

RESEARCH PAPER

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## Cutaneous malignant melanoma incidences analyzed worldwide by sex, age, and skin type over personal Ultraviolet-B dose shows no role for sunburn but implies one for Vitamin D<sub>3</sub>

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### ABSTRACT

Because the incidence of cutaneous malignant melanoma (CMM) was reported to increase with increasing terrestrial UVR (290–400 nm) doses in the US back in 1975 and a recent publication showed no association exists with UVR exposure at all, we set out to fully elucidate the role of UVR in CMM. To achieve this goal, we analyzed the CMM incidences over latitude and estimated the average personal UVR dose in the US and numerous countries (> 50) on 5 continents around the world. Using data from the International Agency for Research on Cancer in 2005, we performed worldwide analysis of CMM over UVR dose by sex, age group (0–14, 15–29, 30–49, 50–69, 70–85+) and Fitzpatrick skin types I–VI. Surprisingly, increasing UVR doses, which represent erythemally-weighted doses comprised primarily of UVB (290–315 nm) radiation, did not significantly correlate with increasing CMM incidence for people with any skin type anywhere in the world. Paradoxically, we found significant correlations between *increasing* CMM and *decreasing* UVB dose in Europeans with skin types I–IV. Both Europeans and Americans in some age groups have significant *increasing* CMM incidences with *decreasing* UVB dose, which shows UVB is not the main driver in CMM and suggests a possible role for lower cutaneous vitamin D<sub>3</sub> levels and UVA (315–400 nm) radiation. CMM may be initiated or promoted by UVA radiation because people are exposed to it indoors through windows and outdoors through some sunscreen formulations. Thus, our findings may explain why some broad-spectrum sunscreen formulations do not protect against getting CMM.

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### Introduction

The incidence of cutaneous malignant melanoma (CMM) has been exponentially increasing over the last several decades in fair-skinned males and females around the world.<sup>1,2</sup> The exponential increase in CMM may be due to the exponential spread of the Human Papilloma Virus (HPV), the declining levels of vitamin D over recent decades primarily from indoor work, and the increasing UVA (315–400 nm) and visible light (400–700 nm) exposures through windows and sunscreens. HPV may explain the exponential increase in CMM over recent decades because it has also been increasing at an exponential rate<sup>3</sup> while vitamin D levels have decreased over the last 5 decades, as reflected by the almost 10-fold increase in the inversely related

parathyroid hormone levels.<sup>4</sup> Vitamin D is important for a variety of reasons in reducing the risk for getting CMM but one of the most essential is for T cell activation in order to kill virally infected and cancerous cells.<sup>5</sup> Low cutaneous vitamin D<sub>3</sub> levels can occur from intermittent sun exposures and people's perception of having a tendency to burn,<sup>6,7</sup> which leads to protective behaviors like avoiding sun exposure and excessive use of sunscreens that increase UVA and visible light doses. Ironically, the sunscreen formulations in the United States (US) that decrease UVB doses and successfully prevent sunburn did not result in a decrease in the incidence of CMM<sup>8</sup> but rather they may have increased CMM in a dose-dependent manner.<sup>9</sup> Although controversial, the reasons US sunscreens were not protective

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against CMM may be because they almost completely annihilate vitamin D<sub>3</sub> production using sun protection factor of 15 or more<sup>10</sup> and they allow people to stay out in the sun longer resulting in higher doses of UVA radiation and visible light. UVA is suspected to be the driver in CMM because the US broad-spectrum sunscreens did not provide enough protection in the longer waveband regions of UVR (> 380 nm) and they did not protect against getting CMM as did the European broad-spectrum sunscreens<sup>11</sup> and the Australian broad-spectrum sunscreens given to study participants which did decrease the incidence of CMM by 50%.<sup>12</sup>

CMM has been exponentially increasing exclusively in European-ancestry populations for over 5 decades as revealed by worldwide temporal analysis by sex, age (0–14, 15–29, 30–49, 50–69, 70–85+) and Fitzpatrick skin types I–VI.<sup>13,14</sup> The observed 2 orders of magnitude increase in the CMM incidence between the 2 youngest age groups, 0–14 and 15–29, exclusively in European-ancestry populations indicates a hormonal event occurs during puberty which dramatically increases the incidence of CMM. Some scientists think this might have occurred because children get 3 times the UVR dose that adults get, but that assumption was proven not to be true; in fact, people get about the same exposures throughout their lives.<sup>15</sup> So, one important risk factor for CMM may be developing androgenic body hair follicles that are immune privileged sites<sup>16</sup> of persistent HPV infection.<sup>17</sup> Additionally, the hormone estrogen, which is involved in hair maintenance,<sup>18</sup> evidently increases the risk for getting CMM because only older women (>40 yr) exclusively of European ancestry have an almost linear rather than a power function increase in the CMM incidence over their lifetime (unpublished results) possibly due to loss of androgenic hair as they age.<sup>19</sup>

Most of the risk factors for CMM were determined by epidemiology studies which showed people with fair skin (Fitzpatrick skin type I–III), numerous nevi, and light hair have significantly higher incidences of CMM.<sup>20</sup> These fair-skinned people also have higher incidences of non-melanoma skin cancers, which unlike CMM occur exclusively on body sites that are chronically exposed to the sun. For example, clinicians have known for over 5 decades that 70–90% of non-melanoma skin cancers occur primarily on body sites chronically exposed to UVR, i.e., the head and neck;<sup>21</sup> whereas, about 75% of CMM occur primarily on body sites not chronically exposed to UVR.<sup>22</sup> Males have

more CMM on their face, neck, and trunk than females who have more CMM on their lower limbs, which corresponds well with the distribution of androgenic body hair<sup>23</sup> that HPV can infect<sup>16</sup> along with nevi and CMM.<sup>24</sup> Light hair color, especially red hair and nevi are major risk factors for CMM<sup>20</sup> probably because they both contain large amounts of pheomelanin and its early precursor molecules like benzothiazine<sup>25</sup> that absorb UVA1 radiation (341–400 nm;  $\lambda_{\text{max}} > 340$  nm).

Because the incidence of CMM was reported to increase with increasing terrestrial UVR doses in the US back in 1975<sup>26</sup> and a recent report found no association with UVR exposure at all,<sup>27</sup> we set out to fully elucidate the role of UVR in CMM. To achieve this goal, we analyzed the CMM incidences over latitude and estimated the average personal UVB dose of males and females in 5 age groups (0–14, 15–29, 30–49, 50–69, 70–85+ yr.) with all skin types I–VI in the US and numerous countries (>50) on 5 continents around the world.

## Materials and methods

### *Analysis of CMM incidence by sex, age, and skin type over personal UVR dose*

We analyzed the age-standardized CMM (ICD-10, C43) incidence rates in 2005 (average of 2003–2007) over estimated average personal UVB doses based on indoor workers' personal ambient exposures and residential latitudes of males and females around the world. We segregated the data by sex and 5 age groups (0–14, 15–29, 30–49, 50–69, 70–85+) with Fitzpatrick<sup>14</sup> skin types I–III, III–IV, IV–V, and V–VI and analyzed it using age-adjusted, world population normalized data obtained from the International Agency for Research on Cancer.<sup>28</sup> White, non-Hispanic white or Caucasian skin is primarily represented by Fitzpatrick skin type I–III; Asian, Latino, Hispanic, and Polynesian skin is primarily represented by Fitzpatrick skin type III–IV; brown to black or African American skin is primarily represented by Fitzpatrick skin type IV–VI;<sup>29</sup> eastern Indian skin is primarily represented by Fitzpatrick skin type IV–V;<sup>30</sup> Mediterranean, olive tone skin is primarily represented by Fitzpatrick skin type III–IV.<sup>31</sup> One possible limitation to this study is that a Fitzpatrick skin type for the primary population of each country had to be assigned if the IARC data did not segregate the populations by skin type, e.g., white, non-Hispanic white, non-Maori, Maori, or

black (African-American). We used data designated as white, other white, or non-Hispanic white for Fitzpatrick skin type I-III whenever available.

The data for the countries shown here are Australia, the US (white, other white, or non-Hispanic white), Europe (24 countries average shown for primarily skin type I-III), China, Japan, South America (6 countries average shown), Italy, India, and the US African-Americans.

We give the details of this analysis including the countries, territories, regions, and states with corresponding latitudes used to calculate the UVB doses in reference 13.

### Personal annual UVR dose calculations

We calculated estimates of the average personal UVR dose after geometric conversion from planar to cylinder measurements<sup>32</sup> using the equation derived from the personal UVR doses known at various latitudes in different countries: Sweden (60°N; 5,200 J/m<sup>2</sup>), Denmark (55°N; 6,800 J/m<sup>2</sup>), the Netherlands (52.5°N; 7,000 J/m<sup>2</sup>), and the US (44°N, 10,000 J/m<sup>2</sup> and 34°N, 12,400 J/m<sup>2</sup>):

$$\text{UVR dose} = -280X + 22000$$

where X is the latitude.<sup>33</sup> One can also use this equation to estimate the average personal UVR doses for people in the southern hemisphere because their estimated personal UVR doses are similar and fall on the same trend line (see Fig. 3 in reference<sup>34</sup>). For example, before geometric conversion, as in the above equation, the average Australian gets about 29,000 J/m<sup>2</sup> of erythemally-weighted UVR each year at 34°S and the average US citizen gets about 28,000 J/m<sup>2</sup> of erythemally-weighted UVR each year at 34°N. In the US, we used the population-centered latitudes for better accuracy, although they were not available for other countries like Australia. To get erythemally-weighted UVR doses, the solar spectra in W/m<sup>2</sup>, wavelength for wavelength from 290–400 nm, is multiplied by the erythemal action spectrum,<sup>35</sup> and then the summated value is multiplied by the number of exposure seconds to get the dose in J/m<sup>2</sup>,<sup>34</sup> which are primarily UVB doses. Note that the residential UVB doses do not include vacations taken at random latitudes, but they give a good estimate of the average populations' dose.

### Statistical analysis

For all the data, we conducted linear regression analysis to compute correlations using Minitab 16.2.4 (Minitab Inc., State College, Pennsylvania) to evaluate the association between personal UVR doses (independent variable) and CMM incidence rate (dependent variable). We consider data to be significance when  $p < 0.05$ .

### Results

In Table 1, we show the p values for all age groups and skin type I-VI populations around the world. Significance ( $p < 0.05$ ) with CMM can correlate either with increasing UVR dose (bold) or with decreasing UVR dose (bold italicized). We observe a significant correlation between increasing CMM and decreasing UVR dose with increasing age of female Europeans, especially in male and female Italians over the age of 29. Note that this is a linear regression analysis and that we plotted the figures as semi-log for visual presentation only.

We began by analyzing the countries with primarily fair-skinned type I-III populations as more UVB radiation can penetrate deeply into their skin increasing its biological effects. Fig. 1 shows the CMM incidences over personal UVB dose (J/m<sup>2</sup>) for the fair-skinned populated territories, states, and countries' on the continents of Australia (20–42°S; left panels), North America (in the US, 21.31–47.4°N; middle panels), and Europe (46–65°N; right panels) for males and females in 5 age groups (0–14, 15–29, 30–49, 50–69, 70–85+ yr.). The analysis reveals only 2 possible significant correlations exist between increasing CMM incidence and increasing personal UVB dose in the southern hemisphere continent of Australia for males in age group 15–29 and 50–69 (Table 1). Note here that 1 in 20 p values might show significance that is due to type I error. The youngest age group in Australia (0–14 y.) does not display a significant increase in the CMM incidence with increasing UVB dose; it only appears that way because the data point near the equator was higher for both males ( $p = 0.204$ ) and females ( $p = 0.132$ ) and the missing 3–4 data points were zero. On the North American continent in the US, no significant correlation exists between increasing CMM incidence and increasing personal UVB dose but it is nearly significance in some age groups with

**Table 1.** P values from the linear correlation of CMM with UVB dose for males and females in the 5 age groups in countries with different Fitzpatrick skin types I-III. We consider p values < 0.05 to be significant. Values in bold show significant correlations between increasing UVB dose and increasing CMM, but those that are bold italicized have significant correlations between decreasing UVB dose and increasing CMM incidence. ND = no data above zero to analyze.

Fig. 1	Australian		US White		European	
Age	Male	Female	Male	Female	Male	Female
0-14	0.204	0.132	0.456	0.312	0.426	0.685
15-29	<b>0.041</b>	0.177	<b>0.008</b>	<b>0.007</b>	0.385	0.067
30-49	0.101	0.381	0.158	<b>0.006</b>	0.313	<b>0.045</b>
50-69	<b>0.030</b>	0.109	0.739	0.054	0.152	<b>0.048</b>
70-85	0.550	0.309	0.472	0.513	0.064	<b>0.012</b>

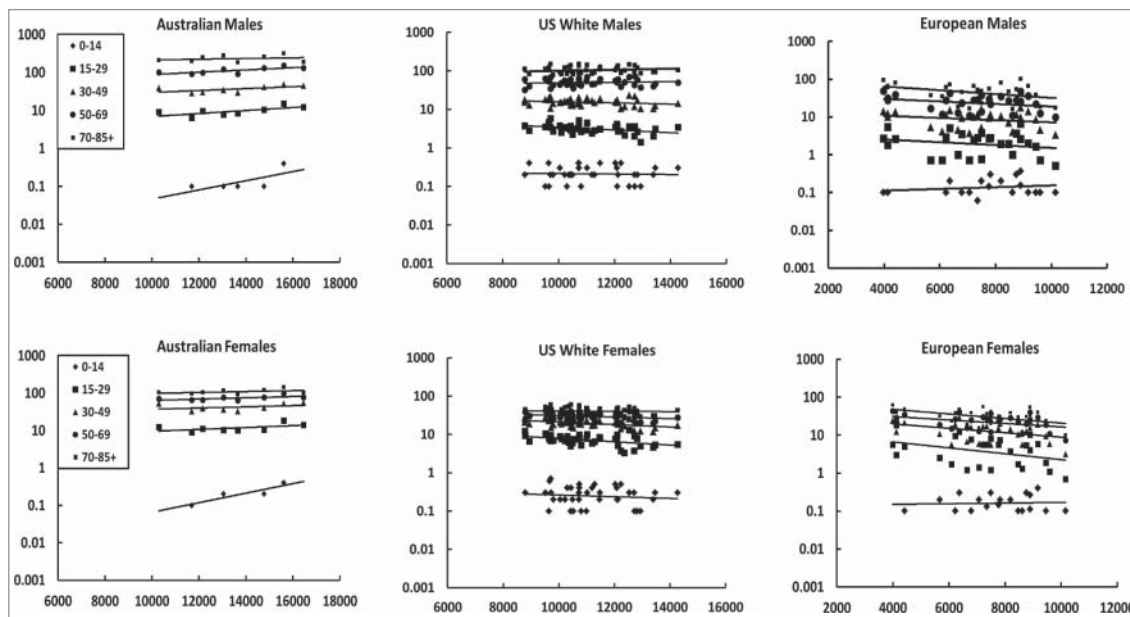
Fig. 2	Chinese		Japanese		South American	
Age	Male	Female	Male	Female	Male	Female
0-14	ND	ND	ND	0.436	0.536	0.281
15-29	0.248	0.369	0.686	0.815	<b>0.001</b>	0.098
30-49	0.952	0.763	0.531	0.879	0.299	0.081
50-69	0.429	0.968	0.130	0.625	0.963	0.365
70-85	0.951	0.057	0.601	0.593	0.894	0.318

Fig. 3	Italian		Indian		US Black	
Age	Male	Female	Male	Female	Male	Female
0-14	0.604	0.454	ND	ND	0.606	0.526
15-29	0.113	0.087	0.664	0.473	0.406	0.140
30-49	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.779	0.675	0.188	<b>0.013</b>
50-69	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.224	0.725	0.056	0.172
70-85	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.248	0.600	0.778	0.074

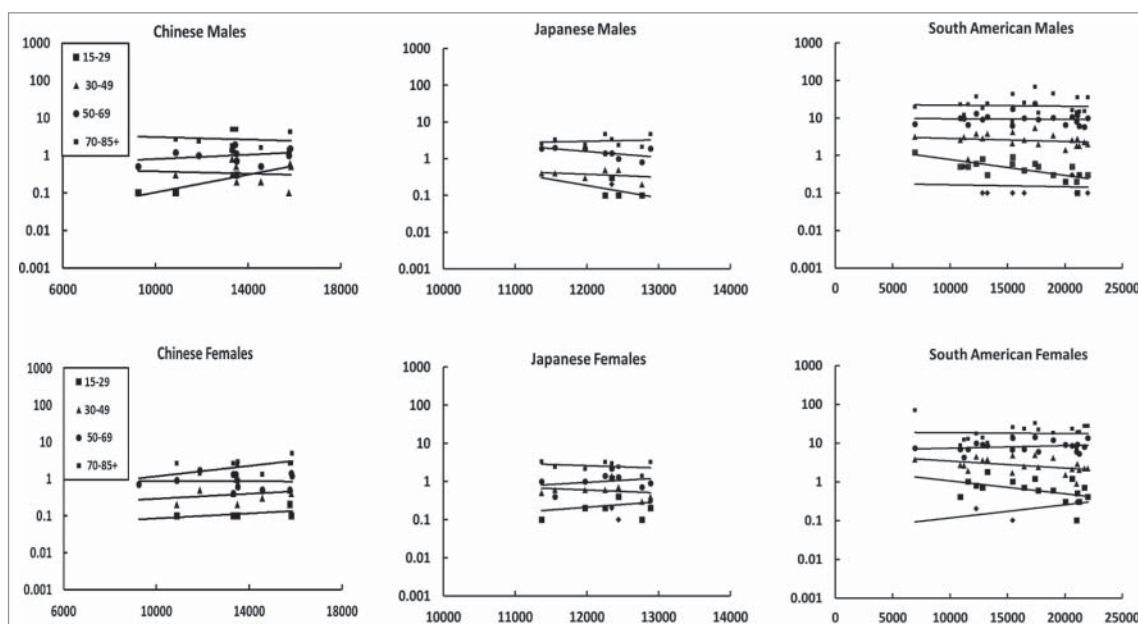
decreasing personal UVB dose, i.e., males and females ages 15-29 and females ages 30-49 (Table 1). However, because there is no apparent trend, this may be due to type I error. Ninety correlations were computed in Table 1 so at the level of significance 0.05 one expects 4.5 type 1 errors and we have 2 positive and 14 negative correlations with UVB dose.

Thus, we have a preponderance of negative correlations or increasing CMM with decreasing UVB dose. In addition, the higher latitudinal countries on the continent of Europe shows a p trend correlation exists between increasing CMM incidences and decreasing personal UVB doses for females in all age groups over 29 y. (Table 1).



**Figure 1.** Age-standardized CMM cases per 100,000 people by personal UVB dose in  $J/m^2$  for males and females with Fitzpatrick skin type I-III. Semi-log plots were chosen for visual presentation only.





**Figure 2.** Age-standardized CMM cases per 100,000 people by personal UVB dose in  $J/m^2$  for males and females with Fitzpatrick skin type III-IV. Semi-log plots were chosen for visual presentation only.

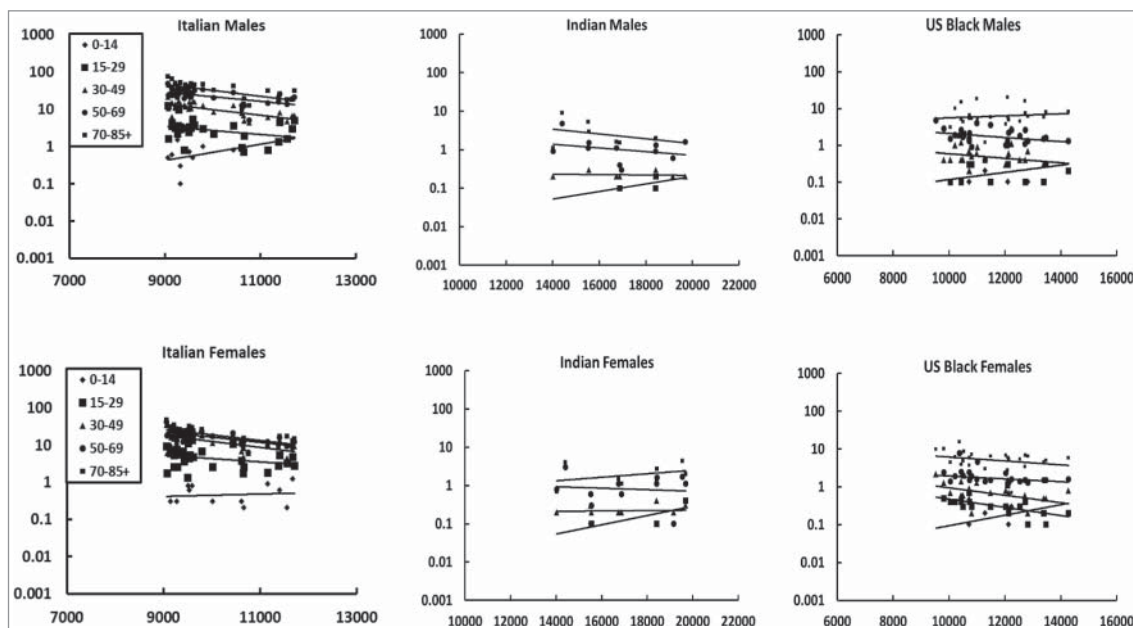
We continued the analysis of CMM incidence by personal UVB dose in Asian and Latino countries with primarily skin type III-IV populations. **Figure 2** shows the results for the countries with primarily skin type III-IV populations with yellow skin tone of males and females of all ages and the *p* values in **Table 1** reveal no correlation exists between CMM incidences and UVB dose in China ( $22.2\text{--}45.7^\circ\text{N}$ ), Japan ( $32.8\text{--}38.2^\circ\text{N}$ ), or South America ( $7.1^\circ\text{N}\text{--}54^\circ\text{S}$ ). The only possible correlation that may exist between decreasing UVB dose and increasing CMM in South American males of age group 15–29; however, again there is no significant trend so this may be due to type I error. These countries cover broad latitudinal ranges in both the northern and southern hemispheres on the continents of Asia and South America.

We finished analysis of CMM incidence by personal UVB dose with the countries having primarily skin type III-IV, IV-V, and IV-VI populations (**Fig. 3**). The male and female skin type III-IV populations with olive skin tone in Italy ( $36.9\text{--}46.4^\circ\text{N}$ ), like the rest of Europe, show significant correlations exist between increasing incidence of CMM and decreasing personal UVB dose (based on residential latitude) over the age of 29 ( $p < 0.001$ ; **Table 1**). But for darker skin types, no significant correlation between the CMM incidences and personal UVB dose exists for people in India ( $8.5\text{--}28.7^\circ\text{N}$ ) having primarily

skin type IV-V or for African-Americans in the US ( $27.8\text{--}44.7^\circ\text{N}$ ) with primarily skin type V-VI.

### Discussion

For the first time, we provide comprehensive worldwide analyses of CMM incidences over personal UVB doses for males and females in 5 age groups (0–14, 15–29, 30–49, 50–69, 70–85+ yr.) with all Fitzpatrick skin types I-VI<sup>14</sup> on 5 continents around the world. Contrary to popular belief, no evidence exists for a significant trend or correlation between the increasing incidences of CMM and increasing personal UVB dose for males or females of any age group or skin type I-VI anywhere in the world (**Fig. 1–3** and **Table 1**). We did find an apparent correlation between CMM incidence and UVB dose in the US back in 1975 (results not shown), in agreement with previous findings.<sup>26</sup> However, those observations may be misleading because IARC only had data for 10 states in 1975 (12 regions; 3 in California) and the previous study only analyzed data for 9 states compared with our analysis here in 2005 for 44 out of 50 states, which confirms another recent US analysis that used another database and approach.<sup>27</sup> Counter intuitively, in Europe, we found a significant correlation exists between decreasing UVB doses and increasing CMM for fair-skinned, skin type I-III females over 29 y.



**Figure 3.** Age-standardized CMM cases per 100,000 people by personal UVB dose in  $J/m^2$  for males and females with Fitzpatrick skin type IV-VI. Semi-log plots were chosen for visual presentation only.

(Fig. 1A right panels;  $p < 0.05$ , Table 1). In addition, we found a very significant correlation exists between decreasing UVB doses and increasing CMM for skin type III-IV Italian males and females over 29 y. (Fig. 3 left panels;  $p < 0.001$ , Table 1). Our European findings here are in agreement with our previous analysis for the year 2000 where we found both sexes in all age groups had a significant correlation between increasing CMM incidence and decreasing UVB dose.<sup>1</sup> Besides decreasing levels of vitamin D<sub>3</sub> with decreasing UVB dose or increasing latitude,<sup>1,2</sup> another explanation may be increasing red hair gene variants of MC1R with increasing latitude. However, a significant increasing CMM incidence with increasing latitude only began to occur in Europe after 1960 with no corresponding change in the population's hair distribution, so that decreasing vitamin D levels appears to be a more feasible explanation for this observation.

The fact that we did not find a correlation between increasing CMM and increasing UVB doses for males or females in any age group or skin type I-VI on any continent or country around the world suggests that unlike non-melanoma skin cancers, UVB radiation does not play an important role in the etiology of human CMM. If UVB were responsible for initiating melanoma, we would expect any sunscreen formulation to protect against getting CMM because they all prevent sunburn by screening out UVB, but we do not

observe this.<sup>11</sup> Thus, our results in combination with the sunscreen findings allows us to rule out a major role for UVB radiation or sunburns initiating human CMM, in agreement with other studies.<sup>27,36</sup> However, we cannot rule out a major role for UVA radiation, for unlike UVB radiation that displays a steep latitudinal gradient<sup>34</sup> and is dramatically reduced by the ozone layer,<sup>37</sup> UVA radiation displays a shallow latitudinal gradient<sup>36,38,39</sup> because it is not reduced by the ozone layer. Sunscreens offer less protection in the longer waveband regions of the spectrum<sup>11</sup> allowing people to stay out longer and accumulate high doses of UVA radiation and visible light and UVA may be effective for initiating melanoma in humans, as first suggested by an action spectrum in fish.<sup>40</sup> Other studies using cell lines,<sup>41</sup> mice,<sup>42</sup> and opossums<sup>43</sup> also suggested a role for UVA initiating CMM and mounting evidence has accumulated over recent years suggesting that UVA may initiate or promote CMM in humans.<sup>1,2,36,38,45,46</sup>

The mechanism by which UVA radiation initiates or promotes CMM may occur via certain chromophore molecules directly absorbing its energy resulting in ROS or free radical molecules that can form adducts with or crosslink chemicals such as 8-methoxypsoralen (PUVA),<sup>47,48</sup> furocoumarins (in common foods),<sup>49</sup> proteins,<sup>50</sup> and amino acids like cysteine (sulfhydryl group) to the thymine bases.<sup>51</sup> Most importantly, the

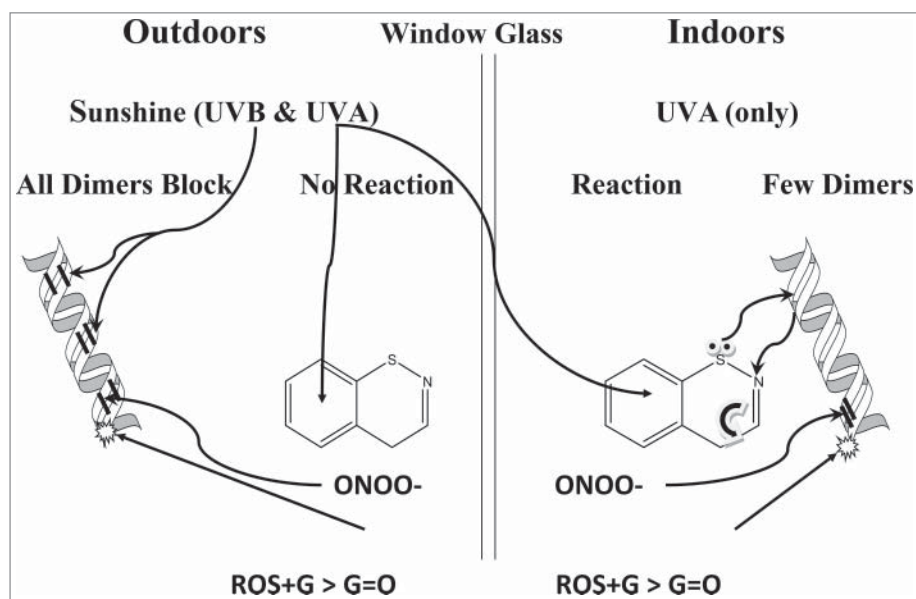
preponderance of UVB signature transition mutations, i.e., CC→TT, or the UVA signature transversion mutations, i.e., G→T and C→A, or the oxidative mutation AT→CG caused by eta polymerase's incorporation of 8-hydroxy-2'-deoxyguanosine into the DNA<sup>52</sup> that are commonly found in non-melanoma skin cancers are rarely found in CMM.<sup>53</sup> Furthermore, the so-called UVB mutation C→T found in CMM may actually be a mutation caused by UVA because UVA produces ROS that can oxidize cytosine, which can subsequently deaminate resulting in a C→T transition mutation.<sup>54</sup> HPV can also create these C→T transition mutations via its E2 protein because it causes production of ROS by adversely interacting with the mitochondria.<sup>55</sup> In addition to transition mutations, clinicians find unique transversion mutations from photoadduct formation<sup>47</sup> in CMM after therapeutic PUVA (8-methoxypsoralen and UVA).<sup>48</sup> CMM's UV nonsignature transversion T→A mutation<sup>53</sup> in BRAF<sup>V600E</sup> may also be UVA-induced from photoadduct formation between a red-pigment pheomelanin precursor molecule like benzothiazine ( $\lambda_{\text{max}} > 340$  nm) crosslinking to a thymine base, similar to how adduct formation occurs during PUVA.<sup>47,48</sup>

Besides the DNA damage caused by chemical crosslinks forming photoadducts and ROS causing deamination of cytosines, epigenetic events like methylation of cytosines can also result in deamination leading to C→T transition mutations.<sup>54</sup> Other than the UV nonsignature transversion T→A mutation in BRAF<sup>V600E</sup>, CMM somatic mutations consist almost entirely of C→T transition mutations occurring predominately at NpCpG trinucleotide sites, which are signature mutations shared with cervical cancer.<sup>57</sup> HPV causes cervical cancer, which is associated with its own signature mutations, specifically the APOBEC3B-mediated cytosine deaminations leading to C→T (or G) mutations.<sup>58</sup> These C→T transition mutations are specific for HPV+ tumors as they are only found in cervical and HPV+ oropharyngeal cancers; they are not found in HPV- oropharyngeal cancer or liver cancers associated with Hepatitis B or C. HPV causes deamination of methylated cytosines leading to C→T transition mutations in CDKN2A, a gene that codes for the tumor suppressor proteins p16 and p14<sup>arf</sup>, which is associated with HPV+ oropharyngeal and cervical cancers.<sup>59,60</sup> Intriguingly, a C→T polymorphism in CDKN2A is also methylated in CMM.<sup>61</sup> These C→T transition mutations appear to be increasing over time

exclusively in the European-ancestry germline.<sup>62</sup> UVR cannot penetrate deeply enough to result in germline mutations while viruses can; and scientists find HPV in sperm.<sup>63</sup> Thus, the so-called UVB signature mutations found in CMM that are almost always C→T transition mutations may actually be created by ROS produced during pheomelanin synthesis, UVA/Visible exposure, or HPV infection, the latter of which can also cause methylation of cytosines resulting in deamination of cytosine and C→T mutations.

Animal studies showing UVB radiation initiates CMM may be deceiving due to the superficial location of their follicular melanocytes (epidermal thickness of about 20 $\mu\text{m}$ ) compared with human (epidermal thickness of about 80 $\mu\text{m}$ )<sup>64</sup> allowing UVB to irradiate their bulge regions but not the human follicular melanocytes located between 362 $\mu\text{m}$  for vellus and 1,161 $\mu\text{m}$  for terminal bulge regions.<sup>65</sup> However, about 1% of the incident UVA1 radiation (341–400 nm) can penetrate into the subcutaneous layer of the skin irradiating all follicular regions including the root bulb of terminal hair (1,000–5,000 $\mu\text{m}$ ).<sup>66</sup> Thus, if UVR plays any significant role in human CMM, it has to be UVA rather than UVB radiation.

In order to help elucidate the potential role of UVA in CMM, we surveyed epidemiology studies for clues. Most epidemiology studies found light haired, especially red-haired people have significantly higher incidences of CMM than darker-haired people do, which meta-analysis confirmed.<sup>20</sup> A recent epidemiology study revealed a significant increase in the CMM risk exists between European people with light blond/red hair and European people with dark brown/black hair ( $p = 5.96 \times 10^{-6}$ ).<sup>67</sup> Meta-analysis of the melanocortin-1-receptor and CMM risk revealed 3 variants associated with only red hair that gave significant increased risks for CMM with odds ratios ranging from 2.99 to 8.10.<sup>68</sup> Moreover, in the absence of UVR exposure, regardless of their epidermal melanocyte status ( $\pm$ ), black and albino C57BL/6 mice on a mutated BRAF<sup>V600E</sup> background developed similarly low rates of CMM after a long latency period, whereas over half the mice with red hair developed melanomas after only a year.<sup>69</sup> Recently, scientists confirmed the human variants of the MC1R red hair gene dramatically increase the risk for getting CMM independent of UVR exposure.<sup>70</sup> Furthermore, both homozygote and heterozygote red hair melanocortin-1-receptor variants are sensitive to UVR exposure<sup>71</sup> and have



**Figure 4.** The consequences of DNA photoproduct damage from exposure to outdoor UVA and UVB radiation versus indoor UVA radiation. Outdoor sunshine has both UVA and UVB radiation vs. indoor sunshine that has only UVA radiation because, unlike UVB, it can pass through window glass. The outdoor UVB radiation causes efficient pyrimidine dimer formation and UVA makes benzothiazine or benzothiazinylalanine and other radicals ( $\lambda_{\text{max}} > 340 \text{ nm}$ ) that cannot react with the pyrimidine dimers formed outdoors because the covalent bonds block the available reaction sites (competitive reaction); whereas, indoor UVA forms few dimers so that many pyrimidine sites are available to react with the benzothiazine radicals it forms. Besides UVA creating ROS that oxidizes deoxyguanosine to 8-oxodG ( $G = O$  in diagram), UVA can create ONOO- radicals that can also make CPD in the dark for several hours post exposure [80].

eumelanin to pheomelanin ratios of only 1.46 and 4.44, respectively, while wild types have 5.81 ( $p < 0.001$ ).<sup>72</sup> These findings highlight the fact that people with dark hair and certain melanocortin-1-receptor variants can also get CMM.<sup>73</sup> Pheomelanin has been detected in normal unexposed skin and its synthesis is markedly increased in dysplastic melanocytic nevi and melanoma cells to the point where high levels of its metabolites have been detected in patients' urine.<sup>25</sup> Unlike eumelanin synthesis, pheomelanin synthesis produces ROS and its cysteine-related precursors like benzothiazine, benzothiazinylalanine and similar precursor molecules that absorb UVA1 radiation ( $\lambda_{\text{max}} > 340 \text{ nm}$ )<sup>74</sup> probably also play significant roles in the etiology of CMM.

The most important evidence that some kind of melanin is crucial for developing melanoma comes from studies showing albino blacks almost exclusively do not get CMM while they do get numerous, early onset non-melanoma skin cancers and they also sunburn easily.<sup>75,76</sup> Albino blacks with white skin and white hair have the same number of melanocytes as normal people with pigmented skin and hair but they do not usually produce any melanin or its precursor molecules revealing 2 important risk factors involved in non-melanoma skin cancers are not involved in

CMM, i.e., skin color and conventional UVR-induced DNA damage such as cyclobutane pyrimidine dimers and 8-hydroxy-2'-deoxyguanosine. Like whites, albino blacks get the same UV-induced DNA damage that keratinocytes accumulate, as shown by their higher rates of non-melanoma skin cancer at younger ages, but those DNA lesions do not transform their melanocytes. The fact that all 4 types of albinos, OCA1 (A&B), OCA2, OCA3, and OCA4 have drastically reduced production of melanin and its precursors<sup>76</sup> and have an almost non-existent incidence of CMM suggests that either melanin or its precursors are probably required for initiating melanoma. Strong evidence that melanoma initiation relies upon melanin content comes from the fact that the action spectrum for melanoma in a fish model (*Xiphophorus*) is identical to the action spectrum for photosensitized radicals that are only produced by the pigmented fish.<sup>77</sup>

## Conclusions

A working hypothesis must explain why the incidence of CMM is similar between outdoor and indoor workers when the former gets 3–10 times the UVR dose that the latter gets (see Fig. 4). Unlike indoor workers, who are only exposed to UVA radiation through



windows,<sup>2</sup> along with visible light, outdoor workers are exposed to UVA and UVB as well as visible light. Because UVB immediately crosslinks adjacent thymine bases, it prevents UVA from crosslinking other molecules like benzothiazine to the thymine bases, which is similar to PUVA's 8-methoxypsoralen bulky adduct that is difficult to repair. Thus, to prevent skin cancer, and especially melanoma, we apparently need sunscreens with not only UVB protection but with better broad-spectrum protection in the UVA and even in the visible waveband region.<sup>78,79</sup>

## Abbreviations

CMM	cutaneous malignant melanoma
HPV	Human Papilloma Virus
PUVA	8-methoxypsoralen and UVA
ROS	reactive oxygen species
US	United States
UVR	Ultraviolet Radiation (290–400 nm)
UVA	Ultraviolet-A (316–400 nm)
UVAI	(341–400 nm)
UVA2	(316–340 nm)
UVB	Ultraviolet-B (290–315 nm)

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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The authors wish to dedicate this paper to the memory of Professor Jan C. van der Leun who passed away on July 6, 2016; his greatest accomplishments include: chairman of UNEP's effects section of the coordinating Committee on the Ozone Layer from 1982 to 1988, head of the Photodermatology Department of Utrecht University (where his group significantly contributed toward developing the SCUPh action spectrum for photocarcinogenesis in 1993), founding co-chair of the Environmental Effects Assessment Panel (EEAP), winner of the UNEP Global Ozone Award in 1995, Finsen Medal award in Photobiology in 1996, UNEP Global 500 Roll of Honor for Environmental Achievement in 1997, European Society for Photobiology Medal Award in 2003, and he was made a Knight in the Order of the Dutch Lion by Queen Beatrix for his "scientific work and for applying the results for the sake of the environment" in 2004. Besides being a great scientist, Professor Jan C. van der Leun was also a great human being that will be dearly missed by multitudes of people worldwide.

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