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SUNBURN, SUN EXPOSURE, AND SUN SENSITIVITY IN THE STUDY OF NEVI IN CHILDREN

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Abstract

Purpose—To examine the joint effect of sun exposure and sunburn on nevus counts (on the natural logarithm scale; log nevi) and the role of sun sensitivity.

Methods—We describe an analysis of cross-sectional data from 443 children enrolled in the prospective study of nevi in children. To evaluate the joint effect, we partitioned the sum of squares due to interaction between sunburn and sun exposure into orthogonal components representing: (i) monotonic increase in log nevi with increasing sun exposure (rate of increase of log nevi depends upon sunburn), and (ii) non-monotonic pattern.

Results—In unadjusted analyses, there was a marginally significant monotonic pattern of interaction (p -value = 0.08). In adjusted analyses, sun exposure was associated with higher log nevi among those without sunburn ($p < 0.001$), but not among those with sunburn ($p = 0.14$). Sunburn was independently associated with log nevi ($p = 0.02$), even though sun sensitivity

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explained 29% (95% CI: 2%-56%, $p = 0.04$) of its effect. Children with high sun sensitivity and sunburn had more nevi, regardless of sun exposure.

Conclusions—A program of increasing sun protection in early childhood as a strategy for reducing nevi, when applied to the general population, may not equally benefit everyone.

Keywords

proportion of effect explained; regression; statistical interaction; sun protection; ultraviolet radiation

Introduction

Total body nevus count is the strongest known phenotypic risk factor for cutaneous melanoma [1, 2]. Epidemiologic studies suggest that sunburn history (hereinafter referred to as sunburn), outdoor sun exposure (hereinafter referred to as sun exposure), and sun sensitivity are each associated with higher nevus counts in childhood and adolescence [3-7]. Sunburn and sun exposure are unique forms of ultraviolet radiation (UVR). It remains unclear how the joint effect of sunburn and sun exposure contribute to nevus development in childhood – particularly whether the effect of sun exposure on nevus counts varies according to sunburn. Sunburn is also determined in part by sun sensitivity. However, it is unclear whether sun sensitivity and sunburn confer independent effects on nevus counts.

In this paper, we use data from the Study of Nevi in Children (SONIC; [7]) to examine these issues by evaluating the joint effect of sunburn and sun exposure on nevus counts and by estimating the proportion of the effect of sunburn explained by sun sensitivity. Clarifying these issues can add to our knowledge of UVR-related pathways of nevogenesis and the putative benefits of increasing sun protection.

Materials and Methods

Study Population

The Study of Nevi in Children (SONIC) is a prospective study investigating risk factors associated with the etiology and evolution of nevi. Details of enrollment and data collection are described elsewhere [7]. Briefly, a study description, consent forms, assent forms, and surveys were mailed to the families of 691 fifth grade students from 10 schools in Framingham, Massachusetts during fall 2004. Informed consent for participation was obtained from the parents of 443 children and assent was obtained from all participants. These children were followed through the eighth grade, and imaging techniques were used to measure the total number of nevi on their back (described below). The children completed an annual survey to provide information on their sunburn history, sun exposure, and sun protection behavior. The study was approved by the Institutional Review Boards at Boston and Harvard Universities.

Here we report an analysis of cross-sectional data ascertained at baseline (year 2004) from 443 children.

Outcome and risk factors

The total number of nevi on the back of each child (trait of interest i.e., outcome) was measured using imaging techniques independently by two study dermatologists. A photograph of the back of each child was obtained using standard photographic equipment (Phase One P25 Camera Back, Hasselblad 503w Camera System, Canfield Scientific, Inc. 1kWatt Studio Flash System). Two transparent reference lines were superimposed on the photograph to divide the back into 4 quadrants. Each quadrant was enlarged 100% and the reviewers tagged each nevus. Total back nevus count of each child was obtained from the respective photograph as the sum of the number of nevi in the 4 quadrants. On the basis of work by other investigators [8, 9], a 2-mm cutpoint was used to determine a nevus versus a freckle. Each child was categorized as having none, mild, moderate, or severe freckling. Due to potential misclassification of nevi as freckles, nevus counts for children with back freckling were validated independently by a second study dermatologist. For analysis, total back nevus count was transformed to the natural logarithm scale and referred to as log nevi [6, 10], and freckling was dichotomized as either absent (no freckling) or present (mild, moderate or severe).

Race/ethnicity [binary factor: non-Hispanic White and other (Hispanic, Asian, Black, Native American)] and gender were obtained from the school records. Sunburn history is response to the question “How many times did your skin stay red or pink for 12 or more hours, after being out in the sun this past summer?” Outdoor sun exposure is response to the question “On a typical day on the week this past summer, how many hours did you spend outdoors between 10am and 4pm?” Sunscreen use is response to the question “When you were outside on a sunny day during the past summer for at least 15 minutes during 10AM–4PM not at the beach or pool, how often did you use sunscreen strong enough to block the sun on your back?” Shirt use is response to the question “When you were outside on a sunny day during the past summer for at least 15 minutes during 10AM – 4PM at the beach or pool, how often did you wear a shirt?”

Sunburn was coded as a binary variable denoting none or at least one instance of redness. Sun exposure was coded as an ordinal variable denoting low (< 3 hours), medium (3 to 4 hours), and high (> 4 hours) exposure. Sunscreen and shirt use were coded as categorical variables denoting never, rarely, sometimes, and often/always.

A single study nurse recorded hair color and skin color. Sun sensitivity was determined using a sun sensitivity index (SSI), a continuous value between 0 and 3, measured as a weighted sum of hair color, skin color, and tendency to burn (0 = high sun sensitivity i.e., light hair and skin colors, and a tendency to burn; 3 = low sun sensitivity i.e., dark hair and skin colors, and no tendency to burn).

Statistical Analysis

Descriptive Analyses—Mann-Whitney test was used to compare the distribution of SSI between individuals with and without sunburn. Kruskal-Wallis test was used to compare the distribution of SSI between individuals with: (i) low, medium, and high sun exposure; and

(ii) freckling absent and present. Fisher's exact test was used to compare the association between: (i) sunburn and sun exposure; and (ii) freckling and sun exposure.

Evaluation of interaction—We estimated the joint effect of sunburn and sun exposure via a regression model by incorporating their additive and interaction effects, adjusting for other variables of interest. Under the standard approach, product terms of the risk factors are included in the model to evaluate interactions. When there are L_1+1 levels of sun exposure and L_2+1 levels of sunburn, the degrees of freedom for testing the null hypothesis of no interactions is L_1L_2 . Certain interactions, evaluated under a practically interpretable scale of the outcome such as log nevi, may exhibit monotonic patterns [11] – for example, log nevi increase with increasing level of sun exposure, but the rate of increase vary according to the level of sunburn. Other interactions can be non-monotonic – for example, for a fixed level of sunburn, log nevi increase with increasing level of sun exposure; but, when the level of sunburn factor is changed, log nevi decrease with increasing level of sun exposure i.e., there is a change in the direction of the effect.

The standard method for evaluating interactions does not distinguish between specific patterns of interactions. When evaluating interactions, investigating specific patterns can be a pragmatic strategy to obtain useful insights about the risk factors. A monotonic pattern of interaction can shed light on how the benefits of therapeutic or preventive interventions (such as increasing sun protection behavior) increase (or decrease) among those with and without a sunburn. Admittedly, such a pattern must be confirmed using controlled experiments before moving forward with further investigations or practical interpretation. A non-monotonic pattern of interaction may be an indication that some yet to be measured risk factors (for example, novel genetic mutations) in a subset of individuals contribute to the change in the direction of the effect, which must also be confirmed using further studies.

Building on our previous works [12, 13], we partitioned the interaction sum of squares of the standard approach into two orthogonal components corresponding to monotonic and non-monotonic interactions, and developed hypothesis tests (see Supplementary Material for details). Briefly, when the interaction is monotonic, all the L_1L_2 interaction terms can be represented using a single parameter. We first estimate this parameter and test the null hypothesis of no monotonic interaction using a single degree of freedom. If there is no monotonic interaction, this parameter will be 0. Next, we calculate a test statistic for non-monotonic interactions using the difference between the sum of squares due to interaction under the standard approach and the sum of squares due to monotonic interaction. This test has $L_1L_2 - 1$ degrees of freedom and can be interpreted as a test for residual interaction after accounting for monotonic pattern. If there is no non-monotonic interaction, this residual will be 0.

Regression models—We developed four linear regression models for log nevi. The first model included sunburn and sun exposure, and did not adjust for any other variable (**Model 1**). We extended Model 1 by adjusting for SSI (**Model 2**). Next, we extended Model 1 by adjusting for race/ethnicity, gender, sunscreen and shirt use and freckling (**Model 3**). Finally, we extended Model 3 by adjusting for SSI (**Model 4**). In all our analyses, the main and interaction effects were specified using orthogonal contrasts. In each model we tested

the null hypotheses of no monotonic interaction and no non-monotonic interaction between sunburn and sun exposure. In our analyses sun exposure had $L_1+1 = 3$ levels and sunburn had $L_2+1 = 2$ levels. We also fitted Models 1 and 4 separately for the subgroups of children with and without sunburn, and tested the statistical significance of the effect of sun exposure on log nevi in each of these subgroups under the two models.

Proportion of effect explained—Denoting $\hat{a}_i$ as the estimated effect of sunburn in Model i ($i = 1, 2, 3, 4$), we estimated $\hat{\pi}_{12} = 1 - \frac{\hat{a}_2}{\hat{a}_1}$ and $\hat{\pi}_{34} = 1 - \frac{\hat{a}_4}{\hat{a}_3}$ as the proportions of the effect of sunburn explained by SSI without and after adjusting for other variables, respectively, and tested the null hypotheses that these proportions are not significantly different from zero ([14, 15]; see Supplementary Material). The measures $\hat{\pi}_{12}$ and $\hat{\pi}_{34}$ are interpretable as proportions when $\hat{a}_2 \neq \hat{a}_1$ and $\hat{a}_4 \neq \hat{a}_3$.

All the analyses were conducted using the R programming language (<http://cran.rproject.org>).

Results

The characteristics of the study subjects are summarized in Table 1.

Association between sunburn, sun exposure, and SSI

Children with sunburn had significantly lower SSI i.e., high sun sensitivity than those without sunburn ($p < 0.0001$) (see Table 1). The distribution of SSI did not differ significantly by levels of sun exposure in these children (mean SSI = 1.3, 1.3, and 1.1 among those with low, medium, and high sun exposure; $p = 0.22$). Among children without sunburn, mean SSI was 2.1, 1.8, and 1.7 in those having low, medium, and high sun exposure, respectively. Thus, highly sun sensitive children who did not have sunburn appeared to seek more exposure to the sun ($p = 0.0003$). Children with freckling had significantly lower SSI than those without freckling ($p < 0.0001$; Table 1). The prevalence of freckling increased with increasing level of sun exposure ($p = 0.004$): 14 (10%) of 142 children with low sun exposure, 35 (19%) of 183 children with medium sun exposure and 30 (25%) of 118 children with high sun exposure had freckling.

Regression analyses

Table 2 shows the analysis of variance summary for Model 1. Both sunburn and sun exposure had significant main effects. The overall test for interaction between sunburn and sun exposure was not statistically significant ($p = 0.12$, 2 degrees of freedom test). However, there was a marginally significant evidence for monotonic interaction ($p = 0.08$); Figure 1 shows the following monotonic pattern: (i) in the absence of sunburn, mean log nevi increased from 1.28 to 1.53 to 1.97 as sun exposure increased from low to medium to high levels, respectively; (ii) in the presence of sunburn, mean log nevi increased from 1.89 to 2.12 to 2.17 as sun exposure increased from low to medium to high levels, respectively; and (iii) mean log nevi were higher in the presence of a sunburn, regardless of sun exposure level. Among those without sunburn, log nevi increased significantly in a dose response manner with increasing level of sun exposure ($p < 0.0001$). In contrast, among those with

sunburn, although mean log nevi increased with increasing level of sun exposure, it was not statistically significant ($p = 0.21$).

This monotonic pattern of the data was observed even after adjustment for race/ethnicity, gender, SSI, sunscreen and shirt use, and freckling (Figure 1), although neither the overall interaction nor its orthogonal components were statistically significant after adjustment for these factors (Table 2). We fitted adjusted models separately for children without and with sunburn since there was a marginally significant monotonic pattern of interaction in the unadjusted analysis. The main effect of sun exposure was statistically significant among children without sunburn ($p < 0.0001$) but not among children with sunburn ($p = 0.14$; detailed results not shown). In particular, among children without sunburn, those having high level of sun exposure had significantly higher log nevi than those with low or medium sun exposure. In contrast, log nevi did not vary significantly with increasing level of sun exposure among those with sunburn (see Figure 1).

Table 3 shows the parameter estimates of Models 1-4. The main effect of sunburn was statistically significant in all the models considered. SSI was significantly associated with nevus counts, and explained 58%

($=100 \times \hat{\pi}_{12} = 100 \times [1 - 0.097/0.230]$, 95% CI: 33% – 83%, $p < 0.0001$) of the effect of sunburn without adjustment for race/ethnicity, gender, sunscreen and shirt use, and freckling. It explained 29%

($=100 \times \hat{\pi}_{34} = 100 \times [1 - 0.093/0.132]$, 95% CI: 2% – 56%, $p < 0.04$) of the effect of sunburn after adjustment.

In adjusted models, freckling explained 4% of the effect of sunburn ($\approx 100 \times [1 - 0.093/0.097]$; 0.093 is the estimated effect of sunburn from Model 4 and 0.097 is the estimated effect of sunburn by adjusting for all the effects as in Model 4 except freckling; 95% CI: -11% - 19%, $p = 0.61$).

Discussion and Conclusion

Our results support previous work that solar UV and subsequent solar-induced sunburn are associated with nevus counts [10, 16-18]. We partitioned the interaction sum of squares to obtain insights into specific patterns of interactions. Separate adjusted analyses of individuals with and without sunburn revealed that increasing level of sun exposure is associated with increasing nevus counts only in children without sunburn.

In our study, sunburn and SSI were independently associated with log nevi (Model 4), and SSI explained a significant proportion (but not all) of the effect of sunburn on log nevi. As variants in the *MC1R* gene are the most widely studied genetic factors predisposing to lighter pigmentation that are hallmarks of sunburn and high sun sensitivity, we reviewed published data on gene expression in *MC1R* mutant and wild type mice in response to ultraviolet B (UVB) exposure [19]. Among *MC1R* wild type mice, UVB exposure led to the under-expression of several genes from important cellular pathways that have been implicated in the etiology of melanoma. In contrast, these genes were under-expressed even in the absence of UVB exposure in *MC1R* mutant mice (see Supplementary Figure). These

observations are consistent with the SONIC results that children with sunburn and high sun sensitivity have more nevi regardless of sun exposure, while nevi increase with increasing level of sun exposure in the absence of sunburn.

In our study, freckling did not explain the effect of sunburn. However, children with freckling had higher sun sensitivity and freckling increased with increasing level of sun exposure. Therefore, although the etiology of freckling may depend upon genetic predisposition and high sun exposure, freckling itself may not be in the UV-related pathway for nevogenesis. Additional work studying the role of freckling on nevogenesis using larger cohorts is needed to confirm these observations.

A key question is whether a program of increased sun protection (such as reducing sun exposure) in childhood can reduce nevus counts. The results of the murine study suggest that children who sunburn easily are likely to have certain molecular-level alterations predisposing them to increased nevus counts regardless of the level of UVB exposure. The murine study and the observed monotonic pattern of interaction in SONIC suggest the hypothesis that reducing sun exposure may not be as effective in reducing total nevus counts in children who sunburn easily. Further, in our study, children with high sun sensitivity have many nevi, regardless of sun exposure level and sunburn. These results suggest that increasing sun protection behavior to reduce sun exposure as a way for reducing nevus counts in childhood, while important, may not equally benefit everyone when applied uniformly to the general population. These observations also align with the results of recent work on gene-sun exposure interaction and the longitudinal evolution of nevi [20].

Two previous studies have examined reduction in nevus counts and sun protection use. In the non-randomized Kidskin study from Western Australia, sun protection use was not associated with nevus reduction in children [21, 22]. In the Vancouver randomized trial, children randomized to the sunscreen arm developed fewer nevi, with a statistically significant, albeit small, reduction seen for children with freckles [23]. In SONIC, sunscreen is not randomized, the children are older than those in the Vancouver and Kidskin studies, and we have used nevus counts measured at a single time point. Nonetheless, our hypothesis that sun protection behavior may not equally benefit everyone (with respect to reducing nevus counts) aligns with the general conclusions of these studies. Additional studies investigating the biologic effects of UV radiation on nevus formation in genetically distinct individuals are needed to understand the benefits of sun protection.

The limitations of our study include cross-sectional data and self-reported questionnaires. We did not comprehensively measure other forms of sun avoidance such as shading during peak sun hours, and do not know how well the children used their sunscreen, which are limitations of any observational study. Whether increasing sun protection behavior beyond age 10 may facilitate nevus reduction and by how much are important issues that cannot be addressed by our cross-sectional data. Ongoing longitudinal follow-up of the SONIC cohort into adulthood and accrual of additional study subjects and genetic information will allow us to refine these findings.

The R computer programs used for testing monotonic and non-monotonic interactions and for estimating the proportion of effect explained are available upon request from the corresponding author.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Mean log nevi as a function of outdoor sun exposure among all 443 children, shown for unadjusted means (dashed line with filled diamond for children with sunburn, and bold line with filled circle for children without sunburn), and means obtained after adjusting for the effects of race/ethnicity, gender, SSI, sunscreen and shirt use, and freckling (dot-dashed line with inverted triangle for children with sunburn, and dotted line with star for children without sunburn).

TABLE 1

Characteristics of all 443 children in the SONIC study.

Sunburn History	Outdoor Sun Exposure ^a	Race/Ethnicity				Freckling (N)		Mean log nevi	Std. error	Mean SSI ^c		
		Non-Hispanic White (N)		Other ^b (N)		All children	By freckling					
		Male	Female	Male	Female		Absent			Present		
No	Low	17	20	24	25	80	6	1.285	0.079	2.1	2.1	2.1
No	Medium	35	17	17	14	72	11	1.538	0.111	1.8	1.9	1.5
No	High	31	10	10	1	43	9	1.971	0.113	1.7	1.7	1.3
Yes	Low	24	15	7	10	48	8	1.892	0.124	1.3	1.4	1.3
Yes	Medium	49	32	16	3	76	24	2.118	0.091	1.3	1.4	0.8
Yes	High	36	24	4	2	45	21	2.166	0.108	1.1	1.3	0.7

^aLow = < 3 hours, Medium = 3-4 hours, and High = >4 hours.

^bOther race/ethnicity includes 91 Hispanic (52 boys, 39 girls), 22 Asian (13 boys, 9 girls), 19 Black/African American (10 boys, 0 girls), and 1 Native American girl.

^cSSI = Sun sensitivity index.

TABLE 2

Analysis of variance table based on Models 1 and 4. Abbreviations: SS = sum of squares, DF = degrees of freedom, MS = mean squared, F = F statistic, p = p-value.

	Model 1 (unadjusted)					Model 4 (adjusted for race/ethnicity, gender, SSI, sunscreen and shirt use, and freckling)				
	SS	DF	MS	F	p	SS	DF	MS	F	p
Main Effects										
Sunburn	31.53	1	31.53	40.24	<0.0001	4.17	1	4.17	7.49	0.006
Sun Exposure	14.50	2	7.25	9.25	0.0001	0.86	2	0.43	0.77	0.46
Interaction[§]										
Overall	3.35	2	1.68	2.14	0.12	1.25	2	0.63	1.12	0.33
Monotonic	2.48	1	2.48	3.17	0.08	0.74	1	0.74	1.33	0.25
Non-monotonic	0.87	1	0.87	1.11	0.29	0.51	1	0.51	0.92	0.34
Residuals	342.44	437	0.78			237.73	427	0.56		

[§]The overall interaction sum of squares is the sum of squares due to interaction under the standard approach. Adding the sum of squares due to monotonic and non-monotonic interactions gives the overall interaction sum of squares.

TABLE 3

Parameter estimates, 95% confidence intervals and p-values for the four regression models based on all 443 SONIC participants.

	Models used for calculating $\hat{\pi}_{12}$			Models used for calculating $\hat{\pi}_{34}$				
	Model 1		Model 2		Model 3		Model 4	
	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p	Estimate (95% CI)	p
Parameter								
Sunburn (Yes vs No)	0.230 (0.145, 0.315)	< 0.0001	0.097 (0.013, 0.181)	0.02	0.132 (0.057, 0.207)	0.001	0.093 (0.016, 0.170)	0.002
Sun Exp. (Medium vs Low)	0.119 (0.021, 0.218)	0.02	0.060 (−0.029, 0.150)	0.19	0.018 (−0.068, 0.104)	0.68	0.003 (−0.080, 0.087)	0.93
Sun Exp. (High vs Medium/Low)	0.120 (0.057, 0.183)	0.002	0.073 (0.016, 0.131)	0.01	0.040 (−0.015, 0.096)	0.16	0.034 (−0.020, 0.088)	0.22
Interaction: Sunburn × Medium Sun Exp.	−0.007 (−0.105, 0.091)	0.89	0.030 (−0.060, 0.119)	0.52	0.008 (−0.077, 0.092)	0.86	0.023 (−0.059, 0.105)	0.58
Interaction: Sunburn × High Sun Exp.	−0.066 (−0.129, −0.003)	0.04	−0.046 (−0.103, 0.011)	0.11	−0.048 (−0.103, 0.007)	0.09	−0.040 (−0.093, 0.135)	0.140
SSI (Linear)	-	-	0.584 (0.241, 0.927)	0.001	-	-	0.551 (0.224, 0.878)	0.001
SSI (Quadratic)	-	-	−0.352 (−0.464, −0.240)	< 0.0001	-	-	−0.268 (−0.379, −0.157)	< 0.0001
Freckling	-	-	-	-	0.401 (0.206, 0.596)	< 0.0001	0.405 (0.211, 0.598)	< 0.0001

The outcome in all the models is log nevi. The predictor variables are:

- Model 1 includes main and interaction effects of sunburn and sun exposure, and is unadjusted for other variables.
- Model 2 expands Model 1 by adjusting for SSI (linear and quadratic effects).
- Model 3 expands Model 1 by adjusting for race/ethnicity, gender, sunscreen and shirt use, and freckling.
- Model 4 expands Model 3 by adjusting for SSI (linear and quadratic effects).

The effects of race/ethnicity, gender, and sunscreen and shirt use are not shown for Models 3 and 4 in the table. Following Oliveria et al [7], we include linear and quadratic effects of SSI in Models 2 and 4. This involves including SSI (linear) and SSI² (quadratic) terms in the model and estimating their effects.