Number of Nevi and Early-Life Ambient UV Exposure Are Associated with *BRAF*-Mutant Melanoma

Nancy E. Thomas,[1] Sharon N. Edmiston,[2] Audrey Alexander,[2] Robert C. Millikan,[2] Pamela A. Groben,[1] Honglin Hao,[1] Dawn Tolbert,[2] Marianne Berwick,[4] Klaus Busam,[5] Colin B. Begg,[6] Dianne Mattingly,[2] David W. Ollila,[3] Chiu Kit Tse,[2] Amanda Hummer,[6] Julia Lee-Taylor,[7] and Kathleen Conway[2]

Departments of [1]Dermatology, [2]Epidemiology, and [3]Surgery, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina; [4]Department of Medicine, Division of Epidemiology, University of New Mexico, Albuquerque, New Mexico; Departments of [5]Pathology and [6]Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York; and [7]Atmospheric Chemistry Division, National Center for Atmospheric Research, Boulder, Colorado

Abstract

Malignant melanomas often contain *BRAF* or *NRAS* mutations, but the relationship of these mutations to ambient UV exposure in combination with phenotypic characteristics is unknown. In a population-based case series from North Carolina, 214 first primary invasive melanoma patients in the year 2000 were interviewed regarding their risk factors. Ambient solar UV exposures were estimated using residential histories and a satellite-based model. Cases were grouped on the basis of *BRAF* and *NRAS* somatic mutations, determined using single-strand conformation polymorphism analysis and radiolabeled DNA sequencing, and the risk profiles of these groups were compared. Mutually exclusive *BRAF*-mutant and *NRAS*-mutant cases occurred at frequencies of 43.0% and 13.6% with mean ages at diagnosis of 47.3 and 62.1 years, respectively. Tumors from patients with >14 back nevi were more likely to harbor either a *BRAF* mutation [age-adjusted odds ratio (OR), 3.2; 95% confidence interval (95% CI), 1.4-7.0] or an *NRAS* mutation (age-adjusted OR, 1.7; 95% CI, 0.6-4.8) compared with patients with 0 to 4 back nevi. However, *BRAF*-mutant and *NRAS*-mutant tumors were distinctive in that *BRAF*-mutant tumors were characteristic of patients with high early-life ambient UV exposure (adjusted OR, 2.6; 95% CI, 1.2-5.3). When ambient UV irradiance was analyzed by decadal age, high exposure at ages 0 to 20 years was associated with *BRAF*-mutant cases, whereas high exposure at ages 50 and 60 years was characteristic of *NRAS*-mutant cases. Our results suggest that although nevus propensity is important for the occurrence of both *BRAF* and *NRAS*-mutant melanomas, ambient UV irradiance influences risk differently based on the age of exposure. The association of *BRAF* mutations with early-life UV exposure provides evidence in support of childhood sun protection for melanoma prevention. (Cancer Epidemiol Biomarkers Prev 2007;16(5):991–7)

Introduction

Melanoma risk factors include increased number of melanocytic nevi, solar UV exposure, including high levels in childhood, and fair phenotypic characteristics (1-4). An increased number of nevi is a very strong risk factor for the occurrence of melanoma (1). Ambient UV irradiance in early life (birth and 10 years of age) has been found by Kricker et al. (5) to be one of the strongest sun-related risk factors for multiple primary melanomas, and these results should theoretically apply to risk of all melanomas (6). Phenotypic factors identified as associated with increased melanoma risk include sun sensitivity, freckles, blond or red hair, and light-colored eyes (3). However, the epidemiology of melanoma is complex (7), a variety of histologic subtypes exist, and the presence of different genetic alterations (8) indicates that melanoma is a heterogeneous disease. A better understanding of melanoma heterogeneity and its relationship to risk factors should assist efforts toward improving prevention.

BRAF mutations have been reported to occur in ~50% of melanomas, predominately in or around codon 600 (accession number NM_004333.2) in exon 15 with the remainder in exon 11 (9). *NRAS* mutations have been reported to occur, exclusive of *BRAF* mutations, in ~15% of melanomas, mainly in codons 12/13 and 61 in exons 2 and 3 (NM_002524; ref. 10). Most of these mutations have been shown to activate the RAS–RAF–mitogen-activated protein kinase/extracellular signal-regulated kinase kinase–extracellular signal-regulated kinase cell signaling pathway and have been implicated in cancer initiation and progression (11, 12).

The objective of this study was to investigate the relationships of *BRAF* and *NRAS* somatic mutations with phenotypic susceptibility and ambient solar UV exposure, including childhood exposure. These relationships were examined for 214 population-based first invasive primary cutaneous melanoma cases from North Carolina in the year 2000 who were interviewed with regard to their risk factors. Erythemal UV irradiance, derived from residential history and satellite-based measures, was used to estimate ambient solar UV exposure. Tumors were screened for *BRAF* and *NRAS* mutations using single-strand conformation polymorphism analysis and DNA sequencing.

Materials and Methods

Study Population and Protocol. The participants in this study were from one center (North Carolina) in the Genes,

Received 12/11/06; revised 2/22/07; accepted 3/8/07.

Grant support: Lineberger Comprehensive Cancer Center grant; Dermatology Foundation grant; and National Cancer Institute grants CA102096, CA103089, CA112243, and CA83180.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Cancer Epidemiology Biomakers and Prevention online (http://cebp.aacrjournals.org/).

Requests for reprints: Nancy E. Thomas, Department of Dermatology, University of North Carolina, 3100 Thurston Bowles Building, CB7287, Chapel Hill, NC 27599. Phone: 919-966-0785; Fax: 919-966-6460. E-mail: nthomas@med.unc.edu

doi:10.1158/1055-9965.EPI-06-1038

Environment, and Melanoma Study (5, 13, 14). Eligible subjects, identified by the population-based North Carolina Cancer Registry, were diagnosed with a first incident cutaneous invasive melanoma between January 1 and December 31, 2000, and resided within 42 counties of North Carolina. All of the dermatologists and hospitals in the 42 counties were responsible for reporting new cases. North Carolina dermatologists were notified of the study and reporting procedures and asked where their histopathology specimens were being sent. The study protocol was approved by the Institutional Review Board of The University of North Carolina at Chapel Hill. Physician approval was sought before contacting ascertained cases, and each participant or their guardian provided informed consent. Of the 582 eligible patients at this study site, 285 (49%) participated. We conducted analyses comparing participants and nonparticipants using information collected from the Central Cancer Registry. There were no significant differences between these groups based on age, gender, histologic type, tumor site, or Breslow thickness.

Participants completed a self-administered questionnaire and 1-h telephone interview regarding demographic characteristics, medical history, and phenotypic factors. Using a glossy-colored guide to aid in differentiating between nevi and other skin lesions, subjects were asked to have the nevi on their backs counted by a family member or friend, and, for the purposes of this article, back nevi counts were categorized based on tertiles in the wild-type group. The presence of nevi was also assessed, as in Marrett et al. (15), by asking the subject to select which of four whole-body diagrams corresponded most closely to his or her nevus density, and the two diagrams representing the most nevi were combined to form the highest category.

Erythemal UV irradiances were calculated as wavelength-integrated spectral irradiance between 250 and 400 nm, weighted by the Commission Internationale de l'Eclairage erythemal sensitivity function (16), which describes the stronger skin-reddening capacity of light at shorter wavelengths. The tropospheric UV-visible model (17) was used to calculate the irradiances as a function of solar zenith angle, ozone column, and surface elevation, after the method of Madronich (18). The model used a discrete ordinates method (19) and a pseudospherical correction (20). Corrections for variations in the Earth-Sun distance and for cloud cover (21) were applied. Ozone column and cloud reflectivity data were obtained from the satellite-borne Total Ozone Mapping Spectrometer (22-24), for November 1978 to June 2000.

For each participant, the location of their home at birth and each decade up to age at diagnosis was recorded. Location-specific erythemal UV dose values from the 1978 to 1989 climatology were applied to all participant exposure dates before 1990, and values from the 1990 to 2000 climatology were applied to exposure dates from 1990 onward. These decadal values were integrated to give lifetime total potential exposure. As in Kricker et al. (5), early-life UV irradiance was defined as the average of UV irradiance at birth and age 10 years, and average annual lifetime UV irradiance was calculated as the lifetime total divided by age.

Participants provided informed consent to obtain diagnostic slides and melanoma recuts. Tumors were reviewed by one dermatopathologist (K.B.). Of the 285 cases, 16 were excluded after histopathologic review based on ineligible diagnoses (12 *in situ*, 1 mucosal and 1 metastatic melanoma, and 2 dysplastic nevi). Eligible cases (N = 269) were classified according to criteria proposed by Clark et al. (25) and McGovern et al. (26). Although in many cases, only biopsies of the melanoma were available for review, the biopsies typically contained the majority of the tumor, except for cases of lentigo maligna melanoma (LMM), but the findings were representative. The diagnosis of LMM was based on the presence of a predominant lentiginous growth pattern of skin with some evidence of sun damage (mild to moderate or severe solar elastosis). Chronic sun damage (CSD) was scored using the 0 to 3+ multipoint scale and dichotomized into non-CSD (level 0 to 2−) and CSD (level 2 to 3+) melanomas, as in Landi et al. (27).

***BRAF* and *NRAS* Mutational Analyses.** Of the 269 participants eligible after slide review, we obtained tissue recuts for 245 of 269 (91%). Of tissues collected, 223 of 245 (91%) had sufficient tissue on the recuts to proceed with analysis, and, of these, 214 of 223 (96%) had successful PCR amplification and sufficient high-quality DNA for complete mutational analysis of *BRAF* exons 11 and 15 and *NRAS* exons 2 and 3. Using information collected from study participants, we compared participants whose tumors were successfully analyzed for *BRAS* and *NRAS* mutations versus participants whose tumors were not analyzed. There were no significant differences based on age, gender, histologic type, site, Breslow thickness, number of back nevi, nevus density, propensity to tan, childhood freckles, hair color, eye color, estimated lifetime average ambient UV, or estimated early-life ambient UV.

When indicated, because of small tumor size or admixture of nonmalignant cells, tumor cells were selectively procured using laser capture microdissection under the supervision of a dermatopathologist (P.G.). Tumor DNA was prepared as previously described (28) and analyzed for mutations in *BRAF* exons 11 and 15 (including codons 466 and 600) and in *NRAS* exons 2 and 3 (including codons 12, 13 and 61), using single-strand conformational polymorphism (SSCP) analysis and radiolabeled sequencing of SSCP-positive samples and 10% of negatives. Radiolabeled, rather than automated fluorescent, sequencing of PCR products was carried out because it was more sensitive in tumor samples with mixed populations of mutant and normal cells (29, 30). All mutations were independently confirmed, and mutational artifacts were eliminated by sequencing a separately amplified aliquot of DNA. Furthermore, we directly compared SSCP and sequencing in the *BRAF* and *NRAS* fragments for a series of 40 specimens. In no case did SSCP fail to detect a mutation that was observable by sequencing. In addition, in the 10% of SSCP-negative samples that we sequenced, we did not observe a failure of SSCP to detect mutations. Experimental details are in Supplementary Information.

Statistical Analysis. The goal of the study was to compare the etiologic profiles of tumors characterized by distinct molecular pathologic features (i.e., the presence of *BRAF* or *NRAS* mutations versus the absence of these mutations) using a case series design (31). In such a design, significant differences in the distribution of risk factors between pathologic subgroups provide evidence of etiologic heterogeneity. Because *BRAF* and *NRAS* mutations were exclusive of each other in all samples, cases were analyzed according to three subclassifications, those containing either a *BRAF* mutation (*BRAF*+), an *NRAS* mutation (*NRAS*+), or neither mutation (wild-type).

The cutoff points for UV exposures were chosen based on the distribution in our sample and previous findings (5) that early-life UV, which was the strongest sun-related risk factor distinguishing cases and controls in the entire Genes, Environment, and Melanoma study, showed an elevated odds ratio (OR) for risk of melanoma. For both lifetime and early-life UV, low UV was defined as the lowest tertile and high UV was defined as the combined upper two tertiles of exposure for the wild-type group.

The ANOVA test was used for comparison of mean ages across mutational subtypes. ORs and accompanying 95% confidence intervals (95% CI) were calculated in logistic regression models in SAS (SAS Institute, 1989) with adjustment for age (continuous). Trend tests were accomplished by modeling each variable as a single quantitative covariate. All significance tests were two-sided and a P value of 0.05 was

Table 1. Spectrum of *BRAF* and *NRAS* mutations in invasive cutaneous melanomas (*N* = 214)

Gene	Mutation	Base change	No. patients	%*
BRAF	V600E	GTG to GAG	60	28.0
	V600K	GTG to AAG	15	7.0
	V600E	GTG to GAA	4	1.9
	V600R	GTG to AGG	2	0.9
	V600D	GTG to GAT	1	0.5
	VKS600-602DT †	GTG-AAA-TCT to GAT-ACT	1	0.5
	K601E	AAA to GAA	2	0.9
	K601N	AAA to AAC	1	0.5
	L597R	CTA to CGA	1	0.5
	D594N	GAT to AAT	1	0.5
	G469A	GGA to GCA	1	0.5
	G466E	GGA to GAA	1	0.5
	G455R	GGG to AGG	1	0.5
	G455E	GGG to GAG	1	0.5
NRAS	Q61K	CAA to AAA	14	6.5
	Q61R	CAA to CGA	10	4.7
	Q61L	CAA to CTA	4	1.9
	Q61H	CAA to CAC	1	0.5

*Percentage of melanomas screened that carry this alteration.
†A deletion of six bases and an insertion of three bases, with a net loss of three bases. Wild-type residue 603/Arg would then follow the Asp-Thr.

considered statistically significant. In addition, models were fit to estimate the independent effects of age at diagnosis, early-life UV exposure, and number of back nevi in their association with *BRAF* and *NRAS* mutations. ORs for *BRAF* and *NRAS* were estimated using separate logistic regression models. Such an approach is simpler and leads to equivalent results compared with polytomous logistic regression modeling (32). The *BRAF* and *NRAS* models were reanalyzed excluding LMM and acral lentiginous melanoma (ALM).

Results

Subject Characteristics. Study cases had a mean age at diagnosis of 52.2 years and were 54.7% male. The study included a high percentage (56.1%) of thin (Breslow thickness <0.75 mm) melanomas. The percentages of histologic subtypes were 79% superficial spreading melanoma, 4.7% nodular melanoma, 10.3% LMM, 0.9% ALM, 1.4% nevoid or spitzoid melanoma, and 3.7% unclassifiable melanoma. The composition of our cases is consistent with other recent population-based studies, reflecting a downward trend for tumor thickness and a trend toward more superficial spreading melanomas over time (33, 34).

***BRAF* and *NRAS* Mutational Frequencies.** *BRAF* and *NRAS* mutations were found in 92 of 214 (43.0%) and 29 of 214 (13.6%) melanomas, respectively (Table 1), and were exclusive of each other, with a low probability of this distribution occurring by chance ($P < 0.001$). Of the *BRAF* mutations, 88 of 92 (95.7%) were in exon 15 in and around codon 600, whereas the other 4 of 92 (4.3%) were in exon 11. Of these *BRAF* mutations, 98.6% have been associated with *in vitro* enhancement of downstream extracellular signal-regulated kinase activity (9, 35, 36). The majority of *BRAF* mutations found in the invasive melanomas analyzed were located at codon V600. Of the V600 mutations, 26.5% (22 of 83) are double base pair (tandem) substitutions and one is a complex deletion-insertion (VKS600-602DT). The V600 mutations are not considered classic UV signature mutations. The other *BRAF* mutations listed in Table 1, which were found at very low frequencies, are opposite pairs of pyrimidine dimers. The *NRAS* mutations, which were all at codon 61, are known to activate several downstream effectors of RAS, including RAF, phosphatidylinositol 3-kinase, and RalGDS (37). A few synonymous *BRAF* and *NRAS* mutations were found (see Supplementary Information) and were considered negative in the analyses.

Clinicopathologic Characteristics. Clinicopathologic characteristics were categorized by mutational subtype, and *BRAF*+ and *NRAS*+ cases were analyzed in reference to wild-type cases (Table 2). The mean ages at diagnosis were 47.3 years for *BRAF*+, 62.1 years for *NRAS*+, and 53.9 years for wild-type cases ($P < 0.001$). As age was clearly associated with mutational status, all further analyses were age adjusted. No significant association was found for gender. Although LMM was less likely to contain *BRAF* mutations before age adjustment (data not shown), there was no statistically significant association of histologic subtype with mutational status after age adjustment. Breslow thickness ≥0.75 mm was much more common in *BRAF*+ cases (54%) and *NRAS*+ cases (59%) than in wild-type cases (29%).

Phenotypic Factors. As shown in Table 3, tumors from patients with increasing number of back nevi were progressively more likely to harbor a *BRAF* mutation ($P = 0.006$).

Table 2. Clinicopathologic characteristics by *BRAF* and *NRAS* mutational status among melanoma cases (*N* = 214)

Characteristic	*BRAF*+ (*n* = 92)	*NRAS*+ (*n* = 29)	Wild-type (*n* = 93)	*BRAF*+ vs wild-type, OR* (95% CI)	*NRAS*+ vs wild-type, OR* (95% CI)
Age at diagnosis (y)					
Mean ± SD, y	47.3 ± 14.1	62.1 ± 12.8	53.9 ± 17.9		
$P < 0.001$ †					
Per 10 y				0.8 (0.6-0.9)	1.3 (1.0-1.7)
Gender, *n* (%)					
Male	48 (52)	17 (59)	52 (56)	1.0	1.0
Female	44 (48)	12 (41)	41 (44)	1.0 (0.5-1.8)	1.0 (0.4-2.5)
Histologic subtype, *n* (%)					
SSM	79 (86)	23 (79)	67 (72)	1.0	1.0
NM	5 (5)	3 (10)	2 (2)	3.3 (0.6-18.2)	2.6 (0.4-17.9)
LMM	5 (5)	2 (7)	15 (16)	0.4 (0.1-1.3)	0.2 (0.4-1.1)
ALM/other ‡	3 (3)	1 (4)	9 (10)	0.3 (0.1-1.1)	0.3 (0.0-2.5)
Breslow thickness (mm), *n* (%)					
<0.75	42 (46)	12 (41)	66 (71)	1.0	1.0
≥0.75	50 (54)	17 (59)	27 (29)	3.2 (1.7-5.9)	3.2 (1.3-7.7)

Abbreviations: *BRAF*+, *BRAF*-mutant; *NRAS*+, *NRAS*-mutant melanoma; Wild-type, melanoma negative for *BRAF* and *NRAS* mutations; SSM, superficial spreading melanoma; NM, nodular melanoma.
*OR values for gender, histologic subtype, and Breslow thickness are adjusted for age as a continuous variable.
†Two-sided ANOVA test for comparison of mean ages across *BRAF*+, *NRAS*+, and wild-type cases.
‡This category includes two ALMs that were negative for *BRAF* and *NRAS* mutations and other melanomas that were nevoid, spitzoid, and unclassifiable.

Table 3. Phenotypic characteristics by *BRAF* and *NRAS* mutational status among melanoma cases (*N* = 214)

Characteristic	*BRAF*+ (*N* = 92)	*NRAS*+ (*N* = 29)	Wild-type (*N* = 93)	*BRAF*+ vs wild-type, OR* (95% CI)	*NRAS*+ vs wild-type, OR* (95% CI)
		n† (%)			
No. nevi on the back					
Median (interquartile range)	14.5 (7-34)	12.0 (4-20)	9.0 (3-19)		
0-4	14 (16)	9 (31)	32 (36)	1.0	1.0
5-14	31 (34)	9 (31)	28 (31)	2.4 (1.1-5.5)	1.2 (0.4-3.7)
>14	45 (50)	11 (38)	29 (33)	3.2 (1.4-7.0)	1.7 (0.6-4.8)
P_{trend}				0.006	0.34
Nevus density diagrams					
None	11 (12)	5 (19)	27 (30)	1.0	1.0
Low	51 (58)	17 (62)	49 (55)	2.3 (1.0-5.2)	2.7 (0.8-8.6)
Medium to high	26 (30)	5 (19)	13 (15)	3.8 (1.4-10.4)	3.3 (0.7-14.9)
P_{trend}				0.009	0.10
Propensity to tan					
Deep tan	20 (23)	5 (19)	11 (13)	1.0	1.0
Moderate tan	34 (38)	13 (48)	35 (41)	0.5 (0.2-1.3)	0.7 (0.2-2.6)
Mild tan	27 (31)	6 (22)	24 (28)	0.7 (0.3-1.8)	0.4 (0.1-1.8)
No tan	7 (8)	3 (11)	15 (18)	0.3 (0.1-0.9)	0.3 (0.1-1.6)
P_{trend}				0.13	0.09
Childhood freckles					
None	50 (56)	13 (45)	48 (52)	1.0	1.0
Few	31 (34)	14 (48)	29 (31)	0.9 (0.5-1.8)	2.0 (0.8-4.8)
Many	9 (10)	2 (7)	16 (17)	0.5 (0.2-1.2)	0.5 (0.1-2.4)
P_{trend}				0.18	0.92
Hair color					
Black or dark brown	28 (30)	12 (41)	28 (30)	1.0	1.0
Light brown or blond	57 (62)	13 (45)	50 (54)	1.2 (0.6-2.3)	0.6 (0.2-1.4)
Red	7 (8)	4 (14)	14 (15)	0.5 (0.2-1.5)	0.6 (0.2-2.4)
Eye color					
Black or brown	26 (28)	7 (24)	26 (28)	1.0	1.0
Hazel, green, gray or blue	66 (72)	22 (76)	67 (72)	1.0 (0.5-2.0)	1.3 (0.5-3.4)

*OR values are adjusted for age as a continuous variable.
†Counts may not sum to the total number of study subjects due to missing data.

Similarly, using nevus density diagrams, patients who reported higher nevus densities had a progressively increased odds of having a *BRAF*+ melanoma ($P = 0.009$). Patients were more likely to have an *NRAS*+ melanoma with increasing number of nevi, when assessed by back nevus counts and by nevus density diagrams, although the trends were not statistically significant.

The ability to develop a tan was modestly associated with both *BRAF*+ and *NRAS*+ tumors, but the trends were not statistically significant. Childhood freckling and hair and eye color were not significantly associated with mutational subtype.

Indicators of Sun Exposure. As shown in Table 4, melanomas on chronically exposed (head, neck, or extremities) sites were less likely to harbor *BRAF* mutations (OR, 0.5; 95% CI, 0.3-1.0) or *NRAS* mutations (OR, 0.4; 95% CI, 0.2-0.9) than those on intermittently exposed (trunk) sites. Chronically exposed sites, when redefined as head, neck, lower arms, and lower legs, remained less likely to harbor a *BRAF* mutation (OR, 0.5; 95% CI, 0.3-0.9) or *NRAS* mutation (OR, 0.5; 95% CI, 0.2-1.3). Evidence of CSD, as assessed by histologic solar elastosis, was less evident for tumors associated with *BRAF* mutation before age adjustment (data not shown); however, this comparison was not significant after age adjustment.

BRAF+ cases were characterized by high estimated lifetime ambient UV irradiance (OR, 2.0; 95% CI, 1.0-4.0) and high estimated early-life ambient UV irradiance (OR, 2.6; 95% CI, 1.2-5.3), whereas *NRAS*+ tumors were similar to wild-type tumors with respect to this factor. By decadal age, *BRAF*+ cases were associated with estimated high UV irradiance at ages 0, 10, and 20 years, but a positive association was not evident for the decade years from age 30 years onward. By contrast, *NRAS*+ cases showed a nonsignificant association with high UV at age 50 years (OR, 2.5; 95% CI, 0.7-8.5), which persisted at age 60 years (OR, 2.0; 95% CI, 0.4-9.8).

Models for *BRAF* and *NRAS* Mutations. We examined the independent effects of age, number of back nevi, and early-life UV irradiance in models analyzing *BRAF*+ and *NRAS*+ cases compared with wild-type cases (Table 5). In the *BRAF* model, adjusting for all three factors, number of back nevi, and high early-life UV irradiance remained significantly associated with *BRAF* mutation, while the association with age was borderline. In the *NRAS* model, older age remained significantly associated with *NRAS* mutation, and number of back nevi had a nonsignificant positive association for >14 versus 0 to 4 nevi. Early-life UV exposure was not associated with presence of an *NRAS* mutation. Reanalyses controlling for Breslow depth in both the *BRAF* and *NRAS* models gave similar results (data not shown).

The *BRAF* and *NRAS* models were reanalyzed excluding LMM, which is generally considered to be related to high levels of chronic sun exposure (38, 39), and ALM, which may be unrelated to sun exposure. In the *BRAF* model, the association of back nevi with *BRAF* mutation was unchanged, whereas the association with high early-life UV irradiance was stronger (OR, 3.7; 95% CI, 1.6-8.7). Excluding LMM and ALM from the *NRAS* model, the association of back nevi with *NRAS* mutations was stronger (5-14 nevi: OR, 1.7; 95% CI, 0.5-5.7; >14 nevi: OR, 2.4; 95% CI, 0.7-7.7).

Discussion

Whiteman et al. (40) proposed that cutaneous malignant melanoma arises through at least two different causal pathways, one associated with aberrant nevus growth and another with chronic sun exposure. *BRAF*+ melanoma, due to its

association with intermittently sun-exposed site (41), has been hypothesized to identify one of these pathways (42). Our study further develops the divergent pathway hypothesis by finding differences in the distribution of risk factors between subgroups based on *BRAF* and *NRAS* mutational status, providing evidence of etiologic heterogeneity. Our data indicate that *BRAF*+ melanoma cases are characterized by young age at diagnosis, increased number of nevi, and high early-life ambient solar UV exposure, whereas *NRAS*+ cases are characterized by late age at diagnosis, increased number of nevi, and later-life ambient UV exposure compared with wild-type cases.

The specific association of *BRAF*+ melanomas with high childhood ambient solar UV exposure, which is apparent from the data in this report, lends further support to the suggestion that error-prone replication of UV-induced DNA damage could underlie the acquisition of *BRAF* mutations in melanocytic tumors (43). Although the most common V600 mutations are not immediately recognized as UV signature mutations, the role of UV-induced DNA damage cannot be excluded at this point (43). It is possible that *BRAF* mutations are produced in melanocytes in childhood as a result of early-life UV irradiance, and these altered cells progress over time to melanoma. This may account for the younger mean age at diagnosis of *BRAF*+ compared with *NRAS*+ melanomas. Nevi, which also frequently contain *BRAF* mutations (44) and have been found to be increased in number with high early-life UV exposure (45), could be causal intermediates for some melanomas and/or arise in parallel. Factors other than or in addition to ambient UV irradiance, such as pattern and timing of sun exposure, phenotypic susceptibility, and/or site-specific populations of melanocytes may influence melanoma location (46, 47).

The differential age association for *BRAF*+ and *NRAS*+ melanomas found in our study with 214 subjects is consistent with other studies despite modest sample sizes and differences in analytic approaches for detecting *BRAF* mutations. A study of 219 subjects using pyrosquencing (48) and another study with 69 patients using direct sequencing (49) found similar associations with age. Two studies (27, 50), each with fewer than 100 cases, did not find *BRAF*+ melanomas to be significantly associated with number of nevi or fair phenotypic characteristics. In contrast, we found *BRAF*+ melanoma to be significantly associated with number of nevi, possibly due to greater statistical power for finding this association in our study. Similar to these other studies, our results do not support a strong association of *BRAF* mutations with fair phenotypic characteristics, but a modest association of *BRAF*+ and *NRAS*+ tumors with ability to develop a tan cannot be excluded.

Concordant with other studies, we found *BRAF*+ melanomas to be inversely associated with chronically sun-exposed sites (41, 48, 51), histologic solar elastosis (reported as CSD; refs. 8, 41), and LMM (52, 53), although the latter two factors were not significantly associated after age adjustment. Our finding of an association of *NRAS* mutations with truncal location contrasts with previous reports of *NRAS*+ melanomas occurring more frequently on habitually than intermittently sun-exposed sites (10, 54), associated with chronic occupational

Table 4. Indicators of sun exposure by *BRAF* and *NRAS* mutational status among melanoma cases (*N* = 214)

Characteristic	*BRAF*+ (*N* = 92)	*NRAS*+ (*N* = 29)	Wild-type (*N* = 93)	*BRAF*+ vs wild-type, OR* (95% CI)	*NRAS*+ vs wild-type, OR* (95% CI)
		n† (%)			
Anatomic site					
Intermittently exposed site (trunk)	54 (59)	17 (59)	37 (40)	1.0	1.0
Chronically exposed sites (head/neck/extremities)	38 (41)	12 (41)	56 (60)	0.5 (0.3-1.0)	0.4 (0.2-0.9)
CSD assessed by histologic solar elastosis					
Non-CSD	75 (82)	17 (59)	59 (63)	1.0	1.0
CSD	17 (18)	12 (41)	34 (37)	0.5 (0.3-1.2)	0.7 (0.3-1.9)
Ambient erythemal UV irradiance, annual total in kJ/m^2/y					
Lifetime (average of all decadal ages)					
Low UV (≤804)	17 (20)	9 (33)	30 (33)	1.0	1.0
High UV (>804)	70 (80)	18 (67)	60 (67)	2.0 (1.0-4.0)	1.1 (0.4-2.7)
Early life (average of ages 0 and 10 y)					
Low UV (≤770)	14 (16)	11 (39)	30 (33)	1.0	1.0
High UV (>770)	75 (84)	17 (61)	60 (67)	2.6 (1.2-5.3)	0.9 (0.4-2.2)
By decade‡					
Birth year					
Low UV (≤751)	17 (19)	11 (39)	30 (33)	1.0	1.0
High UV (>751)	72 (81)	17 (61)	60 (67)	2.0 (1.0-4.1)	0.9 (0.4-2.2)
Age 10 y					
Low UV (≤804)	17 (19)	12 (41)	29 (32)	1.0	1.0
High UV (>804)	73 (81)	17 (59)	61 (68)	1.9 (1.0-3.9)	0.8 (0.3-1.9)
Age 20 y					
Low UV (≤783)	13 (14)	12 (41)	29 (33)	1.0	1.0
High UV (>783)	77 (86)	17 (59)	60 (67)	2.7 (1.3-5.7)	0.8 (0.3-1.9)
Age 30 y					
Low UV (≤806)	25 (31)	14 (48)	30 (36)	1.0	1.0
High UV (>806)	57 (69)	15 (52)	53 (64)	1.0 (0.5-1.9)	0.7 (0.3-1.8)
Age 40 y					
Low UV (≤806)	14 (22)	9 (31)	25 (34)	1.0	1.0
High UV (>806)	49 (78)	20 (69)	48 (66)	1.4 (0.6-3.3)	1.3 (0.5-3.4)
Age 50 y					
Low UV (≤806)	8 (18)	5 (19)	20 (38)	1.0	1.0
High UV (>806)	37 (82)	21 (81)	32 (62)	1.2 (0.4-3.8)	2.5 (0.7-8.5)
Age 60 y					
Low UV (≤807)	3 (17)	4 (25)	14 (38)	1.0	1.0
High UV (>807)	15 (83)	12 (75)	23 (62)	1.1 (0.2-7.0)	2.0 (0.4-9.8)

*OR values are adjusted for age as a continuous variable.
†Counts may not sum to the total number of study subjects due to missing data.
‡Logistic regression models above age 60 y were unstable due to sparse data.

Table 5. Relationship of age at diagnosis, number of back nevi, and estimated early-life ambient erythemal UV irradiance to *BRAF* and *NRAS* mutational status among melanoma cases (*N* = 202)

Characteristic	*BRAF*+ vs wild-type, OR* (95% CI)	*NRAS*+ vs wild-type, OR* (95% CI)
Age at diagnosis (per 10 y)	0.8 (0.7-1.0)	1.4 (1.1-1.9)
No. nevi on the back		
0-4	1.0	1.0
5-14	2.8 (1.2-6.4)	1.1 (0.4-3.3)
>14	3.4 (1.5-7.8)	1.9 (0.6-5.5)
P_{trend}	0.004	0.27
Early-life ambient UV irradiance in $kJ/m^2/y$		
Low UV (≤770)	1.0	1.0
High UV (>770)	2.6 (1.2-5.6)	0.9 (0.4-2.2)

NOTE: Subjects were 202 participants for whom no data were missing for the factors in the model.
*OR values are adjusted for all factors in the model.

exposure (10), and occurring with approximately equal frequencies for CSD and non-CSD anatomic sites (8). However, our population-based study included a higher percentage of thin and superficial spreading melanoma tumors than previous studies and two of these prior studies (10, 54) were done before the discovery of *BRAF* mutations, which would be expected to comprise part of their reference groups.

In our study, we found *NRAS*+ tumors to be similar to wild-type tumors with respect to estimated early-life and lifetime ambient UV irradiance. However, when examined by decade, *NRAS*+ melanomas were positively associated with high ambient UV irradiance at age 50 and 60 years, indicating that occurrence of these cases could be influenced by adult sun exposure nearer to the time of diagnosis, which might explain the older mean age at diagnosis of *NRAS*+ cases. In addition, our data indicate that a nevus-prone tendency may be important for occurrence of *NRAS*+ cases, which is seemingly consistent with previous findings that nevi can contain *NRAS* mutations (55).

Our results are concordant with migration studies that determined melanoma risk to be highest in those exposed to sunlight in early life, even if exposed for a relatively brief period (4). Our data further indicate that one influence of early-life sun exposure may be specifically to increase the risk of *BRAF*+ melanoma. Our finding that early-life UV irradiance and number of nevi are independent predictors of *BRAF* mutation indicates that a nevus-prone tendency also influences the risk of *BRAF*+ melanoma. Others are investigating genetic susceptibility for nevus production (56) and that susceptibility in combination with solar UV exposure may increase risk of *BRAF*+ melanoma.

Strengths of this study include population-based case ascertainment, a quantitative observationally based approach to measuring ambient UV irradiance instead of using a proxy such as latitude, standardized histopathology review, and rigorous screening methodology designed for sensitive detection of all known *BRAF* and *NRAS* oncogenic mutations. Caveats of this study are that we do not know whether the results can be generalized to other geographic areas, and our findings pertain only to cutaneous melanoma, as ocular and mucosal melanomas were excluded. Also, all participants were Caucasian and only two had ALM, so the mutational profile of acral melanomas or melanomas in other racial groups could not be addressed. Individual-level sun exposure was not examined in this analysis and could differ for subjects living in areas of high and low ambient UV. Furthermore, additional somatic genetic alterations that occur in melanomas (57) were not assessed in this study. Mutations and homozygous deletions in CDKN2A, a potential UV target, were associated with *BRAF/NRAS* mutation in one study (58).

We note as a limitation that the association of *NRAS* mutations with nevi and later-life sun exposure were not statistically significant; however, this may simply be due to low statistical power, as the number of *NRAS*+ cases was relatively small. In addition, several sources of bias may have affected this study. We anticipated and attempted to decrease reporting bias, which is thought to be common for melanoma (59), by notifying all North Carolina dermatologists of the study and reporting procedures. In addition, our consent rate was high compared with other studies that collected biological samples. When we compared participants with nonparticipants, there were no significant differences for demographic characteristics, tumor size, or the key risk factors under study. Our block collection and PCR amplification rates were high, minimizing additional sources of selection bias that can occur in molecular epidemiology studies. Recall bias is unlikely to be a major factor because the analyses were based on residential locations rather than more complex sunlight-exposure behaviors.

Self-reported back nevus counts may have resulted in misclassification; however, the instrument we used had a correlation coefficient of 0.57 compared with dermatologist review of photographic documentation (60), and nevus density diagrams resulted in similar associations in our data. Although our finding of an association between *BRAF* and *NRAS* mutations and thicker melanoma could have been affected by selective difficulties in analyzing thin melanomas, we attempted to minimize this possibility by using laser capture microdissection for all small samples. In addition, our high PCR amplification and analysis rate (96%) ensured that a high percentage of tumors were screened for mutations. Furthermore, the associations in our *BRAF* and *NRAS* models did not change when we adjusted for Breslow depth.

Our results suggest that the factors driving the development of melanoma vary for different mutational subtypes. Our data indicate that both early-life UV exposure and nevus propensity contribute to occurrence of *BRAF*+ melanoma, whereas nevus propensity and later-life sun exposure influence the occurrence of *NRAS*+ melanoma. From a public health perspective, the association of *BRAF*+ melanoma with early-life solar UV exposure provides additional support for an emphasis on childhood sun protection in melanoma prevention programs. In addition, our study indicates that the erythemal UV index calculated by the National Weather Service (61) may be useful for predicting when sun exposure should most be avoided for melanoma prevention.

Acknowledgments

We thank Urvi Mujumdar at Memorial Sloan Kettering Cancer Institute for assistance with data management; Sasha Madronich at the National Center for Atmospheric Research (Boulder, CO) for his assistance with model estimation of the erythemal UV irradiances (the National Center for Atmospheric Research is operated by the University Corporation for Atmospheric Research under National Science Foundation sponsorship); Dr. Marila Cordeiro-Stone for useful discussion regarding the manuscript; the two anonymous reviewers for helpful comments on the manuscript; and the following National Cancer Institute–designated core laboratories of the University of North Carolina Lineberger Comprehensive Cancer Center for their support: The Tissue Procurement and Analysis Facility, under the direction of Dr. Lynn G. Dressler, and The Microscopy Services Laboratory's Laser Capture Microdissection Facility, under the direction of Dr. C. Robert Bagnell.

References

1. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005;41:28–44.

2. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005;41:45–60.
3. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer 2005;41:2040–59.
4. Whiteman DC, Whiteman CA, Green AC. Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. Cancer Causes Control 2001;12:69–82.
5. Kricker A, Armstrong BK, Goumas C, et al. Ambient UV, personal sun exposure and risk of multiple primary melanomas. Cancer Causes Control 2007;18:295–304.
6. Begg CB, Berwick M. A note on the estimation of relative risks of rare genetic susceptibility markers. Cancer Epidemiol Biomarkers Prev 1997;6:99–103.
7. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B 2001;63:8–18.
8. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005;353:2135–47.
9. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–54.
10. van Elsas A, Zerp SF, van der Flier S, et al. Relevance of ultraviolet-induced N-ras oncogene point mutations in development of primary human cutaneous melanoma. Am J Pathol 1996;149:883–93.
11. Campbell PM, Der CJ. Oncogenic Ras and its role in tumor cell invasion and metastasis. Semin Cancer Biol 2004;14:105–14.
12. Meier F, Schittek B, Busch S, et al. The RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways present molecular targets for the effective treatment of advanced melanoma. Front Biosci 2005;10:2986–3001.
13. Millikan RC, Hummer A, Begg C, et al. Polymorphisms in nucleotide excision repair genes and risk of multiple primary melanoma: the Genes Environment and Melanoma Study. Carcinogenesis 2006;27:610–8.
14. Begg CB, Hummer AJ, Mujumdar U, et al. A design for cancer case-control studies using only incident cases: experience with the GEM study of melanoma. Int J Epidemiol 2006;35:756–64.
15. Marrett LD, King WD, Walter SD, From L. Use of host factors to identify people at high risk for cutaneous malignant melanoma. CMAJ 1992;147:445–53.
16. McKinlay AF, Diffey BL. A reference action spectrum for ultraviolet induced erythema in human skin. In: Passchier WR, Bosnjakovic BFM, editors. Human exposure to ultraviolet radiation: risks and regulations. Amsterdam: Elsevier; 1987.
17. Madronich S, Flocke S. Theoretical estimation of biologically effective UV radiation at the Earth's surface, in Solar Ultraviolet Radiation-Modeling, Measurements and Effects. In: Zerefos C, editor. NATO ASI Series. Vol 152. Berlin: Springer-Verlag; 1997.
18. Madronich S. Implications of recent total atmospheric ozone measurements for biologically active ultraviolet radiation reaching the Earth's surface. Geophys Res Lett 1992;19:37–40.
19. Stamnes K, Tsay S, Wiscombe W, Jayaweera K. A numerically stable algorithm for discrete-ordinate-method radiative transfer in multiple scattering and emitting layered media. Appl Opt 1988;27:2502–9.
20. Petropavlovskikh I. Evaluation of photodissociation coefficient calculations for use in atmospheric chemical models. PhD Thesis. Boulder: University of Brussels and NCAR/CT-159; 1995.
21. Eck TF, Bhartia PK, Kerr JB. Satellite estimation of spectral UVB irradiance using TOMS derived total ozone and UV reflectivity. Geophys Res Lett 1995;22:611–4.
22. McPeters RD, Bhartia K, Krueger AJ, et al. Earth Probe Total Ozone Mapping Spectrometer (TOMS) data products user's guide, NASA Tech. Pub. 1998-206895. Greenbelt (MD): Goddard Space Flight Center; 1998.
23. McPeters RD, Bhartia K, Krueger AJ, et al. Nimbus-7 Total Ozone Mapping Spectrometer (TOMS) data products user's guide. NASA Reference Publication 1384. Washington (DC): National Aeronautics and Space Administration; 1996.
24. Herman JR, Krotkov N, Calarier E, Larko D, Labow G. Distribution of UV radiation at the Earth's surface from TOMS-measured UV-backscattered radiances. J Geophys Res 1999;104:12059–76.
25. Clark WH, Jr., From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 1969;29:705–27.
26. McGovern VJ, Mihm MC, Jr., Bailly C, et al. The classification of malignant melanoma and its histologic reporting. Cancer 1973;32:1446–57.
27. Landi MT, Bauer J, Pfeiffer RM, et al. MC1R germline variants confer risk for BRAF-mutant melanoma. Science 2006;313:521–2.
28. Thomas NE, Alexander A, Edmiston SN, et al. Tandem BRAF mutations in primary invasive melanomas. J Invest Dermatol 2004;122:1245–50.
29. Ahrendt SA, Halachmi S, Chow JT, et al. Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. Proc Natl Acad Sci U S A 1999;96:7382–7.
30. Conway K, Parrish E, Edmiston SN, et al. The estrogen receptor-α A908G (K303R) mutation occurs at a low frequency in invasive breast tumors: results from a population-based study. Breast Cancer Res 2005;7:R871–80.
31. Begg CB, Zhang ZF. Statistical analysis of molecular epidemiology studies employing case-series. Cancer Epidemiol Biomarkers Prev 1994;3:173–5.
32. Begg CB, Gray R. Calculation of polychotomous logistic regression parameters using individualized regressions. Biometrika 1984;71:11–8.
33. Garbe C, McLeod GR, Buettner PG. Time trends of cutaneous melanoma in Queensland, Australia and Central Europe. Cancer 2000;89:1269–78.
34. Jemal A, Devesa SS, Hartge P, Tucker MA. Recent trends in cutaneous melanoma incidence among Whites in the United States. J Natl Cancer Inst 2001;93:678–83.
35. Garnett MJ, Marais R. Guilty as charged: B-RAF is a human oncogene. Cancer Cell 2004;6:313–9.
36. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:855–67.
37. Repasky GA, Chenette EJ, Der CJ. Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? Trends Cell Biol 2004;14:639–47.
38. Holman CD, Armstrong BK. Cutaneous malignant melanoma and indicators of total accumulated exposure to the sun: an analysis separating histogenetic types. J Natl Cancer Inst 1984;73:75–82.
39. Newell GR, Sider JG, Bergfelt L, Kripke ML. Incidence of cutaneous melanoma in the United States by histology with special reference to the face. Cancer Res 1988;48:5036–41.
40. Whiteman DC, Watt P, Purdie DM, et al. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 2003;95:806–12.
41. Maldonado JL, Fridlyand J, Patel H, et al. Determinants of BRAF mutations in primary melanomas. J Natl Cancer Inst 2003;95:1878–90.
42. Rivers JK. Is there more than one road to melanoma? Lancet 2004;363:728–30.
43. Thomas NE, Berwick M, Cordeiro-Stone M. Could BRAF mutations in melanocytic lesions arise from DNA damage induced by ultraviolet radiation? J Invest Dermatol 2006;126:1693–6.
44. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. Nat Genet 2003;33:19–20.
45. English DR, Milne E, Simpson JA. Ultraviolet radiation at places of residence and the development of melanocytic nevi in children (Australia). Cancer Causes Control 2006;17:103–7.
46. Siskind V, Whiteman DC, Aitken JF, Martin NG, Green AC. An analysis of risk factors for cutaneous melanoma by anatomical site (Australia). Cancer Causes Control 2005;16:193–9.
47. Whiteman DC, Stickley M, Watt P, et al. Anatomic site, sun exposure, and risk of cutaneous melanoma. J Clin Oncol 2006;24:3172–7.
48. Edlundh-Rose E, Egyha Zi S, Omholt K, et al. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res 2006;16:471–8.
49. Goel VK, Lazar AJ, Warneke CL, Redston MS, Haluska FG. Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. J Invest Dermatol 2006;126:154–60.
50. Poynter JN, Elder JT, Fullen DR, et al. BRAF and NRAS mutations in melanoma and melanocytic nevi. Melanoma Res 2006;16:267–73.
51. Deichmann M, Krahl D, Thome M, et al. The oncogenic B-raf V599E mutation occurs more frequently in melanomas at sun-protected body sites. Int J Oncol 2006;29:139–45.
52. Lang J, MacKie R. Prevalence of exon 15 BRAF mutations in primary melanoma of the superficial spreading, nodular, acral, and lentigo maligna subtypes. J Invest Dermatol 2005;125:575–9.
53. Sasaki Y, Niu C, Makino R, et al. BRAF point mutations in primary melanoma show different prevalences by subtype. J Invest Dermatol 2004;123:177–83.
54. Jiveskog S, Ragnarsson-Olding B, Platz A, Ringborg U. N-ras mutations are common in melanomas from sun-exposed skin of humans but rare in mucosal membranes or unexposed skin. J Invest Dermatol 1998;111:757–61.
55. Kumar R, Angelini S, Snellman E, Hemminki K. BRAF mutations are common somatic events in melanocytic nevi. J Invest Dermatol 2004;122:342–8.
56. Falchi M, Spector TD, Perks U, Kato BS, Bataille V. Genome-wide search for nevus density shows linkage to two melanoma loci on chromosome 9 and identifies a new QTL on 5q31 in an adult twin cohort. Hum Mol Genet 2006;15:2975–9.
57. de Snoo FA, Hayward NK. Cutaneous melanoma susceptibility and progression genes. Cancer Lett 2005;230:153–86.
58. Ranjit T, Bloethner S, Ugurei S, et al. Association between the B-RAF/N-RAS mutations, the CDKN2A alterations and the MC1R variants in melanoma. Melanoma Res 2006;16:S30–1.
59. Hall HI, Jamison P, Fulton JP, et al. Reporting cutaneous melanoma to cancer registries in the United States. J Am Acad Dermatol 2003;49:624–30.
60. Wilson JR. A validation study of self-report, digital photography, and clinical examination for quantifying total back nevus counts as a risk factor for melanoma. Master's Thesis. Department of Internal Medicine. Ann Arbor: University of Michigan; 2002. p. 1–36.
61. NOAA/ National Weather Service. National Centers for Environmental Prediction. Climate Prediction Center. Available from: http://www.cpc.ncep.noaa.gov/products/stratosphere/uv_index [Last accessed: November 20, 2006].