Sunburn Associated with Increased Number of Nevi in Darker as Well as Lighter Skinned Adolescents of Northern European Descent

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Abstract
The associations of sun exposure, sunburn, skin color, and other constitutional characteristics with the density of nevi (2 mm or more in diameter) were assessed in a study of 410 secondary school children ages 14–15 years in Tasmania, Australia. Skin color was estimated by using a chromameter that measures across the visible light spectrum (400–700 nm). Skin color and lifetime history of sunburn were significant predictors of nevus density on the arms and legs of girls and boys and on the shoulders and backs of boys. The nevus density ratios between the highest and lowest exposure groups were 2.85 for the arms and legs of girls (P < 0.01), 2.19 for the arms and legs of girls (P < 0.01), and 1.72 (P = 0.03) for the shoulders and backs of boys. The increase in nevus density appeared to occur at lower levels of lifetime sunburn in children with light or medium skin than it did in children with darker skin. Darker-skinned children with a history of many sunburns (≥11 lifetime sunburns) had a similar number of nevi compared with their lighter-skinned peers.

Introduction
The investigation of the relationship between sun exposure and melanoma has been hampered by the time gap between the apparent key exposure period in childhood and disease onset (1). In an attempt to circumvent this barrier, investigators have substituted childhood nevi as a proxy for melanoma, nevi having been suggested as an appropriate marker for the future development of melanoma. The use of nevi in this research setting has been justified on the grounds that nevi appear to share a common causal pathway with melanoma, involving an interplay between constitutional factors and sun exposure. Supporting this is evidence that nevi are more prevalent at lower latitudes (2) and among fairer-skinned people (3), as are melanoma, and evidence that nevus density is the strongest risk factor in case-control studies of melanoma in adults (4).

The attempts to use the nevus model to answer key questions about sun exposure that have arisen in the study of melanoma have thus far yielded fewer answers than might have been hoped for. Several studies have shown that constitutional factors, such as hair (5, 6), eye color (6, 7), and skin color measured by reflectometer (2) and by interviewers using skin tone panels (8), are associated with risk. The findings on history of sun exposure and sunburn have been inconsistent. In Vancouver (British Columbia, Canada), Gallagher et al. (8) found that history of sunburn was related positively to the presence of nevi in children, but Pope et al. (5) in the West Midlands of the United Kingdom and Coombs et al. (9) in New Zealand were unable to find an association. Of the sun exposure variables measured by Pope et al. (5), a history of the number of days on the beach and of summer holidays in a hot location were predictive. In Australia, Green et al. (6) found nonsignificant associations for sun exposure variables. Even where associations have been present for a measure of sun exposure, they have been relatively weak. As a consequence, the understanding of some of the key questions in melanoma research has been advanced little by this field of investigation to date. Is cumulative exposure as important as exposure that causes burning? Is skin color important because it affects the risk of burning? Does the effect of sun exposure or burning increase steadily or diminish at some point?

On the basis of the hypothesis that stronger associations might be masked by the inadequately controlled confounding or modifying effects of skin color, a study was mounted that included not only many of the measurement protocols used previously, but which also used an objective measure of skin color that had not been used previously in this field. The device chosen, a Minolta Chromameter II (10), measures across the visible light spectrum by using the Commission International de’Eclairage color parameters (L*, a*, b*).3

Below, we report the findings from a study of 14–15 year olds in Southern Tasmania (Australia; latitude 43°S). These subjects were drawn from a predominantly Anglo-Celtic population, approximately 90% of which traces its ancestry to the British Isles.

Methods and Materials
Study Measurements. A survey of nevi in grade nine secondary school students was conducted during 1992. The subjects were selected by using a two-stage sampling method. Ten schools were chosen by using systematic sampling, with a probability proportional to the enrollment of students 15 years of age. The second stage of sampling consisted of the random selection of approximately 60 students from each school. A total of 472 students (response rate, 71.8%) agreed to participate in the survey.

The students, wearing medical gowns with back openings, were examined at school by two registered nurses who had been trained by a dermatologist in the recognition of melanocytic

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3 The abbreviations used are: color parameters L*, a*, b*, lightness, redness, and yellowness of the skin, respectively; CI, confidence interval.
nevi. Nevi were identified by using the IARC protocol (11). Countable lesions were defined as brown to black-pigmented macules or papules that were reasonably well defined and were darker in color than the surrounding skin. These countable lesions did not have the features of freckles, solar lentigines, seborrheic keratoses, café au lait spots, or nonmelanocytic lesions.

Nevi were counted in three size categories (by diameter): <2 mm, ≥2 mm but <5 mm, and ≥5 mm. To discriminate size, black open circles on clear plastic with internal diameters of 2 mm or 5 mm were used. Lesions were considered to be at least 2 mm, or at least 5 mm, if the lesion completely filled the applicable circle. Separate counts of palpable lesions were conducted in each of the size categories. Nevi were counted on the left arm, right leg, shoulders, and back.

Freckling and acne on the face were assessed by using charts similar to those used by Dubin et al. (12). Hair color was assessed by using hair color samples to define blonde/light brown, brown, black, and red. In addition, the nurses subjectively categorized skin color at the inner part of the left upper arm as fair, fair/medium, medium/olive, olive, or dark. Puberty was indicated by the presence of underarm hair in boys and by the onset of menarche in girls. Height and weight were measured to calculate body surface area in square meters (13). The proportion of body surface area at the body sites examined was taken to be 7% (one arm), 15.5% (one leg), and 13% (the back and both shoulders) (14). An objective measure of the visual perception of skin color was obtained by using the Minolta Chromameter II, which measures skin color in the 1976 Commission Internationale d’Eclairage L* a* b* color space parameters (15). These measurements were made on the inner part of the left upper arm. Reflectometers that measure in the 650–700-nm spectrum have been preferred in previous research on skin color in this field of investigation on the grounds that this restricted band of measurement more specifically measures melanin by excluding the pigmentary contribution of carotene and hemoglobin (16). Despite this reasoning, reflectometer-assessed skin color was not strongly associated with the presence of nevi in a recent Australian study (2). On the other hand, interviewer-assessed skin color was quite strongly related to nevi in Canadian children, although this measure has been shown to involve input from the entire visible light spectrum (400–700 nm; Ref. 17). On the basis of this information the chromameter was chosen because, like interviewer assessment, it integrates readings from across the entire visible light spectrum. In addition, the device is free of interviewer bias and its use avoids the unconscious inclusion of eye color, hair color, or freckling in the assessment of skin color.

There was a strong relationship between the L*, a*, b* readings and the nurses’ subjective assessments of skin color. The chromameter readings predicted 40% (girls) and 34% (boys) of the variance in the nurses’ assessment. Eye and hair color were also strongly associated with the nurses’ assessment of skin color. The L*, a*, and b* readings were used throughout the study as the measure of skin color because they were so strongly related to visual assessment of skin color and had the advantage of being objective.

Students were given a questionnaire to take home and complete. This allowed them to consult with their parents, particularly on questions of ethnicity and family melanoma history. The questionnaire focused on the subjects’ ethnic and residential history, their skin’s response to sunlight, history of painful sunburn lasting 2 days or more, and their sun exposure. The measures of sun exposure included estimates of the number of hours spent outside during the holidays and weekends, the frequency of sunbathing, the proportion of time spent outdoors, and memorable, specific activities, such as beach attendance and the number of holidays taken overseas or at a sunny location further north in Australia.

For data analysis, all subjects with one or more grandparents born in Asia or in Southern Europe were excluded from the data set, as were those who were not of age 14 or 15 years. Also excluded was one subject with 253 nevi of diameter at least 2 mm and 164 nevi of diameter <2 mm. The final data set included 410 students (212 females and 198 males).

Data Analysis. All nevi, both flat and raised, 2 mm or more in diameter, were included in this data analysis. The distribution of the nevus counts for each sex at each site was positively skewed, with a small number of subjects having many nevi. Median counts are reported. To permit comparison of our results with those of other studies, the nevus density at each site was calculated by dividing the nevus count by an estimate of the subject’s body surface area at that site. Categorical variables were ordered in terms of increasing risk based on a priori grounds. The degree of ordered association between categorical variables was assessed with the gamma statistic.

The association of nevi with skin color and sun exposure or sunburning was examined by using multiple linear regression methods. In each regression model, the predictors included a single measurement of skin type and a single measurement of sun exposure or sunburning. The regression residuals were normally distributed when the dependent variable, nevus density (the nevus count/square meter of body surface area at the six sites examined), was log transformed. Potential confounders of this association were age, sex, socioeconomic status, and constitutional characteristics other than measures of skin type (acne on the face and pubertal status). A putative causal factor was considered to statistically confound the association between log nevus density and a predictor (skin type or sun exposure or sunburning) if it was associated with log nevus density and with the predictor in univariate analysis and if its inclusion in the multivariate model changed the parameter estimate for the predictor by >10% (18). Age (15 versus 14 years) and pubertal status (yes versus no) were entered as dichotomous (1–0) dummy predictors. Socioeconomic status was assessed by using an index of relative socioeconomic disadvantage based on the postal code, with the weightings determined by the Australian Bureau of Statistics; the indices were categorized into tertiles. The L* readings of skin color were categorized into tertiles. A group of dichotomous dummy predictors, one for each category other than the least-risk category, was entered into the regression model for each categorical variable. To compare the nevus densities of groups of subjects cross-classified by skin color and the number of their lifetime sunburns, ratios of the geometric mean of the nevus densities in each group to that of the baseline comparison group (never sunburned, darkest tertile of skin color) were estimated by using dichotomous dummy predictors. The dependent variable was the logarithm of nevus density, and the parameter estimates were exponentiated to obtain the estimate of the ratio.

The data analysis was undertaken by using the SAS statistical package (19). Estimated probability values for the tests of the null hypotheses are quoted, and 95% CIs were estimated for nevus density ratios.

Results

The data relating to the constitutional characteristics of the subjects and their history of sunburn and sun exposure are presented in Table 1 together with the median nevus counts by...
body site. Nearly all of the girls and most of the boys had reached puberty. Nevi on the shoulders and back were more common in boys than in girls (P < 0.01), but the number was similar on the arm and leg.

Fig. 1 is the correlation matrix for ordered categorical variables constructed from questionnaire responses or measurements made by the nurses. Many of the associations in this correlation matrix are as expected. Those subjects with blue eyes were more likely to have fair hair than were those with brown eyes. Those who described themselves as tanning easily were less likely to sunburn and were more likely to spend time in the sun. The complexity of the associations summarized in Fig. 1 highlight the complexity of the interrelationships in this research setting. An apparently important observation is that girls appeared more likely than boys to report that they sunburn rather than tan if they were assessed as having light skin by the chromameter. Possibly associated with this is the stronger inverse relationship for girls than for boys between having light skin and spending time in the sun.

Table 2 provides information on the univariate association between nevus density and factors that might be expected to influence the development of nevi. The associations are presented as ratios of the geometric means of nevus density for groups of subjects falling in the extreme categories of each factor. The associations were generally strongest for the arm and leg for both sexes and weakest for nevi on the back for girls. The highest nevus density ratios were found on the arm and leg when comparing categories of skin color, skin type (kind of tan at the end of summer and skin reaction to the sun), and lifetime sunburn. These ratios were considerably higher than those for hair or eye color or those for sun exposure measurements. Only the L* score associations are presented because they proved to be much stronger than the a* or b* scores.

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Sunburn, Nevi, and Skin Color in Adolescents

Children of depth of tan and skin reaction were highly correlated with the L* reading from the chromameter and with each other. On a priori grounds, we believe that all three measurements assessed a similar property of the skin. We chose to include the L* reading in preference to tanning or skin reaction as an estimate of skin color because we believed it was most likely to be independent of the sunburning and sun exposure measures. It is objective and, therefore, unlike the skin reaction questions, could not influence directly or be influenced directly by the sun exposure/burning questions.

The subjects' lifetime history of sunburn and the L* measure of skin color from the chromameter were independently associated with nevi on the arms and legs of boys and girls and on the shoulders and backs of boys. Adjusting for age, socioeconomic status, acne on the face, or pubertal status did not alter the parameter estimates by >10% and nor did adjusting for eye color or hair color. Our conclusions were qualitatively unchanged when the other measures of skin type (tanning and skin reaction) were allowed to enter the model. The estimate of skin color from the chromameter were independently associated with nevi on the arms and legs of boys and girls and by body site. Comparing lighter-skinned boys who had been sunburned often vs. never sunburned, darker-skinned children with few sunburns. The nevus density ratio increased for boys, this ratio was 2.19 (P < 0.01). The increase in nevus density appeared to occur at lower levels of lifetime sunburn in children with lighter or medium skin than in children with darker skin. There was no evidence of linear trend in skin color effect for children with 11+ sunburns (P = 1.72 for girls; P = 0.27 for boys), suggesting that darker-skinned children who are frequently sunburned develop as many nevi as lighter-skinned children. There was no association of nevi on the shoulders and backs of girls with either skin color or history of sunburning. For boys, the pattern of nevus densities on the shoulders and back appeared to be similar to, but less pronounced than, that on their arm and leg. The nevus density ratio comparing lighter-skinned boys who had been sunburned often with darker-skinned, never-sunburned boys was 1.72 (P = 0.03).

Discussion

The findings from this study highlight the complexity of the interrelationship among constitutional factors, sun exposure, and nevi in children. In addition, they emphasize the need to examine effects separately for boys and girls and by body site and to include appropriate measures of skin color. They show that there are likely to be some differences in the relationships within categories of skin type or color, both for biological reasons and because sun-seeking behavior differs by skin color and gender.

The principal finding of this study is that lifetime history of sunburn predicts presence of nevi in a population of adolescents of primarily Anglo-Celtic origin after taking skin color into account. The results reported here suggest that this is not solely because those who are likely to sunburn have skin which is more susceptible to the development of nevi but instead that sunburn plays a causal role. Nonetheless, the effect of sunburn varies with the relative darkness of the individual's skin.
Fig. 2. Nevus density ratios for history of lifetime sunburns and tertiles of skin color readings. The reference groups were subjects with no sunburns and with the darkest skin color. There were significant linear effects in nevus density for skin color ($P < 0.01$), sunburn on the arms and legs ($P = 0.07$ for girls and $P = 0.02$ for boys), and sunburn on the shoulders and backs of boys ($P = 0.04$). 

These results suggest that only a few sunburns can produce nevi in the light skinned and, indeed, that something less than a painful sunburn might achieve this outcome. It is unlikely that the nevi that have arisen in the group of light-skinned children who report no previous sunburn are entirely unrelated to sun exposure because this group on average have 34 nevi at the sites examined. Their English counterparts, who presumably have similar skin but live in a lower total sun exposure location, have fewer than five nevi on their entire bodies (5, 20).

To explain the sun exposure-nevus link, Nicholls (21) proposed that UV radiation causes cellular damage resulting in mutation of some melanocytes, which in turn may form nevi. If one accepts this explanation, then the data here suggest that lighter-skinned individuals either experience more cellular damage with a given sunburn or that their melanocytes are more susceptible to mutation when irradiated.

The finding that lifetime history of sunburn is more strongly associated with nevi than any measure of sun exposure suggests that sunburning is more important than cumulative sun exposure. We are reluctant to confidently conclude this. Although the questions concerned with sun exposure that we chose had been used previously by others, a comparison of sun exposure and sunburning responses between this survey and a second conducted in 1993 showed that misclassification on those questions was greater for sun exposure than sunburning history. It is unlikely that we or others have yet measured sun exposure with a sufficient degree of precision to estimate its effects well. It is possible that sunburning is simply a surrogate for sun exposure but one that happens to be recalled with less misclassification than responses to any of the sun-exposure questions. However, in our data the associations of sunburning with other variables exhibited a sufficiently different pattern to those for sun exposure. “Lifetime burns” being more strongly associated with “skin reaction” than with “kind of tan” and “time in sun” being more strongly associated with “kind of tan” than with “skin reaction,” for it to be appropriate to treat sunburning as an independent factor. This has been the approach also of other investigators (2, 8).

When the association of nevi with sunburn is examined, there is no evidence of the inverted U-shaped relationship that some have found in the adult data on history of sun exposure and melanoma (22). A linear or log-linear relationship beyond a particular threshold is more consistent with our data. However, these data on nevi and sunburning do not preclude the possibility that the relationship of nevi with sun exposure follows an inverted U-shaped curve. If the association of sun exposure with sunburning had this shape, then nevi and sun exposure would be related in just this way. Although this is plausible, the absence of an inverted U-shaped relationship between sun exposure and sunburning after controlling for skin color in these data suggests that such a relationship in studies of
melanoma (22) and nevi (23) might be produced by the confounding effects of skin color.

The chromometer readings for L* predicted nevi more strongly than similar objective measurements of skin color obtained from reflectometers that have been used previously, which measure in the near infrared region of the spectrum (685 nm; Ref. 2). It is not concluded from this that chromameters are the ideal way to measure skin color but that instruments that estimate skin color across the visible light spectrum appear to have a place in this field of research and that their additional development needs to be pursued. It is possible that instruments such as spectrophotometers, which have the capacity to measure more accurately at specific wavelengths in the range 400–700 nm, may estimate even more precisely the properties of the skin that researchers are seeking to measure.

The data presented here suggest that there is much that can be learned about the sun’s effect on melanocytes in children by studying nevi and that the inferences will help in understanding the etiology of melanoma. The fact that in these subjects an interplay of skin color and sunburning was seen to be most important for those site- and sex-specific locations where melanoma later occurs and least important for the site where melanoma is uncommon (e.g., the back for females), supports the inference that insights gained in studying nevi in children have relevance to understanding the development of melanoma in adults.

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References
Sunburn associated with increased number of nevi in darker as well as lighter skinned adolescents of northern European descent.

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