Interaction of *CDKN2A* and Sun Exposure in the Etiology of Melanoma in the General Population

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TO THE EDITOR

A major goal in cancer prevention is to identify genetic and environmental risk factors and determine if they interact to increase risk. Melanoma is an excellent model because intermittent sun exposure is a well supported environmental risk factor for the development of melanoma and a genetic factor, *CDKN2A*, plays a major role in melanoma etiology. We have sequenced *CDKN2A,* the major familial melanoma gene, in 3,624 melanoma patients from nine centers in four countries (Berwick *et al*., 2006, Orlow *et al*., 2007), and we have

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CONFLICT OF INTEREST

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identified the same patterns of sun exposure in risk for a second primary melanoma (Kricker *et al.*, 2007) as are found for incident, first primary melanomas (Gandini *et al.*, 2005). We therefore wanted to identify whether and how these two important risk factors – sun exposure and *CDKN2A* – might interact to increase melanoma risk.

We identified incident cases of melanoma from eight population-based registries in Australia (New South Wales and Tasmania), Italy (Piedmont area), Canada (British Columbia and Ontario), the United States (New Jersey, North Carolina, and Orange and San Diego Counties) and one hospital center in Michigan, which sees approximately 50% of the melanoma diagnosed in the state. The study design and details of the data collection have been previously published (Begg *et al.*, 2006). Briefly, single primary melanoma (SPM) controls were people diagnosed with an incident first invasive primary melanoma within a defined accrual period of 6 months during the year 2000, and multiple primary melanoma (MPM) cases were people diagnosed with an incident second- or higher-order invasive or *in situ* melanoma during a 3.5 year period from January 1, 2000. Inclusion of *in situ* cases was designed to avoid exclusion of people who could have been diagnosed with an invasive subsequent primary if the *in situ* lesion had not been removed. Participants gave informed consent, donated 4–6 buccal swabs or blood for DNA extraction, and completed questionnaires detailing demographics, phenotypic characteristics, family history of cancer and lifetime sun exposure behavior. The study protocol was approved by the Institutional Review Board of each participating institution. All study procedures adhered to the Helsinki guidelines. Sequencing was conducted for exons 1α, 2 and 3 of the *CDKN2A* gene, which code for the p16 and or p14ARF proteins as previously described (Berwick *et al.*, 2006; Orlow *et al.* 2007).

Functional *CDKN2A* mutations (Orlow et al. 2007) were identified in 30 of 2,469 individuals with single primary melanomas (1.3%) and in 35 of 1,207 individuals with multiple primary melanoma (2.9%) (Berwick *et al.*, 2006). As we previously published, those with *CDKN2A* mutations are significantly younger than those with wildtype *CDKN2A*, more likely to have a family history of melanoma, more likely to have multiple melanomas and more nevi.

We used five measures of sun exposure previously reported by our group as important risk factors for second primary melanomas (Kricker et al., 2007): (1) Ambient erythemal UVR (UVE) exposure at age 10 – a measure of early life sun exposure supported by migrant studies where the effect of ambient sun exposure is greatest in those who migrated before 10–15 years of age, (2) average annual hours on sunny holidays, (3) average annual hours of beach and waterside exposure, (4) lifetime painful or blistering burns, and (5) lifetime painful or blistering burns to the site of the melanoma. The latter four measures reflect an intermittent pattern of sun exposure, usually indicated by recreational exposure, the major form of sun exposure that has been associated with the development of melanoma (Armstrong and Kricker 1993; Gandini et al., 2006). Odds ratios were calculated using logistic regression for unadjusted and adjusted stratified analyses, controlling for age, sex, study center, an age*sex interaction and ability to tan. Multiplicative interactions were assessed using cross product terms. All statistical analyses were undertaken using SAS Statistical Packages Version 9.2 (SAS, Inc, Cary, NC). All statistical tests were two-sided.

We observed no statistically significant multiplicative interactions between any measure of intermittent sun exposure and mutation of *CDKN2A* (Table 1). The stratified analyses show no statistically significant differences in effects of increasing sun exposure between mutation carriers and non-carriers, and the relative risk estimates are generally smaller than for the non-carriers.

These findings suggest that increasing sun exposure may add little to the increased risk of melanoma that is conferred by carriage of a *CDKN2A* mutation, and that those with a mutation are at high risk for the development of melanoma regardless of sun exposure. The adjusted relative risk of melanoma in carriers is 4.2 (95% CI 2.4, 7.7), as reported earlier in Berwick *et al.,* 2006.

Previous efforts to document a gene-environment interaction among *CDKN2A* carriers have reported similar findings. Consistent with the present results, Goldstein *et al*. (1998) did not find that sun exposure (measured as sunburns) was a significant risk factor for melanoma among families with *CDKN2A* mutations. Recently, Cust *et al.* (2011) reported that *CDKN2A* carriers appeared to have the same cumulative risk of melanoma regardless of ambient sun exposure thus suggesting that our observation of risk of second primary melanoma may be generalizable to all primary melanoma. Most studies of *CDKN2A* have focused on smaller populations (e.g., Nielson *et al*., 2010) or on familial studies (such as Goldstein *et al*., 1998; Bishop *et al*., 2002; Cust *et al.,* 2011); GEM is the largest population-based series to sequence *CDKN2A* among individuals diagnosed with melanoma. Clearly, since the prevalence of *CDKN2A* mutations is very low, the ability to analyze the interaction between *CDKN2A* and solar exposure in the general population is necessarily limited, and inferences from such analyses are uncertain.

In summary, our study provides no evidence to suggest that the influences of *CDKN2A* mutational status and sun exposure on melanoma risk are related. In addition, we found little evidence that sun exposure increases the risk of melanoma in carriers, although our sample sizes are too small for a definitive conclusion on this issue. In the absence of further evidence people with CDKN2A mutations should receive at least the same sun protection advice as other people with similar phenotypic risk factors.

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Abbreviations

OR   odds ratio

CI   Confidence Interval

UVR   ultraviolet radiation

UVE   erythemal ultraviolet radiation

KJ/m^2   kilojoules per square meter

MPM   multiple primary melanoma

SPM   single primary melanoma

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Table 1

Analyses of the associations of multiple primary melanoma (MPM) with sun exposure measures stratified by CDKN2A mutational status.

Mutational Status	Exposure	SPM N=2469	MPM n=1207	OR$_{crude}$ (95% CI)	*OR$_{adjusted}$ (95% CI)	p^1	p^2
UVE at age 10 (KJ/m²)							
No mutation	303–912	1230	463	1.00	1.00		
	913–2084	1042	643	1.64 (1.42, 1.89)	1.26 (0.95, 1.67)		
	missing	167	66				
With mutation	303–912	17	15	1.00	1.0		
	913–2084	12	20	1.89 (0.69, 5.12)	1.18 (0.39, 3.54)	0.92	0.83
	missing	1	0				
Sunny Holiday – Average Hours per Year							
No mutation	0–19	1013	406	1.00	1.00		
	20–678	969	454	1.17 (0.99, 1.37)	1.33 (1.11,1.59)		
	missing	457	312				
With mutation	0–19	12	11	1.00	1.00		
	20–678	14	16	1.25 (0.42, 3.69)	0.92 (0.28, 2.99)	0.55	0.24
	missing	4	8				
Beach and Waterside Activities – Average Hours per Year							
No mutation	0–24	1203	570	1.00	1.00		
	25–1857	1164	575	1.04 (0.91, 1.20)	1.20 (1.02, 1.41)		
	Missing	72	27				
With mutation	0–24	7	10	1.00	1.00		
	25–1857	23	25	0.76 (0.25 2.33)	0.87 (0.26, 2.85)	0.59	0.41
	Missing	0	0				
Lifetime Painful or Blistering Sunburns							
No mutation	0–10	1362	613	1.00	1.0		
	11–700	1023	533	1.16 (1.01, 1.33)	1.33 (1.13, 1.56)		
	missing	54	26				
With mutation	0–10	18	21	1.00	1.00		
	11–700	12	14	1.00 (0.37, 2.71)	0.99 (0.33, 2.91)	0.59	0.25
	missing	0	0				

Mutational Status	Exposure	SPM N=2469	MPM n=1207	OR$_{crude}$ (95% CI)	* OR$_{adjusted}$ (95% CI)	p^1	p^2
Lifetime Painful or Blistering Burns to the Site of the Melanoma							
No mutation	None	1310	522	1.00	1.00		
	Any	945	544	1.45 (1.25, 1.67)	1.49 (1.27, 1.77)		
	missing	184	106				
With mutation	None	13	22	1.00	1.00		
	Any	11	13	0.69 (0.25, 1.94)	0.57 (0.19, 1.74)	0.09	0.09
	missing	6	0				

*
Analyses are adjusted for age, sex, age–sex interaction, center and ability to tan.

[1]
Multiplicative interaction using categories of sun exposure.

[2]
Multiplicative interaction using continuous values of sun exposure.