC simultaneously and observed similar patterns of sunlight exposure to be associated with BCC and SCC risk. Specifically, history of blistering sunburn (a marker of intermittent sunlight exposure) and occupational sunlight

exposure (i.e. having a job in the sun for ≥3 months for >10 years) were both associated with BCC and SCC. The major differences in patterns of sunlight exposure between BCC and SCC were observed for sunlight exposure in one's thirties when adjusting for skin susceptibility factors. Additionally, sunlight exposure in one's twenties was associated with SCC, regardless of pattern of exposure; similar associations were not observed for BCC. Measures of timing of sunlight exposure consistently demonstrated that childhood/adolescent sunlight exposure was statistically significantly more important for SCC than BCC. Specifically, having ≥10 moles on one's forearms and entire body (a marker of increased childhood sunlight exposure), younger age at blistering sunburn and tanning bed use were associated with SCC. Among BCC cases, the only statistically significant association observed was for younger age at blistering sunburn. However, despite differences in statistical significance in sun-related factors between BCC and SCC, case-only analyses demonstrated that the observed ORs were not significantly different in magnitude between BCC and SCC for measures of patterns and timing of sunlight exposure. This additional information supports the observation that patterns of sunlight exposure are more similar than different between BCC and SCC.

It has been hypothesized that intermittent patterns of sunlight exposure and exposure in childhood are important for BCC while continuous, lifelong sunlight exposure is import for SCC. However, the current study did not support clear differences in the exposure response relationships between patterns or timing of sunlight exposure for BCC and SCC. Understanding how sunlight exposure response differs for BCC and SCC is important for better educating the public in sunlight safe behaviors. Simply advising a reduction in sunlight exposure will not help reduce the incidence of NMSC if changes in sunlight exposure patterns are related to skin cancer development. For example, applying sunscreen while on vacation may decrease BCC risk associated with

intermittent sunlight exposure, but may not impact the risk of SCC, which may be more strongly related with continuous sunlight exposure. Further studies are needed to highlight differences in the exposure-response relationship of patterns and timing of sunlight exposure with BCC and SCC. Furthermore, standardized methods for measuring sunlight exposure should be established to enable comparisons across different study populations.

Despite not observing clear differences in patterns and timing of sunlight exposure between BCC and SCC, measures of sunlight related factor were associated with BCC and SCC in our study population. As mentioned above, incidence rates of BCC and SCC continue to rise each year in the U.S. despite the growing knowledge of the harm caused by UVR exposure and increasing use of sunscreen products and other sun safe behaviors. Therefore, there is a need to identify potential co-factors in the relationship between UVR and NMSC, such as skin sensitivity to sunlight exposure and cutaneous HPV infection.

If sunscreen use has the potential to protect against skin cancer development, we would expect to observe a reduced risk in BCC and SCC among individuals that frequently use sunscreen products when exposed to UV radiation. We investigated the association between self-reported sunscreen use with sun protection factor (SPF) ≥15 and NMSC stratified by skin sensitivity to 1 hour of sunlight exposure in the mid-day sun and tanning ability after repeated sunlight exposure (see Appendix 2). The study had insufficient power to conduct stratified analyses and therefore it's difficult to make inferences based on the observed results. However, despite this limitation, evidence from the study (Appendix 2) suggests that skin reaction to sunlight exposure may modify the associations between sunscreen use and NMSC.

Previous studies have demonstrated that cutaneous HPV infection may be associated with NMSC, especially SCC. It has also been hypothesized that UVR and cutaneous HPV may interact in a synergistic manner in NMSC development. Within the same case-control study, potential modifying effects of cutaneous HPV seroreactivity on the associations between sunlight related skin cancer risk factors and BCC and SCC were investigated. Specifically, interactions were tested between cutaneous sensitivity to sunlight exposure, tanning ability, history of blistering sunburn, and cumulative sunlight exposure and seroreactivity to cutaneous HPV types in genera alpha, beta, gamma, mu, and nu.

As expected, the sunlight related skin cancer risk factors listed above were associated with an increased risk for both BCC and SCC in our study population. NMSC cases were more likely to be seropositive for cutaneous HPV antibodies compared to controls and individuals with sun sensitive skin (i.e. tendency to burn) were more likely to be seropositive for HPV compared to those with a tendency to tan. Additionally, cutaneous HPV seroreactivity modified the effects between sunlight related factors and NMSC. Specifically, propensity to sunburn was more strongly associated with BCC among individuals that were seronegative for genus gamma HPV types. Poor tanning ability was more strongly associated with both BCC and SCC among individuals seropositive for HPV types in genera alpha and beta.

The proposed study has some limitations. Case-control studies are often subject to recall bias because cases tend to think about their exposures more carefully as they might relate to their current cancer diagnosis. Sample sizes for stratified analyses were limited, reducing poser to detect statistically significant interactions. Despite the limitations, several strengths should also be noted. The current study was the first case-control study to formally evaluate measures of patterns and timing of sunlight exposure

in NMSC in a high risk U.S. population as well as present findings simultaneously for both skin cancer types, BCC and SCC. This presentation allowed for direct comparisons of patterns and timing of sunlight by skin cancer type. Additionally, the current study presents cutaneous HPV genus-specific associations outside of genus alpha and beta in a U.S. population. It was also the first study to investigate interaction effects between genus-specific HPV seropositivity and multiple measures of sunlight exposure as they relate to both BCC and SCC in a U.S. population. In addition, the measurement of antibodies to HPV was not subject to problems with recall bias. The use of Dr. Pawlita's assay to test for seropositivity to all identified cutaneous HPV types is a great strength of the proposed study. Dr. Pawlita's laboratory has been used in most of the seroepidemiologic studies of cutaneous HPV published to date(29, 34-37, 78), including the two studies published from the U.S. in New Hampshire(34, 72). This will allow us to directly compare our results to those observed in New Hampshire where levels of UV radiation exposure are significantly lower compared to Florida.

UV radiation exposure remains the most important environmental risk factor for NMSC despite the increased use of sunscreen products as well as increasing knowledge of the harms of sunlight exposure. The annual incidence of NMSC continues to rise each year in the U.S., therefore creating a need to better understand the mechanisms of this complex relationship as well as to identify cofactors that may interact with UV radiation exposure to increase the risk of NMSC so novel prevention strategies can be developed. Clear differences in measures of patterns and timing of sunlight exposure between BCC and SCC were not observed in our study population. However, there is a need to assess multiple factors when studying sunlight related risk factors in skin cancer. More qualitative research studies need to be conducted to better understand how constitutional factors, as well as cumulative ambient solar radiation,

recreational activities, clothing worn when exposed to UVR, and geographic residence all influence the frequency of sunscreen use and its potential protective effects in NMSC. Additionally, knowledge of how sunscreen use may relate to patterns and timing of sunlight exposure is important for educating the public on better sun safe behaviors. We observed an interaction between poor tanning ability and genus-specific HPV seroreactivity. However, the precise relationship between one's skin reaction to sunlight exposure and cutaneous HPV infection as they related to NMSC development require further investigation. Evidence in the published literature investigating the association between cutaneous HPV and NMSC is limited and more epidemiologic studies are needed to better understand the association between UV radiation exposure and cutaneous HPV infection as they relate to NMSC development. A majority of the studies investigating the association between cutaneous HPV seropositivity and NMSC only included cutaneous HPV types from genus beta and their associations with SCC. Additional research studies are need to identify how differences in sunscreen use, sunlight exposure, and cutaneous HPV infections influence the development of BCC and SCC with the intent of better characterizing individuals at high risk. This information is pertinent in developing novel prevention strategies to reduce the incidence and burden of NMSC.

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APPENDICES

Appendix 1: Scientific Literature Review

1. BACKGROUND AND PUBLIC HEALTH SIGNIFICANCE

1. a. Epidemiology of non-melanoma skin cancer

Descriptive epidemiology of non-melanoma skin cancer

Non-melanoma skin cancer (NMSC), comprised of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), is the most common cancer in Caucasians, with more than one million new cases diagnosed annually in the United States alone(1). The occurrence of BCC is four times more common than SCC, accounts for 75-80% of skin cancers, and rarely metastasizes to other organs(12, 79). In white populations in the U.S., the annual incidence of BCC increases by more than 10% each year, and the estimated lifetime risk is 28-33%(12, 79). SCC accounts for 20% of skin cancers, and its incidence increases by approximately 3-10% per year. SCC has the rare potential to spread to the lymph nodes and other organs leading to an increased risk of death among SCC patients compared to BCC patients(12, 80). The lifetime risk of developing SCC among fair-skinned persons in the US is 7-11% *(9-14% in men and 4-9% in women)*(12). Additionally, NMSC is more common among males compared to females with a ratio of 2 to 1 for BCC and 3 to 1 for SCC. In 1994 it was estimated by Miller and Weinstock that NMSC accounts for 1300 to 2300 deaths per year, mostly from metastatic SCC(54). While the mortality associated with NMSC is low(2), patients with multiple NMSC's may experience substantial morbidity, and treatment costs for NMSC are high at the national level. In 1995, treatment for NMSC and its precursors accounted

for $852 million in Medicare costs, equivalent to 90% of the costs associated with breast cancer treatment(55). Furthermore, a history of NMSC has been consistently associated with increased risk of subsequent primary cancers of other sites in studies from both the U.S. and Europe(3-11).

Risk factors for non-melanoma skin cancer

Identified risk factors for BCC and SCC include older age, male sex, light eye (blue, green, or hazel), hair (red or blonde), and skin (fair) color, and immunosuppression(12). Organ transplant recipients have a 50 to 100 fold increased risk of NMSC compared to the general population(81, 82). In organ transplant recipients SCC occurs more frequently than BCC (4:1) and has a higher incidence of metastasis compared to the general population(80). In addition, long-term use of systematic glucocorticoids, a type of immunosuppressive therapy, has been shown to increase the risk of developing both SCC and BCC(83). Rare genetic disorders are also associated with NMSC, including Epidermodysplasia Verruciformis (EV), which is characterized by multiple flat warts and macular skin lesions that often progress to SCC(84). Lifestyle factors such as smoking have also been proposed as risk factors for NMSC, mainly SCC, although findings are inconsistent across studies(13-28).

Ultraviolet radiation (UVR) exposure has been implicated in the etiology of skin cancer and is considered the most important environmental risk for both BCC and SCC development. However, the precise relationship between UVR and the risk of NMSC is complex, and the relationship may differ by skin cancer type. Evidence from previous studies suggest that intermittent sunlight exposure is important for the pathogenesis of BCC, whereas cumulative sunlight exposure is important for SCC, but the exact relationship between the amount, patterns and timing of UVR exposure and risk of BCC

and SCC still remain unclear. In addition to sunlight exposure, epidemiologic studies have demonstrated that cutaneous human papillomavirus (HPV) infection may be a risk factor for developing NMSC. However, the pathway by which cutaneous HPV is associated with NMSC remains unclear. It is hypothesized that UVR exposure may interact synergistically with cutaneous HPV in NMSC development.

1. b. Ultraviolet radiation exposure in non-melanoma skin cancer

Ultraviolet-A (UVA) and ultraviolet-B (UVB) radiation are responsible for causing DNA damage to the skin, leading to all types of skin cancer. When using epidemiologic approaches to study the relationships between ultraviolet radiation (UVR) and NMSC, it is difficult to separate the effects of UVA versus UVB exposure. Therefore, epidemiologic studies tend to identify UVR as "sunlight" exposure as a whole. Several lines of evidence from epidemiologic studies support the association between sunlight exposure and NMSC. This evidence includes higher NMSC incidence among: 1) persons living in geographic areas with higher ambient solar radiation; 2) persons with sun sensitive skin (fair-skinned); 3) frequently sun exposed anatomical sites; 4) persons experiencing frequent sun exposure and 5) persons with other sun related skin conditions. In addition, incidence rates are lower among persons who practice sun safe behaviors (e.g. sunscreen use).(85) However, despite epidemiologic and molecular evidence supporting the causal relationship between sunlight exposure and NMSC, the exact biological mechanism underlying the association remains unclear. The way in which the amount, pattern, and timing of sunlight exposure effects skin cancer development still remains to be answered.

1. c. Sunscreen use as prevention for basal cell and squamous cell carcinoma

As mentioned previously, a majority of epidemiologic studies measure exposure to “sunlight” (UVR as a whole) and are unable to distinguish different components of the UV spectrum responsible for the induction and promotion of BCC or SCC. Sunscreens were originally formulated to protect against UV-induced sunburns, thought to be caused mostly by UVB radiation. Laboratory studies in rodents revealed that sunscreens had the potential to reduce UV-induced skin cancer, such as SCC(86). Animal models have not supported similar findings for BCC or melanoma. However, despite the lack of evidence that sunscreens can protect against BCC or melanoma occurrence, sunscreen products are advocated for the prevention of all types of skin cancers. Currently available sunscreens provide broad spectrum coverage (UVA and UVB). However, observational studies have found sunscreen use to be associated with increased risk for BCC and an increased incidence of melanocytic nevi among children and adolescents. Randomized controlled trials demonstrated that sunscreens had the ability to reduce the occurrence of solar keratoses (precursors for SCC) and of SCC, however, no effect was observed for BCC(87-89).

Thompson et al. conducted a trial among residents from Victoria, Australia of at least 40 years of age with a history of sun-induced skin damage(87). Subjects either applied a sunscreen cream or a base cream (placebo) that did not contain any active ingredients of the sunscreen. Results from the trial showed the ability of daily sunscreen use to reduce the rate of new solar keratosis by 40% (RR=0.62; 95% CI=0.54-0.71), compared to using the placebo. Additionally, the average remission of solar keratosis was 28% and 20% for the sunscreen group and base cream group, respectively, and the estimated likelihood of remission of lesions present at baseline was greater in the sunscreen group compared to the placebo group (OR=1.53; 95% CI=1.29-1.80).

The Nambour Skin Cancer Prevention Trial began in 1992 among a random selection of residents living in Nambour, a township of Queensland, Australia, who were ages 20 - 69 in 1986. The trial aimed to test the effectiveness of regular sunscreen use on the head, neck, hands, and forearms to prevent against SCC and BCC occurrence. Among participants being followed between 1992 and 1996, a reduction in SCC incidence was observed for daily sunscreen use, compared to no sunscreen use, among individual participants (RR=0.88; 95% CI=0.50-1.56), as well as for the overall number of SCC tumors (RR=0.61; 95% CI=0.46-1.81) that developed during the four year study follow-up period(88). However, these differences were not statistically significant. No reduction of BCC was associated with daily sunscreen use among this study population. Van der Pols and colleagues continued to follow participants from the Nambour Trial for eight years after its completion to test the potential latent effect of sunscreen use to prevent BCC and SCC(89). A reduced rate of SCC occurrence was observed among daily sunscreen users (RR=0.62; 95% CI=0.43-0.98), compared to no daily sunscreen use, but a similar reduction in BCC incidence was not observed (RR=1.02; 95% CI=0.75-1.37). Additionally, daily sunscreen use was associated with the development of fewer skin cancer tumors compared to the number of tumors for no daily sunscreen use. However, this association was stronger for SCC (RR=0.62; 95% CI=0.38-0.99) than BCC (RR=0.89; 95% CI=0.64-1.25). The Nambour Trial was conducted in Queensland, Australia, in a population with both high ambient solar radiation year round and the highest incidence of skin cancer worldwide.

In contrast to evidence provided by randomized controlled trials, observational studies have not demonstrated protective effects of sunscreen use against either type of NMSC. However, a majority of the published studies focused on the effects for BCC only. Two cohort studies observed increased risk of BCC and SCC associated with

sunscreen use. Results from a cohort study of female registered nurses 30 to 55 years of age living in the United States concluded that sunscreen use while outdoors was related to an increased risk of BCC(19). More specifically, a statistically significant decreased risk was observed for BCC among participants that reported *not using sunscreen* at baseline (RR=0.70; 95% CI=0.60-0.82) compared to *usually using sunscreen* when outdoor in the summer for at least 8 hours per week. Among actinically damaged individuals, ages 21 - 85 years, living in Arizona, sunscreen use was associated with increased risk for BCC and SCC, though none of the associations were statistically significant(68). Compared to never use at baseline, using sunscreen more than half of the time over the five year follow-up period was associated with RRs of 1.14 (95% CI=0.67-1.95) and 1.23 (95% CI=0.66-2.29) for BCC and SCC, respectively. Participants reporting always using sunscreen had RRs of 1.55 (95% CI=0.94-2.54) and 1.42 (0.79-2.55) for BCC and SCC, respectively, compared to not using sunscreen during study follow-up.

Results from case-control studies of the associations between sunscreen use and NMSC have also been inconclusive. A study conducted among U.S. women from Nashville, Tennessee between 20 and 40 years of age showed no association between sunscreen use and BCC (p=0.563)(14). A dermatological hospital based case-control study from Italy showed a 40% reduced likelihood of BCC among those using sunscreen always or often versus never (OR=0.6; 95% CI=0.3-1.4), but this association was not statistically significant(16). In contrast, use of sunscreen sometimes or rarely, compared to never, showed an increased likelihood for BCC (OR=1.2; 95% CI=0.6-2.7), but once again this association was not statistically significant. Among participants from the Geraldton Skin Cancer Prevention Survey, the use of sunscreen (on the site of BCC diagnosis) with a SPF of at least 10 half of the time or more compared to never or less

than half of the time when sun exposed was associated with an increased risk of BCC: the ORs for 1 - 9 and ≥10 years of sunscreen use half of the time or more were 1.92 (95% CI=1.17-3.13) and 1.25 (95% CI=0.82-1.90), respectively, compared to using sunscreen never or less than half of the time when sun exposed(52). Additionally, using sunscreen with a minimum 10 SPF half of the time or more compared to never or less than half of the time 1 to 9 years prior to diagnosis was associated with an almost 80% increased likelihood for BCC (OR=1.77; 95% CI=1.09-2.87). Among the same study population from Western Australia, English and colleagues observed no statistically significant associations for any use of SPF 10 compared to no use and SCC for any age interval: 8-14 (OR=0.61; 95% CI=0.08-4.7), 15-19 (OR=1.9; 95% CI=0.82-4.4), and 20-24 (OR=0.99; 95% CI=0.44-2.2) years(53).

The difficulty in demonstrating a protective effect of sunscreen use on BCC and SCC from observational studies may be explained by prolonged sun exposure with sunscreen use, and therefore an increase in UV-induced skin damage and/or sunburn occurrence, when sunscreens are used. Additionally, the increased risk of BCC or SCC associated with using protective measures in the recent past may be due to high risk individuals adopting protective behaviors. Therefore, sunscreen use for prevention against skin cancer remains a controversial topic as NMSC rates continue to rise despite increased sales and use of sunscreen products.

1. d. Patterns and Timing of sun exposure in basal cell and squamous cell carcinoma

Beginning in the late 1950s, researchers began to conduct case-control studies to identify risk factors for NMSC, including total (cumulative) outdoor sun exposure hours and sun exposure on working and non-working days(19, 47-49). These studies

demonstrated that BCC and SCC have different exposure-response relationships with sun exposure. However, few epidemiologic studies have formally evaluated the relationship between patterns and timing of sun exposure in BCC and SCC. Patterns of exposure refer to whether sun exposure was experienced continuously (chronic exposure) or sporadically (intermittent exposure). For example, persons working outdoors, such as farmers, or living in geographic regions with a high annual UV index, such as Florida, are classified as having had chronic sun exposure. Alternatively, intermittent sun exposure refers to persons working indoors and experiencing most of their sun exposure on the weekends or persons living in northern latitudes with a low UV index being exposed to high doses of sun exposure while on vacation to regions with high UV index. Continuous or chronic sun exposure has been observed to be associated with the development of SCC, whereas intermittent sun exposure has been observed to be associated with BCC. Timing of sun exposure refers to what period in life the majority of a person's sun exposure was received, in early childhood, adulthood or both. Others have speculated that a high level of sunlight exposure in childhood is more strongly associated with SCC while exposure in adulthood is more strongly associated with BCC.

1. e. Patterns of sun exposure in basal cell and squamous cell carcinoma

In 1990, Vitasa and colleagues published results from a case-control study investigating the relationship between UVB radiation and SCC, BCC, and actinic keratosis (AK) among white, male watermen of at least 30 years of age residing in the Eastern Shore or Maryland(49). It was observed that subjects with SCC and AK experienced higher annual UVB radiation exposure (11% and 8%, respectively) compared to their age matched controls, while BCC cases had about 8% less UVB radiation exposure compared to their age matched controls between 15 and 60 years of

age. Participants whose cumulative UVB radiation exposure exceeded the third quartile were more than two times likely to have SCC (OR=2.53, 95% CI=1.18-5.40) compared to those with lower UVB exposure. However, no statistically significant associations were observed for BCC (OR=1.11, 95% CI=0.50-2.44) or AK (OR=1.48, 95% CI=0.99-2.22).

One of the first studies to formally evaluate the association between patterns and timing of sun exposure and NMSC was a population-based nested case-control study by Kricker and colleagues(52), among residents of Western Australian between 40 and 64 years of age from the Geraldton Skin Cancer Prevention Survey(90). The nested-case control study aimed to investigate the association between intermittent sun exposure and BCC development(52). The primary measure of intermittent sun exposure was estimated by the amount of sun exposure experienced on "*non-working*" days relative to the amount experienced during the rest of the week. "Sun exposure on non-working days was considered to be potentially intermittent only if subjects reported 2 days or less per week of non-working time"(52). Additionally, markers of intermittent sun exposure included participation in outdoor recreational activities, sun exposure received on holidays, and history of painful and blistering sunburns. An odds ratio of 3.86 (95%CI=1.93 – 7.75) was observed for 100% intermittency of sun exposure and an odds ratio of 1.82 (95% CI=1.01-3.28) was observed for 59-99% intermittency of sun exposure compared to 0-40% intermittency of sun exposure in late teenage (15-19 years) for risk of BCC. BCC was also positively associated with increasing intermittency of sun exposure among participants 15-19 years old (p for trend = 0.001). Similar patterns of intermittent sun exposure were not observed for participants in the 20-24 or 25-39 year old groups. Kricker et al. also assessed intermittent sun exposure in the ten years prior to diagnosis and an increased risk for BCC was positively associated with 25-49%

(OR=1.75; 95% CI=1.15-2.66) and 50-99% (OR=2.10; 95% CI=1.25-3.54) intermittency. An odds ratio of 1.22 was observed for 100% intermittency in the 10 years prior to BCC diagnosis but was not statistically significant. Increasing hours of sun exposure between 9am and 5pm on the site of skin cancer diagnosis during holiday (vacation) was associated with increasing risk of BCC, especially at 15 – 19 years of age (p for trend = 0.01). No associations between outdoor recreational activities and risk of BCC were observed among this study population.

In 1998, the same research group conducted a nested case-control study of patterns and timing of sun exposure with risk for SCC(53), using the same study population based on the Geradton Skin Cancer Prevention Survey(90), of residents of Western Australia ages 40 - 64 years. Similar to the investigations for BCC, the research group aimed to quantify the relationship between the risk of SCC by the amount and pattern of sun exposure(53). The *amount* of sun exposure was examined by estimating the total lifetime ambient solar irradiance experienced. The amount was calculated by measuring the average daily global UV radiance and the average daily hours of bright sunlight over the participant's lifetime residential history. The highest odds ratio observed for accumulated (*lifetime*) hours of bright sunshine and SCC was 5.2 (95% CI=1.6-16) for the **second** highest category of exposure (150,700 to 170,499 hours), whereas a lower odds ratio of 3.5 (95% CI=0.97-12) was observed for the highest category of exposure (170, 500 plus hours). Additionally, a lower *average* daily global UV radiance was significantly associated with a 2 fold increased risk for SCC (OR=1.9, 95% CI=1.1-3.4), however, lower insignificant risks were observed for higher levels of daily UV radiance.

English and colleagues(53) also assessed the effect of *patterns* (continuous vs. intermittent) of sunlight and SCC by three methods: *first*, by analyzing sunlight exposure

on working and non-working days; *second*, by calculating sunlight exposure on non-working days for the site of SCC diagnosis; and *third* by examining history of sunburn, sunbathing, vacations and outdoor leisure activities. To assess an intermittent pattern of sun exposure the investigators measured the amount of sun exposure to the site of SCC diagnosis on non-working days across age intervals from 15 to 39 years of age. For anatomic sites usually exposed to sunlight on **working days**, no statistically significant observations with SCC were observed for any category of sun exposure hours, but the most elevated odds ratio was observed for the intermediate (OR=1.7, 95% CI=0.81-3.8), not the highest (OR=1.3, 95% CI=0.58-2.8) category of sun exposure. For sun exposure on a usually exposed anatomic site during **non-working days**, a decreasing trend in the magnitude of the odds ratios for SCC was observed from the lowest (OR=2.0, 95% CI=0.89-4.4) to highest (OR=1.3, 95% CI=0.57-2.9) levels of sun exposure. A continuous effect of sun exposure on working days and SCC was investigated by examining exposure on working days across specific age intervals. The strongest association between the amount of sun exposure on working days and SCC was observed for individuals 15-19 years of age (OR=2.2 for 22 hours per week of sun exposure). The authors report that the maximum odds ratios for hours of sun exposure on working days and SCC were lower for the other age groups but the corresponding estimates were not reported. With the exception of frequent gardening (OR=1.8; 95% CI=1.0-3.2) and field sports (OR=1.7; 95% CI=1.1-2.8), no associations were observed between outdoor recreational activities and SCC. Additionally, no associations were observed between lifetime frequency of sunbathing or number of hours of exposure to the site of SCC on holidays (vacations), when site of diagnosis was sun exposed.

From 1989 to 1993, Rosso and colleagues undertook a case-control study to investigate the potential risk factors, including hours of sun exposure during different

activities and time periods, for BCC and SCC among south European populations ranging in age from 20 to 70 years old(51). Investigators calculated lifetime hours of sun exposure based upon duration and type of activity, as well as period of life (childhood, adolescence, adulthood, retirement) and sun exposed body parts. Sun irradiation was considered by taking into account the season individuals participated in the activities (i.e. outdoor work, sports and recreational activities, and holidays). Associations of BCC and SCC with lifetime sun exposure were estimated for outdoor work, outdoor sports and holidays. With less than 7200 hours of lifetime sun exposure as the reference group, no statistically significant associations were observed between any quartile of lifetime sun exposure during outdoor work and BCC or SCC. However, statistically significant linear trends with SCC for increasing lifetime sun exposure hours were observed for all participants (including unexposed, i.e. <7200 sun exposed hours) (p=0.029) and among exposed participants only (i.e. >7200 sun exposed hours) (p=0.008). Similar trends were not observed for BCC.

Similar to results observed among participants from the Geraldton Skin Cancer Prevention Survey(52, 53), sun exposure during holidays showed statistically significant associations with BCC, but not with SCC. Specifically, sun exposure hours in the second (280-1323 hours) and fourth (>3398 hours) quartiles were associated with ORs of 1.26 (CI=1.01-1.56) and 1.47 (CI=1.18-1.83), respectively, for BCC compared to never experiencing sun exposure on a holiday. Additionally, statistically significant linear trends for increasing sun exposure hours on a holiday and BCC was also observed (p=0.036). Though sun exposure during holidays was not associated with SCC across any of the quartiles, a linear trend (with borderline significance) for increasing sun exposure was observed for SCC (p=0.047). The association between BCC and sun exposure during holidays was reinforced by restricting analyses to include beach

holidays only. Similar to previous observations, statistically significant elevated odds ratios between BCC and lifetime sun exposed hours were observed for the second (OR=1.25, 95% CI=1.00-1.54; for 184 to 831 hours) and fourth (OR=1.58, 95% CI=1.27-1.96; for ≥2464 hours) quartiles of sun exposure hours. In contrast, lifetime sun exposure in the second (184 to 831 hours) and third (832 to 2464 hours) quartiles on a beach holiday appeared to be protective for SCC, with ORs of 0.59 (95% CI=0.36-0.96) and 0.47 (95% CI=0.27-0.80), respectively. However, a linear trend for increasing number of sun exposed hours was associated with BCC (p<.001), but not with SCC (0.128), for holidays at a beach. Associations between lifetime sun exposure during outdoor sports and BCC and SCC varied by activity. Taking into account all outdoor sport activities, no statistically significant associations or trends were observed for either BCC or SCC. However, when stratified by type of outdoor activity, the number of hours spent outdoors participating in water sports (for the second, third, and fourth quartiles of exposure) was associated with BCC (p for trend = <0.001), but not SCC (p for trend = 0.567), while sun exposure during sports in the mountains or air were not associated with either BCC or SCC.

1. f. Sunburn and basal cell and squamous cell carcinoma

Epidemiologic studies investigating risk factors for NMSC have reported associations with history/frequency of blistering and/or painful sunburns with mixed results. In the published literature, history of blistering sunburn is regarded as a marker of intermittent sun exposure and consequently is hypothesized to be associated with BCC. However, evidence from the literature supports a stronger relationship between a history of blistering sunburn with SCC than BCC. For example, two case-control studies from the Geraldton Skin Cancer Prevention survey of Western Australian residents investigated independent associations of blistering sunburns and painful sunburns with

BCC and SCC(52, 53). Kricker and colleagues(52) observed that 3 to 10 **painful** sunburns at the site of diagnosis was associated with an almost 2 fold increased likelihood of BCC (OR=1.75; 95% CI=1.08-2.85) compared to those with no history of sunburn. Smaller effects were observed for the frequency of **blistering** sunburn at the site of BCC diagnosis and did not reach statistical significance. Specifically, the OR for 1 to 2 blistering burns was 1.6 (95% CI=0.92-2.79) and 3 or more blistering sunburns was 1.24 (95% CI=0.69-2.24) compared to never experiencing a sunburn(52). English and colleagues(53) observed that a history of **blistering** sunburn to the site of SCC diagnosis was more strongly associated with SCC compared to a history of **painful** sunburns only. For example, an odds ratio of 2.1 was observed for either 1 to 2 (95% CI=1.0-4.6) or 3 + (95% CI=1.0-4.3) **blistering** burns compared to none, while a history of only a **painful** sunburn compared to none showed no association with SCC (OR=1.1; 95% CI=0.67-1.8)(53). A case-control study of young women from the U.S.(14) reported no association between the average number of blistering sunburns and BCC compared to women without a similar history (p=0.06). A nested case-control study of participants from the Nurses Health Study of registered female nurses from the U.S.(67) observed statistically significant associations between increasing numbers of severe sunburns that blistered and SCC (p=0.04) but similar associations were not observed for BCC (p=0.15). Among actinically damaged adults from the U.S.(68) participating in a chemoprevention trial, having a history of severe sunburns that blistered compared to no sunburn at baseline was not significantly associated with BCC (RR=1.26; 95% CI=0.90-1.77) or SCC (RR=0.86; 95% CI=0.58-1.27).

Four additional studies in the published literature investigated the associations between severe and/or painful sunburns with BCC and SCC. Results from a case-control study conducted in Saskatchewan, Canada(18), showed that having a history of

severe sunburns was significantly associated with SCC compared to individuals that never experienced a severe sunburn (p=0.001). Among participants from the Helios I study(91), a multi-center case-control study of southern Europe, a dose-response association was observed with BCC for increasing number of lifetime sunburns compared to never experiencing a sunburn (p=0.03). However, similar associations were not observed with SCC (p=0.53). Similarly, a prospective cohort study of men health professionals from the U.S.(27) observed statistically significant increasing risks for BCC with an increasing number of sunburns compared to no sunburns over a lifetime (p=<.0001). A prospective cohort study of U.S. women(19) also demonstrated increasing risk for BCC with an increasing number of lifetime sunburns compared to no sunburns (p=<.001).

1. g. Tanning bed use and basal cell and squamous cell carcinoma

Six studies from the published literature report on the association between tanning bed use and BCC and SCC. Four of the six case-control studies(14, 16, 67, 92) reported no association between ever versus never using a sunlamp or tanning bed and BCC or SCC, including studies from Canada, the U.S. and Italy. However, Aubry and colleagues(13) conducted a hospital based case-control study of SCC in Montreal, Canada and observed a statistically significant association between ever versus never use of a sunlamp and SCC (OR=13.42; 95% CI=1.38-130.48). A population based study from New Hampshire (NH)(93) observed statistically significant associations between ever versus never using a tanning device for both BCC (OR=1.5; 95% CI=1.1-2.1) and SCC (OR=2.5; 95% CI=1.7-3.8). Additionally, compared to never users, age at first tanning bed use (less than 20 years old) and time since last tanning bed use (greater than 20 years) was significantly associated with BCC and SCC among the participants from the NH study.

1. h. Timing of sun exposure in basal cell and squamous cell carcinoma

As mentioned previously, increasing levels of intermittent sun exposure and increasing hours of sun exposure during holidays (vacation) in late teenage years (15 to 19) was associated with BCC among participants from the Geraldton Skin Cancer Prevention Survey in Western Australia(52). Among the same study population, statistically significant associations were observed between total site specific sun exposure and SCC for age intervals 8 to 14 (OR=5.1), 15 to 19 (OR=3.8), and 20 to 24 (OR=2.4) years(53). For age intervals 25 to 34 and 35 to 39 years, no statistically significant associations were observed between total sun exposure and SCC.

Several epidemiologic studies in the published literature investigated the associations between age (timing) of sunburn and risk for BCC and SCC. Results from the Leiden Skin Cancer Study(94), a case-control study from Sweden, demonstrated that compared to no history of painful sunburns, experiencing painful sunburn and any age prior to 13 years of age was significantly associated with BCC. However, for SCC, the only significant association observed was among participants reporting painful sunburns between 6 and 12 years of age. A hospital based case-control study from Italy(16) did not observe statistically significant associations between history of sunburn before or after 20 years of age, compared to no history of sunburn, and BCC. However, the mean number of weeks per year spent at the beach for summer holidays was significantly associated with BCC. A dose-response relationship was observed for spending 3 to 4 (OR=1.8; 95% CI=0.8-4.4), 5 to 8 (OR=3.7; 1.5-9.0) or more than 8 (OR=4.5; 95% CI=1.9-10.5) weeks, compared to 0 to 2 weeks per year (p for trend = 0.01) at the beach for summer holidays before the age of 20 years. The number of weeks per year spent at the beach after the age of 20 years was not significantly associated with BCC. A case-control study from Spain of SCC of the lip in men(24)

observed a significant protective effect for experiencing first sunburn after 15 years of age (OR=0.1; 95% CI=0.003-0.6) compared to no history of sunburns. An OR of 14.6 was observed for lip SCC among males experiencing sunburn prior to 15 years of age; however, this association was not statistically significant. Results from the Helios I study(91), a multi-center case-control study of southern Europe, demonstrated a statistically significant positive association between age at first sunburn and BCC, but not SCC. Individuals experiencing their first sunburn younger than 15 years of age were more likely to have BCC compared to individuals that reported experiencing their first sunburn after 15 years of age or never at all (OR=1.68, CI=1.17-2.39). Results from the Helios II study(51) demonstrated that the amount of sun exposure experienced in childhood during a holiday at the beach was associated with BCC, but not SCC. The highest quartile of lifetime sun exposed hours (>2079 hours) during a beach holiday was associated with an OR of 1.43 (CI=1.09-1.89) compared to individuals never experiencing beach holiday. No statistically significant observations were observed for SCC across any quartile of sun exposure hours. Additionally, a dose-response relationship for increasing hours of sun exposure during childhood while on holiday at the beach was associated with BCC (p=0.005) but not with SCC (p=0.782).

1. i. Acquired melanocytic nevi

Many epidemiologic studies have investigated the association between sun exposure in early childhood and nevus development. Prospective studies in the published literature provide evidence that increasing sun exposure in early years of life is associated with melanocytic nevus development. Since most nevi develop by the age of 10, their presence in adulthood may be considered an indicator of high UV exposure in childhood.

The SONIC study is an ongoing four year follow-up study of the natural history of nevi development among public and parochial school 5th graders (10 to 11 year olds) from Framingham, Massachusetts. Oliveria and colleagues(64) reported baseline findings after 1 year of follow-up and observed a slight increased risk for nevi development for spending 5 to 6 hours outdoors between 10am and 4pm (RR=1.13; 95% CI=1.00-1.28), compared to <1 to 2 hours (baseline), on a typical summer day. No differences in nevi development were observed between participants spending 3 to 4 hours outdoors (RR=0.93; 95% CI=0.83-1.04) compared to baseline.

Pettijohn et al(65) investigated the relationship between waterside vacations and nevus count among lifetime residents of Colorado at age 7. Results showed that with each additional waterside vacation taken one or more years prior to the skin exam received at age 7, the total number of nevi increased by 5% (p=0.01). The investigators also measured the total UV dose received on waterside vacations as well as the duration of waterside vacations but these factors were not significantly related to the presence of nevi among this young population. Additionally, *non*-waterside vacations were not significantly associated with nevus count.

In 2008, Harrison et al(63) published results looking at the association between sun exposure and incidence of melanocytic nevi among children 1 to 6 years of age and lifetime residents of Townsville, Australia. After one year of follow-up, a positive dose response relationship was observed between the daily average number of sun exposure hours and the median incidence rate of nevi development (p=0.012). The median incidence rate for less than one hour per day of sun exposure was 8.3 and increased steadily up to 4 or more hours of sun exposure per day with a median incidence rate of 13.0 for melanocytic nevi development per one year of follow-up. Additionally, the median incidence rate of melanocytic nevi development also increased with increasing

doses of UVR exposure needed to cause a sunburn while spending time outdoors during the one year of follow-up (p=0.034).

A longitudinal study conducted among German children 2 to 7 years of age from public nursery schools(60) observed a high incidence of total body nevus counts associated with increasing hours per day of sun exposure during holidays (intermittent, high sun exposure) (Regression coefficient=0.040; 95% CI=0.022-0.059) as well as with increasing hours per day of sun exposure during activities at home (chronic, moderate sun exposure) (Regression coefficient=0.043; 95% CI=0.012-0.075). Thus, cumulative sun exposure appears to be an important risk factor for nevi development in this German study population.

Among school children from Brisbane, Australia (12 and 13 years old at enrollment) followed for five years(61), spending all of time in the midday sun during lunchtime was associated with a means ratio for whole body nevi counts of 1.62 (95% CI=1.15-2.29) compared to children that spent very little time in the sun during their lunch period. Means ratios for spending most of the time and some of the time in the midday sun during lunchtime were 1.15 and 1.53, respectively, but failed to achieve statistical significance. Additionally, spending more than 4 weeks at the beach per year during childhood was associated with a 59% higher whole body nevi count (Ratio of means=1.59; 95% CI=1.20-2.10) compared to children spending less than 1 week at the beach per year.

A population based study conducted in Hamburg, Germany of 5 to 6 year old primary school children(62) demonstrated that the number melanocytic nevi was associated with the number of holidays (vacations) in Southern Europe, a time when children would experience intermittent exposure with higher doses of UV exposure

compared to the daily sun exposure in Hamburg. The mean numbers of melanocytic nevi were 14.2 (95% CI=13.7-14.7) and 12.8 (95% CI=12.5-13.2) for ≥2 and 1 holiday, respectively, in Southern Europe, compared to a mean number of melanocytic nevi of 10.9 (95% CI=10.7-11.2) for no holidays in Southern Europe.

1. j. Cutaneous human papillomavirus in non-melanoma skin cancer

As mentioned previously, the number of NMSC cases increases each year in the United States alone. Current prevention strategies, such as limiting the number of hours of sunlight exposure per day or applying sunscreen more frequently has not been effective in reducing the annual incidence of NMSC for either BCC or SCC. Therefore, there is a need to identify potential co-factors that may interact with UV radiation to increase the risk of NMSC. Epidemiologic studies have demonstrated a potential role for cutaneous HPV infections in NMSC development, so that novel prevention strategies may be developed. Furthermore, it has been hypothesized that cutaneous HPV may interact synergistically with UV radiation exposure in NMSC development.

Human papillomaviruses belong to a large family of more than 100 genotypes, with genus alpha comprising types that infect predominantly mucosal epithelia (including “high-risk” types associated with cervical cancer and “low-risk” types inducing benign mucosal lesions), and types that infect cutaneous epithelia(50). HPV types that infect cutaneous epithelia have also been identified from genera beta, gamma, mu, and nu(50).

Cutaneous HPV types in genus beta were identified from patients with Epidermodysplasia Verruciformis, a skin condition characterized by flat warts and macular skin lesions, which are suspected to be associated with SCC in these patients. The E6 and E7 oncoproteins encoded for by genus beta type HPV38 can interfere with

the tumor suppressor activities of both p53(95) and pRb(96) and immortalize keratinocytes through impairment of the telomerase system(97). HPV38 E6 and E7 have also displayed transforming properties in vivo(41). Keratinocytes expressing the E7 oncoprotein encoded for by HPV8, another genus beta type, acquire the ability to penetrate basement membranes(39) and overexpress matrix metalloproteinases that may play a role in HPV8-associated carcinogenesis(39, 98), and the development of cutaneous NMSC has been documented in HPV8-transgenic mice(99, 100). In addition, the E6 proteins of cutaneous HPV types have been shown to inhibit UV-radiation-induced apoptosis(45), supporting a role for cutaneous HPV as a cofactor in skin carcinogenesis. Seventy-five percent of NMSC's occurring in organ transplant recipients contain cutaneous HPV DNA(101), and estimates of HPV DNA prevalence in NMSC tissues from immunocompetent individuals range from 20-48%(32, 33, 101-103).

Presence of antibodies against one or more of the genus beta HPV types has been associated with SCC in several epidemiologic studies (see Appendix Table E). In an Australian case-control study, having antibodies to any of the genus beta HPV types tested was associated with a statistically significant four-fold increased risk of SCC (OR=3.9, 95% CI=1.4–10.7)(73). The single published study from the U.S. was conducted in New Hampshire and reported a 50% increased risk of SCC among individuals seropositive for any of 16 genus beta HPV types tested, with those who had antibodies to more than one HPV type being at greater risk of SCC (OR=1.8, 95% CI = 1.3-2.7) than those with antibodies to only one type (OR=1.5, 95% CI=1.0-2.1)(34). An SCC risk of similar magnitude was also observed in association with seropositivity to one or more cutaneous HPV types in a Dutch population (OR=1.4, 95% CI=0.8-2.5)(30), although no association was observed in a Swedish population (OR=1.13, 95% CI=0.61-2.12)(71) or a British population(29) for one beta HPV type (OR=0.5, 95% CI=0.1-1.7) or

2 or more beta HPV types (OR=1.0, 95% CI=0.4-2.5) compared to not being seropositive to any beta HPV types.

Results from epidemiologic studies of cutaneous HPV and BCC are less consistent (see Appendix Table E). Among genus beta HPV types tested in the Dutch population, seropositivity to any of the types was not significantly associated with BCC (OR=1.3, 95% CI=0.8-2.1), although statistically significant increased risks of BCC were associated with antibodies to HPV 8 (OR=14.7, 95% CI=1.4-154) and HPV 20 (OR=3.5, 95% CI=1.1-11.6)(30). Neither of the studies from New Hampshire(34) or Sweden(71) observed increased risks of BCC associated with cutaneous HPV antibodies. The other studies that investigated SCC did not include BCC, and there have been no studies of cutaneous HPV and BCC only.

The published literature focuses on the associations between NMSC and cutaneous HPV types from genus beta. However, a few more recent studies have presented results for cutaneous HPV types outside of genus beta(29, 34, 71). No statistically significant associations between BCC and SCC with seropositivity to cutaneous HPV types in genus alpha were observed in studies among residents of New Hampshire(34), and Sweden(71). Casabonne et al(29) also presented results for the associations between SCC and cutaneous HPV seropositivity for types from genuses alpha, gamma, mu, and nu but once again no statistically significant associations were observed.

Most of the increased risks of SCC have been associated with seropositivity to the genus beta types as a group, although cutaneous HPV type-specific associations have also been observed. For example, in the Australian population, antibodies against HPV 8 demonstrated the strongest association with SCC of 9.3 (95% CI=1.9–45.6)(73).

Antibodies against HPV 8 have also been associated with SCC in four other case-control studies(30, 32, 73, 101). In the Italian population, increased risks of SCC were observed with seropositivity to HPV 15 (OR=2.8, 95% CI=1.1-7.1), HPV 17 (OR=2.6, 95% CI=1.01-6.5) and HPV 38 (OR=3.0,95% CI=1.2-7.9)(37). Antibodies to HPV 38 were also associated with SCC in the Dutch population (OR=3.0, 95% CI=1.1-8.4)(30).

1. k. UV radiation exposure and cutaneous HPV infection in relation to squamous cell carcinoma

Several lines of evidence suggest that UV radiation exposure is associated with cutaneous HPV infection, and that these two factors may play a synergistic role in the development of cutaneous SCC. UV radiation produces distinct mutations in DNA, and tandem mutations, specifically CC→TT transitions in the TP53 gene (thymine dimers), are a hallmark of UV-induced DNA damage in SCC(42). UV-B radiation can also stimulate the promoter activity of HPV 5 and 8(39). In turn, the E6 proteins of genus beta HPV types have been shown to inhibit UV radiation-induced apoptosis through p53-independent pathways(45, 46), and cells expressing the E6 protein of HPV type 5 have reduced capacity to repair UV radiation-induced thymine dimers(43). In addition, HPV 38 E6 and E7 can alter the regulation of cell cycle checkpoints activated by UV radiation(41).

Epidemiologic evidence also supports the association between UV radiation exposure and cutaneous HPV infection. For example, individuals from the Leiden Skin Cancer study with a history of painful sunburns as a teenager were more likely to have EV-HPV DNA in plucked eyebrow hair samples than individuals without a similar history(104) (OR=1.74, 95% CI=1.04-2.91). Results from two case-control studies of SCC suggest that skin sensitivity to UV radiation and cutaneous HPV infection as

measured by seropositivity may interact synergistically. Among genus beta HPV-seronegative individuals in the New Hampshire study, individuals who reported getting a severe sunburn with blistering had a non-significant two-fold risk of SCC as compared to those who tanned without a sunburn(34) (OR=2.0, 95% CI=0.8-4.9). However, for those who were genus beta HPV-seropositive, having skin sensitive to UV radiation was associated with a statistically significant 4.6-fold risk of SCC(34) (OR=4.6, 95% CI=1.2-18.0). Similarly, statistically significant joint effects were observed between genus beta HPV seropositivity and risk factors for SCC such as skin color, propensity to sunburn, and intensity of sun exposure for risk of SCC among residents from Queensland, Australia(44). Genus beta HPV seropositive participants with fair skin color were more likely to have SCC (OR=26.9, 95% CI=6.6-111) compared to being HPV seronegative with olive-medium skin color. Having a high propensity to sunburn (always or most of the time) when sun exposed and being genus beta HPV seropositive was strongly associated with SCC (OR=8.5, 95% CI=2.4-29.3) compared to being genus beta HPV seronegative and experiencing a sunburn never, rarely, or sometimes when sun exposed. Additionally, high UV exposure and genus beta HPV seropositivity was strongly associated with SCC (OR=10.8, 95% CI=1.1-103) compared to participants with low UV exposure who were genus beta HPV seronegative.

1. l. Seroprevalence of cutaneous human papillomavirus infection

Cutaneous HPV seroprevalence among healthy persons varies across studies. For example, among the eight studies reporting genus-specific seroprevalence for beta HPV types, seroprevalence ranged from 12.3 and 12.5% in the Netherlands(30, 104), to 13% in Australia(73), to 24.7% in New Hampshire (NH) (34), to 26.3% in Germany(35), to 41% in Sweden and Austria(71), to 58% in the UK(29), and up to 70.8% in Florida(78). Genus alpha HPV seroprevalence was 11.2 % in Germany(35) compared

to 48.4% in Florida(78). Among the three studies reporting estimates for genus gamma and genus mu HPV types, the seroprevalence was highest in Florida with 53.3% and 44.3%(78) followed by 51% and 28% in the UK(29) and 26.8% and 27.6% in Germany(35), respectively, for being seropositive to at least one HPV type belonging to genus gamma or genus mu. For the single HPV type belonging to genus nu, seroprevalence ranged from 7.4% in Germany(35), to 14.0% in the UK(29), to 16.3% in Florida(78) to 27.0% in Italy(37).

There are several possible explanations for the observed differences in cutaneous HPV type-specific seroprevalences across studies. First, the genus-specific seroprevalence estimates are based on different numbers of HPV types. Second, there may be differences resulting from the use of different laboratory techniques for antibody detection and non-standardized cut-off definitions. However, there is substantial variation in estimates even among studies that used similar techniques. For example, genus beta seroprevalences ranged from 12.3% to 41% in studies that used ELISA assays for antibody detection(30, 71, 73, 104) and from 24.7% to 70.8% in studies that used Luminex techniques(29, 34, 35, 78). Additionally, variation in cutaneous HPV seroprevalence across studies may be due in part to differences in the underlying distribution of factors associated with HPV infection, such as age and sun exposure. For example, the single case-control study published from the U.S. was conducted in NH, where antibodies to genus beta HPV types overall were detected in 25% of controls(34). Data from our own study of healthy volunteers in Florida using the same laboratory methods as the NH study indicate that seroprevalence for the same HPV types is much higher in Florida(78) (57%), where sun exposure is greater. As described above in section 1.k., a statistically significant association was observed between cutaneous HPV

seropositivity and SCC among participants with skin sensitive to sun in the NH study(34).

1. m. Additional factors related to cutaneous human papillomavirus infection

Among the case-control studies of NMSC, only a few studies report independent findings of cutaneous HPV positivity in association with demographic and skin cancer risk factors among the control subjects. Factors associated with cutaneous HPV positivity may differ in individuals with a history of NMSC compared to individuals without a similar history. For example, (as previously mentioned in section 1.k.), in the Dutch population from the Leiden Skin Cancer Study(104), it was observed that control subjects with a history of painful sunburns between ages 13 to 19 years were almost two times more likely to be positive for cutaneous HPV DNA in eyebrow hairs (OR=1.74, 95% CI=1.04-2.91; p=0.04), but similar associations were not observed among SCC cases (OR=1.18, 95% CI=0.54-2.60; p=0.68). Increasing age showed a small significant increase in risk for cutaneous HPV DNA among the controls (OR=1.04, 95% CI=1.01-1.06; p=0.01) but not in the SCC cases (OR=1.01, 95% CI=0.96-1.06; p=0.63). A significant inverse association was observed among control subjects with high levels of lifetime sun exposure compared to individuals without a history of frequent sun exposure. For example, control subjects with a medium level or high level of sun exposure were 46% and 55%, respectively, less likely to be positive for cutaneous HPV DNA (medium level: OR=0.54, 95% CI=0.30-0.98; high level: OR=0.45, 95% CI=0.22-0.92; p=0.057). No associations between lifetime sun exposure and cutaneous HPV DNA positivity were observed among SCC cases. When looking at seropositivity in the same Dutch population, sun exposure related factors were not observed to be associated with cutaneous HPV infection in the control subjects. Among the SCC cases, fair skin compared to dark type (as defined by the Fitzpatrick classification of skin type)

was independently associated with higher cutaneous HPV seroprevalence (OR=3.45, 95% CI=1.18-10.0). No significant associations with age, sex, or other sun related factors were observed for cutaneous HPV seropositivity among control subjects or SCC cases.

Among Swedish individuals, Andersson and colleagues conducted multivariate analyses to investigate the independent associations between several demographic and skin cancer risk factors, such as age, sex, skin type, history of sunburns, smoking, and diagnosis (benign, actinic keratosis, SCC, and BCC) with cutaneous HPV seropositivity(71). Increased seropositivity for genus beta types was observed for fair skinned individuals (OR=2.47, 95% CI=0.91-6.69) compared to dark skinned individuals, but this association was not statistically significant. Smokers and persons who always sunburned were 1.43 and 1.36 times, respectively, more likely to be positive for cutaneous HPV antibodies compared to non-smokers and persons that do not sunbathe, but neither of these associations was statistically significant. Age, sex, and diagnosis showed no associations with genus beta HPV seropositivity. However, cutaneous HPV seroprevalence was observed to be higher among individuals that were male, older in age, fair skinned, smokers, had a high propensity to sunburn, and a diagnosis of SCC.

1. n. Limitations in literature

There are several limitations of the current literature that should be addressed. Evidence supporting the use of sunscreen to protect against skin cancer comes from two randomized controlled trials (RCT) conducted among Australian residents, where the amount, frequency, and formulation of sunscreen being used are controlled. Observational studies have not provided evidence of the protective effect of sunscreen use against NMSC but instead demonstrated increased risk for NMSC with sunscreen

use, possibly due to prolonged sun exposure while using sunscreen (i.e. intentional sun exposure), a factor not adjusted for in most of the analyses presented, as well as changes in sun exposure behaviors (i.e. using sunscreen more frequently) after first diagnosis of skin cancer. Furthermore, the sunscreen formulation being applied by participants enrolled in observational studies is not the same being applied by participants on a RCT. Additionally, most analyses presented in the literature did not consider site of sunscreen use compared to site of NMSC diagnosis. Further observational studies are needed to better understand the effectiveness of sunscreen products in the general population. This will allow better education to consumers for the use of sunscreen and other sun safe behaviors to protect against sunlight exposure and prevent/reduce NMSC.

Studies investigating the associations between amount, patterns, and timing of sun exposure and NMSC are few in number and have been limited to populations outside of the United States(51-53), with the exception of the study conducted by Vitasa et al among watermen from Maryland. However, Vitasa and colleagues measured cumulative exposure to UVB while the other studies(51-53) conducted among residents from Southern Europe and Australia used indirect measurements of sunlight exposure such as hours spent outdoors. Measuring lifetime sun exposure is difficult and measurement methods have varied across studies making it difficult to compare results. Additional limitations of case-control studies, such as recall bias and inability to adjust for time dependent variables, also permit for measurement errors. This error may lead to biased estimates of the effect being measured. For example, difficulty in remembering the number of hours of sunlight exposure during one's teens, 20s, and 30s when older in life (i.e. 60s – 70s) allows for measurement error not only in calculating the number of hours of sun exposure over a lifetime or on working or non-working days but in

estimating the association between patterns and timing of sun exposure and NMSC as well. Additionally, inability to adjust for time dependent variables presents challenges in identifying the true association between two factors. For example, changes in sun exposure behaviors, such as an increase in the frequency of sunscreen use after being diagnosed with skin cancer for the first time allows for measurement error in estimating the potential protective effects of sunscreen use against NMSC; a possible explanation for the associations observed in previous studies where sunscreen use appears to be a risk factor for NMSC.

Evidence in the published literature investigating the association between cutaneous HPV and NMSC is limited and more epidemiologic studies are needed to better understand the association between UV radiation exposure and cutaneous HPV infection as they relate to NMSC development. A majority of the studies investigating the association between cutaneous HPV seropositivity and NMSC did not include cutaneous HPV types in genera other than beta and did not present stratified analyses by factors, such as sun exposure, that may explain the variability observed across study populations. This is important because the findings from the studies conducted in New Hampshire (NH) (34) and Australia(44) showed differences between cutaneous HPV seroprevalence and SCC by sun related factors, such as level of UV exposure, skin color, and propensity to sunburn. Australian residents receive high ambient solar radiation and experience the highest incidence of skin cancer worldwide, very different from what is experienced by residents in NH. Furthermore, the associations between cutaneous HPV seropositivity and BCC and SCC presented in section 1.j. are based on different numbers of cutaneous HPV types tested, limiting the comparability of results across studies. For example, the NH(34) study tested 8 types in genus beta; where as study conducted on the Italian(37) population tested 15 HPV types in genus beta and the

study from the UK(29) tested 16 HPV types. Comparing estimates based on a different number of genus beta HPV types tested may explain the variability observed across studies.

1. o. Public health significance

Non-melanoma skin cancer (NMSC), comprised of squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), is the most frequently occurring cancer among U.S. men and women. Exposure to Ultraviolet (UV) radiation is an established risk factor for NMSC, but despite the current knowledge about the harm of sun exposure, and increased use of sunscreen, NMSC incidence rates continue to increase, emphasizing the critical need to better understand the role of sunscreen use in preventing NMSC and differences in sunlight exposure response relationships for BCC and SCC. Furthermore, it's important to identify additional risk factors for NMSC that may better characterize individuals at high risk and aid in the development of novel prevention strategies.

Evidence demonstrating the preventative capabilities of sunscreen use and SCC has been provided by randomized controlled trials and observational studies have focused on the associations between sunscreen use and BCC. Additional observational studies are needed to better understand the effectiveness of sunscreen and its association with SCC. Further studies are also needed to understand how anatomical site specific sunscreen use relates to anatomical site of NMSC diagnosis. Many epidemiologic studies provide evidence for the role of UV radiation exposure in the etiology of all types of skin cancer. However, few studies have formally evaluated the association between patterns and timing of sunlight exposure as they relate to BCC and SCC. Understanding how sunlight exposure response differs for BCC and SCC is important for better educating the public in sun safe behaviors. Simply advising a

reduction in sunlight exposure will not help reduce the incidence of NMSC if changes in sun exposure patterns are related to skin cancer development. For example, reducing continuous sunlight exposure (i.e. high doses of daily sunlight exposure) may decrease the incidence of SCC but not BCC if intermittent sun exposure, as received on holidays and vacations, is still received in high doses. Epidemiologic studies conducted in several countries have demonstrated an association between cutaneous HPV infection and NMSC, particularly SCC, and there is limited evidence to support the interaction between sunlight exposure and cutaneous HPV seropositivity as they relate to SCC. There is growing interest in utilizing a vaccine approach to preventing cancers caused by HPV, such as NMSC. However, much remains to be understood regarding the epidemiology of cutaneous HPV infections and their relationship with UV radiation exposure and NMSC development before such an approach can be incorporated into public health practice.

The goal of the research study was to better understand the relationships between sunscreen use and differences in sunlight exposure responses for BCC and SCC. Furthermore, the research project had the potential to provide evidence for the interaction between sunlight exposure and cutaneous HPV seropositivity as it relate to BCC and SCC. Individuals who reside in Florida have a significantly increased risk of developing NMSC compared to residents of northern US states(34) due to higher levels of UV radiation exposure. As mentioned earlier NMSC incidence rates continue to rise, emphasizing the public health importance of this highly prevalent cancer and highlighting the need for an increased understanding of its etiology and control.

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Appendix 2: Supplementary Tables

Sunscreen use as prevention for basal cell and squamous cell carcinoma

Sunscreens were originally formulated to protect against ultraviolet (UV)-induced sunburns, thought to be caused mostly by UV-B radiation. However, through laboratory studies in rodents, it was revealed that sunscreens had the potential to reduce UV-induced skin cancer, such as SCC(86). Animal models have not supported similar findings for BCC or melanoma. Despite the lack of evidence that sunscreens can protect against BCC or melanoma occurrence, sunscreen products are advocated for the prevention of all types of skin cancers. Currently available sunscreens provide broad spectrum coverage (UV-A and UV-B). However, observational studies have found sunscreen use to be associated with increased risk for BCC and an increased incidence of melanocytic nevi among children and adolescents. Randomized controlled trials from the published literature demonstrated that sunscreens had the ability to reduce the occurrence of solar keratoses (precursors for SCC) and of SCC, however, no effect was observed for BCC(87-89).

Evidence supporting the use of sunscreen to protect against skin cancer comes from two randomized controlled trials (RCT) conducted among Australian residents, where the amount, frequency, and formulation of sunscreen being used are controlled. Observational studies have not provided evidence of the protective effect of sunscreen use against NMSC but instead demonstrated increased risk for NMSC with sunscreen use, possibly due to prolonged sun exposure while using sunscreen (i.e. intentional sun

exposure), a factor not adjusted for in most of the analyses presented, as well as changes in sun exposure behaviors (i.e. using sunscreen more frequently) after first diagnosis of skin cancer. Additionally, skin sensitivity to sunlight exposure may influence the amount of time exposed to sunlight and in turn the frequency of sunscreen use.

Results presented in Tables A1 through A4 demonstrated the influence skin sensitivity and tanning ability to sunlight exposure have on the associations between sunscreen use and NMSC. Associations between sunscreen use and BCC/SCC stratified by skin reaction to one hour of sunlight exposure in the mid-day sun are presented below in tables A1 and A2. Despite the lack of power to detect statistically significant associations, information in the tables below provide evidence that using sunscreen either some of the time or rarely/never when exposed to sunlight exposure increased the risk of both BCC and SCC among individuals with a tendency to burn. In contrast, among individuals reporting no change in skin color or with a lesser tendency to tan from sunlight exposure, applying sunscreen some of the time or rarely/never when in the sun, increased the risk for BCC and SCC, compared to applying sunscreen often or always.

Table A1. Associations between sunscreen use and basal cell carcinoma stratified by cutaneous sensitivity to sunlight exposure for 1 hour in the mid-day sun

	No change in skin color						Tan with no sunburn or mild burn that turns to a tan						Sunburn with or without blisters					
	Controls		BCC				Controls		BCC				Controls		BCC			
	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI
Apply SPF[2] ≥15																		
Often/always	13	(30.2)	6	(30.0)	1.00	(reference)	52	(36.1)	27	(41.5)	1.00	(reference)	59	(47.6)	47	(36.7)	1.00	(reference)
Sometimes	13	(30.2)	3	(15.0)	0.25	(0.04- 1.63)	54	(37.5)	15	(23.1)	0.39	(0.17- 0.89)	29	(23.4)	40	(31.3)	1.71	(0.88- 3.31)
Rarely/Never	17	(39.5)	11	(55.0)	0.35	(0.06- 2.03)	38	(26.4)	23	(35.4)	0.61	(0.27- 1.38)	36	(29.0)	41	(32.0)	1.13	(0.60- 2.12)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and gender

[2]SPF = sun protection factor

Table A2. Associations between sunscreen use and squamous cell carcinoma stratified by cutaneous sensitivity to sunlight exposure for 1 hour in the mid-day sun

	No change in skin color						Tan with no sunburn or mild burn that turns to a tan						Sunburn with or without blisters					
	Controls		SCC				Controls		SCC				Controls		SCC			
Variable	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI
Apply SPF[2] ≥15																		
Often/always	13	(30.2)	5	(23.8)	1.00	(reference)	52	(36.1)	11	(22.4)	1.00	(reference)	59	(47.6)	35	(38.9)	1.00	(reference)
Sometimes	13	(30.2)	6	(28.6)	0.84	(0.17- 4.01)	54	(37.5)	21	(42.9)	1.36	(0.54- 3.40)	29	(23.4)	29	(32.2)	1.18	(0.55- 2.54)
Rarely/Never	17	(39.5)	10	(47.6)	0.85	(0.19- 3.67)	38	(26.4)	17	(34.7)	1.08	(0.40- 2.89)	36	(29.0)	26	(28.9)	0.73	(0.35- 1.55)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and gender

[2]SPF = sun protection factor

Similar associations were investigated between sunscreen use and BCC/SCC stratified by tanning ability to repeated sunlight exposure. As demonstrated in Table A3, differences in tanning ability did not appear to alter the observed associations between frequency of sunscreen use and BCC risk. With the exception of applying sunscreen some of the time by individuals that reported being unable to tan from repeated sunlight exposure, frequency of sunscreen use and SCC (Table A4) risk did not appear to differ by one's ability to tan.

Table A3. Associations between sunscreen use and basal cell carcinoma stratified by tanning ability to repeated sunlight exposure

	Tans easily						Tan if you work at it						Unable to tan					
	Controls		BCC				Controls		BCC				Controls		BCC			
Variable	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI
Apply SPF ≥15																		
Often/always	68	(37.0)	37	(35.9)	1.00	(reference)	42	(41.2)	33	(35.5)	1.00	(reference)	12	(54.5)	7	(46.7)	1.00	(reference)
Sometimes	56	(30.4)	22	(21.4)	0.52	(0.26- 1.05)	36	(35.3)	35	(37.6)	1.14	(0.56- 2.31)	3	(13.6)	1	(6.7)	0.47	(0.03- 8.58)
Rarely/Never	60	(32.6)	44	(42.7)	0.77	(0.40- 1.47)	24	(23.5)	25	(26.9)	0.92	(0.42- 2.03)	7	(31.8)	7	(46.7)	1.33	(0.26- 6.68)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and gender

[2]SPF = sun protection factor

Table A4. Associations between sunscreen use and squamous cell carcinoma stratified by tanning ability to repeated sunlight exposure

	Tans easily						Tan if you work at it						Unable to tan					
	Controls		SCC				Controls		SCC				Controls		SCC			
Variable	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI	n	%	n	%	OR[1]	95% CI
Apply SPF ≥15																		
Often/always	68	(37.0)	16	(26.2)	1.00	(reference)	42	(41.2)	24	(32.4)	1.00	(reference)	12	(54.5)	12	(48.0)	1.00	(reference)
Sometimes	56	(30.4)	23	(37.7)	0.95	(0.42- 2.18)	36	(35.3)	27	(36.5)	0.88	(0.39- 2.02)	3	(13.6)	5	(20.0)	2.15	(0.26- 17.8)
Rarely/Never	60	(32.6)	22	(36.1)	0.65	(0.28- 1.54)	24	(23.5)	23	(31.1)	0.94	(0.39- 2.27)	7	(31.8)	8	(32.0)	0.97	(0.20- 4.68)

[1]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and gender

[2]SPF = sun protection factor

If sunscreen use has the potential to protect against skin cancer development, we would expect a reduced risk in BCC and SCC among individuals using sunscreen products always or often and possibly some of the time when exposed to sunlight. However, in addition to a person's skin reaction to sunlight exposure, factors such as cumulative ambient solar radiation, recreational and vacation activities, clothing worn when exposure to UVR, and geographic residence, need to be considered simultaneously when investigating the potential preventive effects of sunscreen use in skin cancer development, especially in observational studies. Due to a small sample and therefore, lack of power to detect statistically significant associations, it's difficult to make conclusions on the associations between sunscreen use and BCC/SCC in the current study population. We were not able to control for the factors listed above and we were not able to identify whether or not individuals that reported skin sensitivity and rarely or never using sunscreen as also limiting sunlight exposure.

With the knowledge about the harmful effects of UV radiation, the use of sunscreens has increased. However, the incidence of NMSC continues to rise, perhaps because individuals who use sunscreen spend more time in the sun under the assumption that they are protected from the harmful effects of UV radiation. There is experimental evidence that suggests sunscreens are protective against SCC, but similar results have not been observed for BCC. Additionally, no observational studies have been able to formally evaluate the relationship between sunscreen use and NMSC, partly due to small sample sizes that do not allow for stratified analyses and simultaneous assessment of multiple factors that influence sunscreen use and its potential protective effects.

ABOUT THE AUTHOR

Michelle R. Iannacone was born in New York in 1979. She received her BA in Spanish American Literature from New York University in 2001 and PhD of Public Health in Epidemiology from the University of South Florida in 2011. She has successfully authored and co-authored peer reviewed manuscripts in the scientific literature and has presented her work at many scientific meetings, including the 5th International HPV and Skin Cancer Conference in Heidelberg, Germany and the 33rd Annual American Society for Preventive Oncology conference held in March 2009 in Tampa, Florida.