Etiologic and Other Factors Predicting Nevus-Associated Cutaneous Malignant Melanoma

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Abstract

Cutaneous malignant melanomas with histologic evidence of an associated nevus (N+) may have a different risk factor profile from that of melanomas without it (N−). To address this question, a case-only analysis of 932 people with cutaneous malignant melanoma was done to identify etiologic and other factors associated with N+ melanoma. Evidence of an associated nevus was found in 36% of melanomas. N+ melanomas were thinner (P_{trend} = 0.0009) and more likely to be of the superficial spreading type than other types of melanoma. Subjects with N+ melanomas were younger (P_{trend} < 0.0001) and reported a higher nevus density on their skin than subjects with N− melanomas [odds ratio (OR), 3.1; 95% confidence interval (CI), 1.6-6.0, for high nevus density versus no nevi]. Indicators of high accumulated sun exposure were less prevalent among subjects with N+ melanomas (OR, 0.3; 95% CI, 0.2-0.4, for melanoma location on the head and neck versus location on trunk; OR, 0.2; 95% CI, 0.1-0.4, for severe solar elastosis adjacent to the melanoma versus no elastosis; OR, 0.2; 95% CI, 0.1-0.4, for lentigo maligna melanoma subtype versus superficial spreading subtype). With the exception of solar elastosis and age, all of the aforementioned variables remained significantly associated with N+ melanomas in multivariate analyses. No associations with self-reported measures of sun exposure, sunburn, or pigmentation phenotype were apparent. Our findings provide some support for the hypothesis of etiologically separate pathways for melanoma, with N+ melanomas appearing less likely to develop in the presence of characteristics suggesting high accumulated sun exposure than N− melanomas. However, it is possible that high UV exposure causes involution of nevi, thus reducing the density of nevi in exposed skin and thereby the probability of N+ melanoma. (Cancer Epidemiol Biomarkers Prev 2005;14(8):2015–22)

Introduction

Melanocytic nevi (''moles'') are benign pigmented lesions made up of nests of melanocytes in the epidermis and dermis of the skin. Light-skinned people with many nevi are at high risk of cutaneous malignant melanoma (1-4). Some 20% to 30% of melanomas have been reported to be in histologic contiguity with a nevus (5) and larger proportions of patients with melanoma give a history of an apparently stable precursor pigmented lesion at the site of the melanoma (6). The observation of a melanoma in histologic contiguity with a nevus does not seem to be simply coincidence (7-9); for example, in one study a histopathologic review of malignant skin lesions found nevi associated with 51% of reviewed melanomas but none of 40 basal cell carcinomas reviewed (9).

Recent epidemiologic findings suggest there is etiologic heterogeneity among melanomas depending on whether or not there is an associated nevus (10-17). A hypothesis of divergent pathways has been proposed: that high cumulative sun exposure is necessary for progression of some melanomas whereas for others ''pigment cell instability,'' manifested by a propensity to develop many nevi, is sufficient to drive progression (11, 17). These findings are consistent with the observation that lentigo maligna melanomas (a subtype of melanoma commonly diagnosed at sun-damaged sites) and melanomas arising on habitually sun-exposed anatomic sites are less likely to have evidence of nevus remnants than other melanomas (13, 15, 18-21).

Only two studies have reported on differences in risk factors between melanomas with (N+) and melanomas without (N−) a histologically contiguous melanocytic nevus, and there is little consistency in their findings (16, 18). Interpretation of these findings is limited by the fact that both investigations had relatively crude measures of sun exposure and low statistical power to detect differences between N+ and N− melanomas. Only one analysis included statistical tests comparing the risk factor distributions between these groups.

To address this question with sufficient study power, we have conducted a case-only analysis of data from a multicenter study of melanoma—the International Study of Genes, Environment, and Melanoma (GEM)—to investigate whether N+ and N− melanomas have different risk factor distributions.

Materials and Methods

Study Population. GEM is a collaborative project of nine centers in four countries (Australia, Canada, Italy, and the United States) investigating interactions between sun exposure, pigmentation phenotype, and genes involved in cell-cycle control (*CDKN2A*), melanin synthesis (*MC1R*), and

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DNA nucleotide excision repair in the etiology of melanoma. In it, subjects with a second or subsequent primary malignant melanoma are compared with those with a first primary only (22).

To investigate whether N+ melanomas have a different risk factor profile from N− melanomas, a case-only analysis was done using GEM participants with a first invasive primary melanoma from Ontario and British Columbia (BC), Canada and New South Wales (NSW), Australia. Eligibility criteria included age at diagnosis of 18 years or older and a diagnosis date between January 1, 2000 and June 30, 2000 (August 30, 2000 for Ontario). Subjects diagnosed with acral lentiginous melanoma were excluded, as were individuals who could not complete a telephone interview for reasons of cognitive or language difficulty. All eligible GEM participants from Ontario and BC were included in the analysis. Data from 450 eligible NSW subjects whose melanomas had been reviewed histopathologically by a study dermatopathologist at the time of data analysis were also included in the analysis; four of these subjects were later excluded (three found to have *in situ* lesions; one with insufficient tissue to assess evidence of a coexisting nevus). Local institutional review boards for each study center (University of Toronto, University of British Columbia, British Columbia Cancer Agency, and New South Wales Cancer Council) approved the project.

Data Collection. Eligible patients were ascertained from pathology reports received by the Ontario Cancer Registry, the British Columbia Cancer Registry, and the New South Wales Central Cancer Registry. Physicians caring for them were contacted by study staff to obtain permission to approach their patients. In NSW, eligible patients were then approached by the Cancer Registry for permission to give identifying and contact details to the investigators. All participants returned signed forms indicating their informed consent to participate in this study.

A self-administered mailed questionnaire was sent to each subject; it sought information on pigmentation phenotype and nevus density and residence and occupation at each decade year (i.e., at ages 10, 20, etc.) for use in a later telephone interview. A computer-assisted telephone interview collected information on lifetime exposure to sunlight, sun sensitivity phenotype, family history of melanoma and other cancers, and demographic characteristics. This telephone interview was adapted from the interview questionnaire used in the Geraldton Skin Cancer Prevention Survey (23). The Geraldton instrument was developed to improve the accuracy of recalled lifetime sun exposure through the inclusion of information from a self-completed personal calendar as noted above in which subjects recorded their residences and jobs for each year of life. It has shown high test-retest reliability (24) and an adapted version has been used successfully in a study of sun exposure and ocular melanoma (25). Information from the calendar was used in memory prompts for a series of structured questions on personal sun exposure during outdoor activities and other sun-related behaviors at the decade years. The telephone interview took between 25 and 60 minutes to complete.

Histopathologic Review. Slides from each melanoma were reviewed by a study dermatopathologist to record their histopathologic characteristics, including evidence of an associated nevus. The identification of N+ melanoma was made from the presence of cytologically benign nevus cells in the epidermis or dermis immediately adjacent to or below the melanoma cells.

A single dermatopathologist (L.F.) reviewed the diagnostic slides for all Ontario and BC subjects and for 388 of the 447 NSW subjects (the remaining 59 NSW specimens were reviewed by one other dermatopathologist in Australia). Whenever possible, the original diagnostic slides were reviewed; in some cases, recut sections were used. The slides for 36 Ontario and BC subjects were blindly rereviewed by one dermatopathologist (L.F.) to assess intrarater reliability.

Data Analysis. Putative predictors of N+ melanoma were assessed from among measures of pigmentation phenotype (ethnicity, skin, hair, and eye color, skin propensity to burn, skin tendency to tan, freckling as a child, and nevus density), tumor characteristics (histologic subtype, Breslow thickness, and Clark level), and sun exposure. An estimate of the total hours of sun exposure experienced during decade years was calculated from data collected from the telephone interview. Other measures estimating the amounts of different patterns of sun exposure were also calculated. Total sun exposure on working days was estimated as an indicator of the amount of continuous, occupationally related exposure, whereas sun exposure on nonworking days was calculated as an estimate of intermittent-type sun exposure. Information on the frequency with which the melanoma site was covered by clothing when outdoors was used to calculate a weighted estimate of total hours of sun exposure received at the melanoma site. Also included in the analysis were a history of sunburn at the site of the melanoma (both any sunburns and blistering sunburns only) and the number of vacations to sunny places. Three indirect but more objective markers of high accumulated sun exposure [body site of the tumor (head/neck versus other sites), previous diagnosis of non-melanoma skin cancer, and solar elastosis in the tissue adjacent to the melanoma] were also included in the analysis.

All data analyses were done using SAS software (26). Odds ratios (OR) with accompanying 95% confidence intervals (CI) were calculated using maximum-likelihood estimates from unconditional logistic regression to describe the association of each independent variable with evidence of N+ melanoma, with adjustment for age, sex, and study center. Factors found to be associated with N+ melanoma were included in a single multivariable model to estimate their independent effects. Continuous and ordinal variables were categorized for the purpose of the analysis to enable visualization of any nonlinear trends; tests for linear trend were also done using the Wald test by modeling each variable as a single quantitative covariate. All tests of statistical inference employed an α level of 0.05. Tests of two-way interaction were done for country of residence, tendency to tan on repeated sun exposure (1 = dark/moderate tan, 0 = mild/no tan), age (<55, 55+), and anatomic location (trunk, limbs, head/neck) using the likelihood ratio test.

A variety of subanalyses were done to provide further insight into factors associated with N+ melanoma. A reanalysis stratified by type of associated nevus (common acquired, dysplastic, other) was done to investigate whether etiologic factors predicted the presence of a particular type of nevus. In assessing histologic evidence of an associated nevus, thick melanomas are not necessarily informative, as a nevus may have been obliterated by the growing tumor. To address this issue, a reanalysis was done restricting the study sample to lesions with a Breslow thickness ≤1 mm. A reanalysis excluding lentigo maligna melanomas was also done.

Results

In Ontario and BC, there were 934 individuals with a first primary melanoma ascertained for the GEM study. Of these, 518 (55%) participated in GEM; a histopathologic review of diagnostic tissue was done for the lesions of 497 (96%) of them. There were 1,150 individuals eligible for GEM in NSW, of whom 725 (63%) participated; 446 participants whose histopathologic specimens had been reviewed for evidence of a coexisting nevus at the time of this analysis were included in

the study. Of the 943 specimens that were reviewed, 932 (99%) had sufficient tissue to assess the presence or absence of an associated nevus. There was evidence of a nevus in 339 (36%) of these 932 lesions. A rereview of 36 specimens by the study dermatopathologist suggested a fair to high reliability in scoring evidence of a nevus (κ, 0.64; 95% CI, 0.38-0.89).

Some demographic characteristics were found to be associated with N+ melanoma (Table 1). The prevalence of N+ melanoma decreased with increasing age at diagnosis ($P_{trend} < 0.0001$). Men were more likely than women to have an N+ melanoma (OR, 1.4; 95% CI, 1.0-1.8); its prevalence did not vary significantly with study center or ethnicity.

N+ melanomas differed from N− melanomas with respect to some tumor characteristics (Table 1). Lesions classified as N+ were significantly more likely to be superficial spreading melanomas than N− lesions. Lentigo maligna melanomas were found to be particularly underrepresented among N+ lesions (OR, 0.2; 95% CI, 0.1-0.4). Increasing Breslow thickness was found to be associated with a decreasing probability of finding an associated nevus ($P_{trend} = 0.0009$). Melanomas with a higher Clark's level (denoting a greater extent of invasion) were also less likely to be N+, although this association did not remain after adjustment for Breslow depth (data not shown).

The associations of skin pigmentation characteristics with N+ melanomas are summarized in Table 2. Subjects reporting many moles on their body were more likely to be diagnosed with an N+ melanoma than those with few ($P_{trend} = 0.004$ for the measure involving body diagrams; $P_{trend} = 0.02$ for the self-reported count of nevi on the back). Freckling, skin propensity to burn, skin tendency to tan, and other measures of pigmentation phenotype (skin, hair, and eye color; data not shown) were not associated with N+ melanoma.

Table 3 summarizes the associations of measures of past sun exposure with N+ melanoma. Melanomas arising on an anatomic site other than the trunk were significantly less likely to be N+. Tumors on the head and neck had a particularly low probability of an associated nevus compared with tumors on the trunk (OR, 0.3; 95% CI, 0.2-0.4). Melanomas with increasing severity of solar elastosis in adjacent tissue had a progressively lower probability of being N+ ($P_{trend} < 0.0001$). N+ melanoma was not associated with a previous diagnosis of nonmelanoma skin cancer, measures of self-reported lifetime sun exposure, history of severe sunburn, or number of vacations to sunny places (data not shown).

A multivariable model was fit to estimate the independent effects of variables found to be significantly associated with N+ melanoma (Table 4); study center and skin tendency to tan were also included in the model to adjust for potential confounding. Histologic subtype, anatomic location of melanoma, and nevus density remained significantly associated with N+ melanoma on adjusting for all factors. ORs for Breslow thickness and evidence of solar elastosis were weaker and no longer statistically significant. Study center, sex, age, and skin tendency to tan were not associated with N+ melanoma.

The aforementioned findings did not materially change on exclusion of lentigo maligna melanoma and tumors with a thickness >1 mm. The results did not vary across types of associated nevus (common acquired, dysplastic, other) or level of skin tendency to tan. However, the associations of N+

Table 1. Demographic and tumor characteristics and association of a nevus with cutaneous malignant melanoma (*N*= 932)

Characteristic	n_{N-}* (%)	n_{N+}* (%)	OR_{crude} (95% CI)	OR_{adj}† (95% CI)
Study center				
Ontario	240 (63)	142 (37)	1.0	1.0
British Columbia	66 (63)	38 (37)	1.0 (0.6-1.5)	1.0 (0.6-1.5)
New South Wales	287 (64)	159 (36)	0.9 (0.7-1.2)	1.0 (0.7-1.3)
P			0.90	0.99
Sex				
Female	293 (66)	150 (34)	1.0	1.0
Male	300 (61)	189 (39)	1.2 (0.9-1.6)	1.4 (1.0-1.8)
P			0.13	0.03
Age				
<40	84 (58)	60 (42)	1.0	1.0
40-54	165 (59)	117 (42)	1.0 (0.7-1.5)	0.9 (0.6-1.4)
55-69	178 (64)	103 (37)	0.8 (0.5-1.2)	0.7 (0.5-1.1)
70+	166 (74)	59 (26)	0.5 (0.3-0.8)	0.5 (0.3-0.7)
P_{trend}			0.0005	0.0001
Ethnicity				
British	349 (65)	191 (35)	1.0	1.0
Other Northern European	55 (64)	31 (36)	1.0 (0.6-1.7)	1.0 (0.6-1.7)
Southern/Eastern European	24 (52)	22 (48)	1.7 (0.9-3.1)	1.6 (0.8-2.9)
Mixed	120 (65)	66 (35)	1.0 (0.7-1.4)	1.0 (0.7-1.4)
Other/Don't know	34 (59)	24 (41)	1.3 (0.7-2.2)	1.2 (0.7-2.2)
P			0.99	0.99
Breslow depth (mm)				
≤0.75	286 (58)	204 (42)	1.0	1.0
0.76-1.50	175 (67)	86 (33)	0.7 (0.5-0.9)	0.7 (0.5-0.9)
1.51-4.00	104 (71)	42 (29)	0.6 (0.4-0.9)	0.6 (0.4-0.9)
>4.00	28 (80)	7 (20)	0.4 (0.2-0.8)	0.4 (0.2-0.8)
P_{trend}			0.0008	0.0009
Histologic subtype				
SSM	388 (57)	288 (43)	1.0	1.0
NM	69 (72)	27 (28)	0.5 (0.3-0.8)	0.7‡ (0.4-1.2)
LMM	77 (88)	11 (13)	0.2 (0.1-0.4)	0.2‡ (0.1-0.4)
NOS/Other	59 (83)	12 (17)	0.3 (0.2-0.5)	0.3‡ (0.2-0.7)
P			<0.0001	<0.0001

Abbreviations: Adj, adjusted; P_{trend}, *P* value, test of trend; SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; NOS, not otherwise specified.

*Counts may not sum to the total number of study subjects due to missing data.

†Adjusted for study center, sex and age.

‡Adjusted for study center, sex, age and Breslow thickness.

melanoma with study center and age were found to differ between head/neck melanomas and melanomas arising on other sites (Table 5). There was little or no difference between study centers or age groups in the prevalence of N+ in melanomas of the trunk or limbs. Among melanomas diagnosed on the head and neck, however, the prevalence of N+ melanomas was lower for older (OR, 0.25; 95% CI, 0.09-0.70) relative to younger subjects and for residents of NSW (OR, 0.62; 95% CI, 0.23-1.67) relative to those of Ontario/British Columbia. These associations were stronger when lentigo maligna melanoma was excluded from the analysis (age > 55: OR, 0.18; 95% CI, 0.04-0.73; NSW residence: OR, 0.13; 95% CI, 0.03-0.69).

Discussion

This case-only study was done to investigate the relationship between etiologic and other factors and the presence of an associated nevus in cutaneous malignant melanoma. Evidence of an associated nevus was detected in 36% of the 932 melanomas included in this analysis. Our analysis identified statistically significant, independent, positive associations of N+ melanoma with high nevus density on the skin, location of melanoma on the trunk, and the superficial spreading melanoma type of melanoma. These associations seemed to largely explain initially observed associations with N+ melanoma for male sex, younger age at diagnosis, thinner melanoma, and solar elastosis in adjacent tissue.

Previous studies have found, as we did, that superficial spreading melanoma was more likely than other histologic subtypes of melanoma to be N+ (19, 21). In addition, many studies have reported a lower probability of finding an associated nevus with thicker melanomas (5, 15, 21, 27-29). That histologic type rather than thickness seemed to be the primary factor may indicate that superficial spreading melanoma is more likely to arise in a nevus than other types of melanoma and not simply that progressive horizontal expansion and vertical growth of melanoma obliterate an associated nevus. That a partial biopsy only was available for histologic review for more melanomas classified as not otherwise specified (NOS) or other (91%) than other types (24%) may also have been an influential factor. The small amount of tissue available from a partial biopsy limits the ability both to assign a specific histologic subtype and to identify evidence of nevus involvement.

Lentigo maligna melanomas had the lowest prevalence of an associated nevus. This observation is unlikely to be confounded by tumor growth, as lentigo maligna melanoma was found in our study to be typically diagnosed at a more superficial stage than other subtypes (data not shown). Other studies have also found lentigo maligna melanoma to have the lowest probability of an associated nevus among all subtypes (9, 19). Lentigo maligna melanoma possesses clinical characteristics and epidemiologic patterns that are distinct from other melanoma subtypes. They usually arise later in life on habitually sun-exposed body sites and are commonly adjacent to skin with signs of chronic sun damage (30). These observations could suggest that lentigo maligna melanoma arises along a causal pathway driven by accumulated sun exposure as distinct from arising in association with a nevus. Alternatively, it has been argued that associations of lentigo maligna melanoma with indicators of accumulated sun exposure may be an artifact of the inclusion of adjacent sun damage in the pathologic definition of lentigo maligna melanoma (31, 32).

We found increased nevus density to be positively associated with having an N+ melanoma. One of the two previous studies comparing nevus density between N+ and N− melanomas reported a similar relationship (18); the other reported null findings (16). A positive association between nevus density and N+ melanoma is to be expected, given that a propensity for skin to develop nevi is necessary for observing melanoma with an associated nevus. Aside from nevus density, no other indicator of skin pigmentation was associated

Table 2. Skin pigmentation characteristics and association of a nevus with cutaneous malignant melanoma (*N*= 932)

Characteristic	n_{N-}* (%)	n_{N+}* (%)	OR_{crude} (95% CI)	OR_{adj}† (95% CI)
Nevus density (body diagrams)				
No nevi	174 (75)	57 (25)	1.0	1.0
Low	299 (60)	198 (40)	2.0 (1.4-2.9)	1.8 (1.3-2.6)
Moderate	92 (63)	54 (37)	1.8 (1.1-2.8)	1.5 (0.9-2.4)
High	22 (47)	25 (53)	3.5 (1.8-6.6)	3.1 (1.6-6.0)
P_{trend}			0.0001	0.004
Nevi on back				
0-3	209 (72)	81 (28)	1.0	1.0
4-10	151 (61)	95 (39)	1.6 (1.1-2.3)	1.5 (1.1-2.2)
11-25	123 (61)	79 (39)	1.7 (1.1-2.4)	1.4 (1.0-2.1)
>25	106 (56)	83 (44)	2.0 (1.4-3.0)	1.7 (1.1-2.5)
P_{trend}			0.0004	0.02
Facial freckles at age 10				
Few	404 (62)	247 (38)	1.0	1.0
Moderate	160 (68)	77 (33)	0.8 (0.6-1.1)	0.8 (0.6-1.1)
Many	26 (65)	14 (35)	0.9 (0.5-1.7)	0.8 (0.4-1.6)
P_{trend}			0.13	0.07
Skin propensity to burn				
Severe burn with blistering	48 (68)	23 (32)	0.9 (0.5-1.5)	1.0 (0.6-1.7)
Severe burn, followed by peeling	208 (61)	133 (39)	1.2 (0.9-1.6)	1.2 (0.9-1.6)
Mild burn, followed by tan	272 (65)	149 (35)	1.0	1.0
Tan, no burn	45 (64)	25 (36)	1.0 (0.6-1.7)	1.0 (0.6-1.8)
P_{trend}			0.78	0.53
Skin tendency to tan				
Dark tan	92 (60)	62 (40)	1.0	1.0
Moderate tan	225 (60)	149 (40)	1.0 (0.7-1.4)	1.0 (0.7-1.5)
Mild tan	196 (70)	85 (30)	0.6 (0.4-1.0)	0.7 (0.5-1.1)
No suntan or freckling	56 (64)	32 (36)	0.9 (0.5-1.5)	1.0 (0.6-1.8)
P_{trend}			0.06	0.25

*Counts may not sum to the total number of study subjects due to missing data.
†Adjusted for study center, sex, and age.

Table 3. Indicators of sun exposure and association of a nevus with cutaneous malignant melanoma (*N*= 932)

Characteristic	n_{N-}* (%)	n_{N+}* (%)	OR_{crude} (95% CI)	OR_{adj}† (95% CI)
Anatomic location of melanoma				
Trunk	207 (51)	201 (49)	1.0	1.0
Head/neck	98 (82)	21 (18)	0.2 (0.1-0.4)	0.3 (0.2-0.4)
Upper limb	127 (73)	47 (27)	0.4 (0.3-0.6)	0.4 (0.3-0.6)
Lower limb	142 (70)	60 (30)	0.4 (0.3-0.6)	0.4 (0.3-0.6)
Other/NOS	19 (66)	10 (35)	0.5 (0.3-1.2)	0.5 (0.2-1.1)
P			<0.0001	<0.0001
Solar elastosis in adjacent tissue				
None	228 (56)	180 (44)	1.0	1.0
Mild/moderate	216 (63)	127 (37)	0.8 (0.6-1.0)	0.7 (0.5-1.0)
Marked	115 (85)	20 (15)	0.2 (0.1-0.4)	0.2 (0.1-0.4)
P_{trend}			<0.0001	<0.0001
History of nonmelanoma skin cancer				
No	444 (62)	269 (38)	1.0	1.0
Yes	134 (68)	62 (32)	0.8 (0.6-1.1)	0.9 (0.6-1.2)
P			0.12	0.37
Total hours of sun exposure during decade years				
Overall				
0-2,200	149 (61)	95 (39)	1.0	1.0
2,201-3,600	154 (64)	87 (36)	0.9 (0.6-1.3)	0.8 (0.6-1.2)
3,601-5,400	146 (65)	79 (35)	0.8 (0.6-1.2)	0.9 (0.6-1.4)
>5,400	133 (65)	72 (35)	0.8 (0.6-1.2)	1.0 (0.6-1.5)
P_{trend}			0.38	0.90
On nonworking days only				
0-1,100	141 (61)	90 (39)	1.0	1.0
1,101-1,600	134 (62)	81 (38)	0.9 (0.6-1.4)	0.9 (0.6-1.3)
1,601-2,300	151 (64)	84 (36)	0.9 (0.6-1.3)	0.8 (0.5-1.2)
>2,300	156 (67)	78 (33)	0.8 (0.5-1.1)	0.8 (0.5-1.2)
P_{trend}			0.18	0.32
On working days only				
0-900	180 (62)	109 (38)	1.0	1.0
901-1,700	142 (66)	74 (34)	0.9 (0.6-1.4)	0.8 (0.6-1.2)
1,701-3,000	129 (64)	74 (36)	0.9 (0.6-1.3)	1.0 (0.7-1.5)
>3,000	131 (63)	76 (37)	0.8 (0.5-1.1)	1.1 (0.7-1.7)
P_{trend}			0.89	0.59
To the melanoma site				
0-800	126 (59)	89 (41)	1.0	1.0
801-2,000	141 (59)	99 (41)	1.1‡ (0.7-1.6)	1.0‡ (0.7-1.5)
2,001-3,800	139 (63)	82 (37)	1.0‡ (0.7-1.6)	1.0‡ (0.7-1.5)
>3,800	176 (74)	63 (26)	0.9‡ (0.6-1.3)	0.9‡ (0.6-1.4)
P_{trend}			0.48	0.61

*Counts may not sum to the total number of study subjects due to missing data.
†Adjusted for study center, sex, and age.
‡Adjusted for anatomic location of melanoma.

with N+ melanoma. This was true also when we restricted our sample to thin tumors, in which obliteration of nevus remnants by tumor is less likely. Misclassification in the measurement of skin pigmentation characteristics and nevus density may be relatively high given that these data were self-reported by subjects. Consequently, we cannot rule out the possibility that associations with pigmentation characteristics may have been obscured by the effects of measurement error. However, the consistency across these measures in suggesting no association with N+ melanoma argues against this possibility. The studies referred to above each reported associations between N+ melanoma and some aspect of pigmentation phenotype, but the findings were inconsistent across studies for every factor (16, 18). This lack of consistency, coupled with the null findings of this study, does not support the idea that N+ melanomas differ from N− melanomas with respect to the distribution of pigmentation characteristics other than density of nevi.

The anatomic site of melanoma was strongly associated with N+ melanoma; melanomas on the trunk were most likely and those on the head and neck were least likely to be N+. This pattern has been consistently reported in other studies (13, 15, 19, 20). It cannot simply be ascribed to the association of lentigo maligna melanoma lesions with skin of the head and neck, as the association remained on adjustment for histologic type and on exclusion of lentigo maligna melanoma from the analysis. It is also unlikely to be due to the anatomic distribution of nevi because nevus density, as a function of surface area, has been reported to be higher on the face than on the back (33). Melanomas diagnosed at older age and in the presence of solar elastosis, a histologic marker of chronic sun damage, were also less likely to be N+. However, neither association persisted after adjustment for location, nevus density, and histopathologic type. In addition, no such relationship was found for other indicators of high cumulative sun exposure (self-reported total hours of lifetime sun exposure and previous diagnosis of nonmelanoma skin cancer). The failure to observe associations among such measures may be due to the effects of measurement error as well as nonspecificity in capturing sun exposure at the melanoma site.

Findings from our separate analyses of trunk melanomas and head/neck melanomas suggest the importance of accounting for site specificity in detecting a relationship between high cumulative UV exposure and N+ melanoma. Both age > 55 and residence in NSW were associated with a reduced probability of N+ melanoma among melanomas arising on the head and neck, but not among tumors of the trunk or limbs. In general, age and residence in a region of high ambient UV irradiance, such as NSW, represent potentially informative surrogate measures of high cumulative UV dose; however, their strength as proxies of high cumulative exposure at a

Table 4. Variables independently predicting association of a nevus with cutaneous malignant melanoma (*N*= 932) in a multivariable analysis

Characteristic	Category	OR* (95% CI)	*P*	P_{trend}
Study center	Ontario	1.0	0.80	—
	British Columbia	1.0 (0.6-1.6)		
	New South Wales	1.1 (0.8-1.5)		
Sex	Female	1.0	0.80	—
	Male	1.0 (0.7-1.5)		
Age	<40	1.0	0.79	0.41
	40-54	1.1 (0.7-1.7)		
	55-69	1.1 (0.7-1.8)		
	70+	0.9 (0.5-1.6)		
Breslow thickness	≤0.75	1.0	0.25	0.17
	0.76-1.50	0.7 (0.5-1.0)		
	1.51-4.00	0.9 (0.6-1.5)		
	>4.00	0.5 (0.2-1.4)		
Skin tendency to tan	Dark tan	1.0	0.15	0.84
	Moderate tan	1.0 (0.7-1.5)		
	Mild tan	0.7 (0.5-1.2)		
	No suntan/freckling	1.3 (0.7-2.5)		
Histologic subtype	SSM	1.0	0.008	—
	NM	0.6 (0.3-1.0)		
	LMM	0.4 (0.2-0.9)		
	Other/NOS	0.5 (0.2-1.3)		
Nevus density (body diagrams)	No nevi	1.0	0.05	0.06
	Low	1.5 (1.1-2.3)		
	Moderate	1.2 (0.7-2.1)		
	High	2.3 (1.1-4.8)		
Anatomic location of melanoma	Trunk	1.0	0.0009	—
	Head/neck	0.6 (0.3-1.1)		
	Upper limb	0.5 (0.3-0.8)		
	Lower limb	0.4 (0.3-0.7)		
	Other/NOS	0.5 (0.2-1.3)		
Solar elastosis in adjacent tissue	None	1.0	0.18	0.11
	Mild/moderate	0.9 (0.6-1.2)		
	Marked	0.5 (0.2-1.0)		

*Adjusted for all variables shown in table.

given anatomic site is dependent on the frequency with which that site is uncovered when outdoors. Our finding that age > 55 and NSW residence are negatively associated with N+ melanoma only among head/neck melanomas may reflect the fact that these variables are adequate surrogates for high cumulative UV exposure at this site, but not for skin on the trunk or limbs.

Our findings are reasonably consistent with the hypothesis advanced by Whiteman et al. (11) that there are two pathways for genesis of melanoma: one in which melanocytes of nevus-prone individuals require sunlight only in the early stages of tumorigenesis, with host factors driving tumor progression thereafter, and another in which melanocytes of people with a low propensity to develop nevi require long-term accumulation of sun exposure for melanoma to develop. In our study, location on the trunk, superficial spreading melanoma histologic type, high nevus density, and N+ melanomas indicate the first pathway; location on the head and neck

Table 5. Study center, age at diagnosis, and association of a nevus with cutaneous malignant melanoma; stratified by melanoma location (trunk, limbs, head/neck)

Characteristic	Melanoma location									$P_{interaction}$
	Trunk			Limbs			Head/neck			
	n_{N-}*	n_{N+}	OR (95% CI)	n_{N-}	n_{N+}	OR (95% CI)	n_{N-}	n_{N+}	OR (95% CI)	
Study center										
All melanomas:										
Ontario/British Columbia	113	112	1.0†	141	55	0.39 (0.25-0.60)	44	12	0.30 (0.15-0.60)	0.59
New South Wales	94	89	1.00 (0.67-1.48)	128	52	0.42 (0.27-0.65)	54	9	0.19 (0.09-0.40)	
Excluding LMM:										
Ontario/British Columbia	111	112	1.0	131	55	0.41 (0.27-0.64)	21	11	0.53 (0.24-1.15)	0.03
New South Wales	88	87	1.01 (0.68-1.50)	112	51	0.46 (0.30-0.71)	34	2	0.06 (0.01-0.27)	
Age										
All melanoma:										
<55 y	97	99	1.0‡	124	62	0.48 (0.31-0.75)	19	10	0.52 (0.23-1.17)	0.06
55+ y	110	102	0.92 (0.62-1.36)	145	45	0.30 (0.19-0.47)	79	11	0.14 (0.07-0.27)	
Excluding LMM										
<55 y	95	99	1.0	120	62	0.49 (0.32-0.77)	14	9	0.62 (0.25-1.50)	0.03
55+ y	104	100	0.93 (0.62-1.39)	123	44	0.34 (0.22-0.54)	41	4	0.09 (0.03-0.28)	

*Counts may not sum to the total number of study subjects due to missing data.
†Adjusted for sex and age (<55, 55+).
‡Adjusted for sex and study center (Ontario/British Columbia, New South Wales).

particularly, but other nontruncal sites as well, lentigo maligna melanoma histologic type, and N− melanomas indicate the second. Whereas nodular melanomas fall somewhat in the middle, it is possible that these lesions can arise from either pathway through superficial spreading melanoma or lentigo maligna melanoma (31). The possibility of two pathways, one characterized by an association with nevi and one with long-term accumulation of sun exposure, is also supported by the finding of Bataille et al. (34) that presence of nevi and presence of solar keratoses (lesions believed to be caused by long-term accumulated sun exposure) were positively and independently predictive of melanoma risk but *negatively* associated with one another. Findings from a subsequent case-only study by Whiteman et al. (17) were also consistent with this dual pathway hypothesis.

Both Whiteman's study (11) and another related study conducted by our group[6] suggest that p53 immunostaining of melanoma cells may be an additional characteristic of the N− pathway. A recent study of predictors of *BRAF* mutation in a melanoma case series also offers evidence of a genetic change that could underlie two pathways for the genesis of melanoma. Maldonado et al. found *BRAF* mutations in 23 of 43 melanomas occurring on skin subjected to intermittent UV exposure, but in only 1 of 12 melanomas arising on skin exhibiting chronic sun damage ($P < 0.001$; ref. 35). Given that *BRAF* mutations have been reported to occur in a large majority of nevi, the investigators explored the frequency with which a nevus was associated with *BRAF* mutations in melanoma. They found melanomas with an associated nevus to have only a moderately and not statistically significantly higher prevalence of *BRAF* mutations than other melanomas (55% versus 43%).

There is an alternative explanation to the dual pathway hypothesis for the association of indicators of higher sun exposure with N− melanomas: high cumulative sun exposure may reduce the density of nevi and thus the probability that any melanoma that arises in heavily sun exposed skin will be nevus associated. There is evidence that high cumulative sun exposure plays a role in the involution of nevi, although the evidence linking cumulative sun exposure to nevus density in adults is equivocal (36-38). The possibility that high cumulative sun exposure may reduce the density of nevi, and thus the probability of N+ melanoma, in heavily sun exposed skin is supported by the evidence that higher age and residence in NSW, both indicators of high exposure to solar UV radiation, are associated with a substantially reduced prevalence of N+ melanoma on skin of the head and neck, which is usually exposed to the sun when people are outdoors.

We have considered whether biases in sample selection may explain our findings. Although our participation rate was low, we believe it is unlikely that our findings have been influenced by selection bias. For such bias to be present, the relationship between study participation and exposure status would have to differ according to evidence of a coexisting nevus. This is highly unlikely. Subjects from NSW included in this analysis were generally comparable to other GEM subjects from NSW. However, melanomas included in this sample were significantly more likely to have been classified as superficial spreading melanoma (58% versus 41%) and less likely to have been unclassified (15% versus 25%) at diagnosis. This difference may reflect the fact that diagnostic slides were first requested from laboratories that had reviewed specimens from multiple GEM subjects. Such laboratories may differ from other laboratories in pathologist expertise (pathologists less experienced with melanoma may be less likely to assign a histologic subtype) or in the type of tissue sent to them (histologic subtype is more difficult to ascertain from biopsies than wide excisions). It is unlikely that any such differences between samples would have introduced selection bias into this study. For bias to be introduced, the relationship between selection into the study sample and exposure status would have to differ according to evidence of a coexisting nevus. This is implausible.

This case-only analysis indicates that people with nevus-associated melanomas differ from those with melanomas apparently arising in the absence of a nevus with respect to nevus density, location of the melanoma (possibly indicating amount of accumulated sun exposure), and histologic type of melanoma. These results do not provide unequivocal support for the hypothesis of etiologic heterogeneity of melanoma but do indicate the need for further investigation of this issue.

[6] Purdue MP, From L, Kahn HJ, Armstrong BK, Kricker A, Gallagher RP, McLaughlin JR, Klar NS, Marrett LD. Etiologic factors associated with p53 immunostaining in cutaneous malignant melanoma. In press.

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Appendix A. The Genes, Environment, and Melanoma Study Group

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