Is 2,3,5-Pyrroleticarboxylic Acid in Hair a Better Risk Indicator for Melanoma than Traditional Epidemiologic Measures for Skin Phenotype?

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This study aims to assess type of melanin as a risk indicator for skin tumors, in a sample of melanoma cases and controls within a larger multicenter study (Helios 2), held in Europe and South America in 2001–2002. In each case and control, the melanin content in hair was assessed by three methods: 1) the amount of 2,3,5-pyrroleticarboxylic acid (PTCA); 2) the absorbance ratio with ultraviolet spectroscopy; and 3) the spectra of near-infrared spectroscopy. Statistical analysis was performed in a Bayesian setting, defining priors for confounders and effect modifiers from the larger study data set. Subjects with values of PTCA of less than 85 ng/mg carried an increased risk (26 vs. seven discordant pairs: odds ratio = 4.4, 95% confidence interval: 1.52, 14.54), adjusted by hair color, eye color, and number of nevi ($n \geq 40$). The absorbance ratio showed a weaker and nonsignificant odds ratio of 1.5. After correction by misclassification, near-infrared spectroscopy was associated with an odds ratio of 2.3 (95% confidence interval: 1.36, 4.22). The amount of PTCA is thus a strong and independent risk indicator for melanoma. Incorporating PTCA determination into epidemiologic studies is therefore recommended.

melanins; melanoma; skin neoplasms; spectroscopy, near-infrared

Abbreviations: HPLC, high-performance liquid chromatography; NIR, near-infrared; PTCA, 2,3,5-pyrroleticarboxylic acid.
Research into the etiology of skin tumors has generated numerous epidemiologic studies, leading to consistent scientific agreement on the role of certain phenotypic, behavioral, and environmental risk factors. Results of case-control studies on cutaneous melanoma in different Caucasian populations across America, Europe, and Oceania were summarized in meta-analyses focusing on skin pigmentation (1), nevi, (2), sun exposure (3, 4), and exposure to ultraviolet radiation during childhood (5). Odds ratios were in the order of 2–3 in persons with fair complexions (light blonde or red hair) (1), with a high number of nevi and freckles (2), and in those having experienced intermittent exposure to sun (3, 4). The role of the molecular and genetic features postulated to underlie the increased risk of melanoma in such groups of people was investigated in a subsequent study that compared several genetic markers in cases with multiple melanoma versus controls with nonmultiple melanoma (6). However, the number of genes and polymorphisms possibly involved is rather high, with complex interaction and the involvement of several different metabolic pathways. An intermediate approach would be restricting measurements to those biologic markers generated at the end of the response sequence to ultraviolet stimulation. A class of molecules has been advocated for this role: the melanin. Controlled by the melanocortin 1 receptor gene (MCIR), their production in melanocytes is stimulated by ultraviolet exposure (7) through a complex interaction of metabolic pathways (8). Polymorphism or unrepressed gene damage leads to ineffective forms of melanin (the pheomelanin) or an insufficient amount of eumelanin, the effective form of melanin. The difficulty in extracting a measurable amount of melanin in human tissues has been overcome by use of human hair, where melanin concentrates. Eumelanin is not directly isolated from hair, and its amount is determined through degradation; only the structural markers undergo a quantitative analysis. The most representative of these is 2,3,5-pyrolyetricarboxylic acid (PTCA), which is formed after oxidative degradation of the pigment eumelanin (9, 10).

The present case-control study intends to assess the extent to which eumelanin, measured with different methods, is a risk indicator for melanoma. Data were retrieved from a larger study (Helios 2) (11).

MATERIALS AND METHODS

Cases and controls

Helios 2 was a multiple case-control study on melanoma and squamous and basal cell carcinomas. Cases and controls were recruited in 14 centers mainly in Europe, to ensure a wide range of different phenotypes, from various populations (11). The sample was restricted to Caucasian men aged 20–75 years, since in a previous pilot study (12) we had found a high proportion of women with dyed hair that causes permanent alterations in the structure of hair and therefore modifies the results of biochemical analysis of melanin. Cases were identified and first approached in hospitals by clinicians and subsequently contacted by trained interviewers. Controls were chosen among patients admitted at the same hospitals with nondermatologic diseases during the same period. We excluded patients with orthopedic injuries from emergency rooms, orthopedic clinics, and surgical centers, because their injuries could have been caused by outdoor sports activities. All subjects were interviewed between the autumn of 2001 and spring of 2002.

Melanin was intended to be determined through three different methods, one of which was high-performance liquid chromatography (HPLC). This method turned out to be lengthy and expensive. Therefore, it was decided to limit the analyses to two random samples, respectively, of 100 out of 214 melanoma cases and 100 out of 349 controls. The latter were matched to the cases by age (±5 years) and recruitment center of cases.

Risk factors and sun exposure assessment

A questionnaire was administered to all interviewed subjects, gathering information on pigmentation (hair color and eye color), skin reaction to sun exposure, tendency to tan, past experience of sunburn, lifelong exposure to sunlight during different recreational activities, occupational histories, cosmetic habits such as sunscreens and sun lamps, exposure to artificial ultraviolet sources for therapies, and dermatologic history. Hair color and eye color were assessed by the interviewers who compared reference samples of hair and reference high-quality photographs for eye color. A whole-body nevi count was assessed with a visual chart presenting four body schemes with different densities of nevi approximately corresponding to none, less than 20, between 20 and 40, and more than 40. A visual chart was also proposed to assess the appearance of freckles on the face when young, covering a range from none to heavy freckling in six increasing levels.

Sun exposure history was assessed with structured questions arranged by life periods, places of residence, and type of outdoor activity: work, holidays, sports, or other outdoor recreational activities. Whenever a subject reported an outdoor exposure, he was asked about calendar years of activity, most frequent season of exposure (warmer and cooler months), hours of exposure (amount and distribution during daylight), and usual clothing during such activities. Further details on ascertainment methods of skin characteristics and sun exposure are described elsewhere (11).

Melanin determination

A hair sample, approximately 50–200 mg of hair, was taken from the occipital part of the scalp and was freed of any nonpigmented hair. It was divided into three subsamples for separate analysis of melanin metabolites through HPLC (10 mg), ultraviolet spectroscopy (4 mg), and near-infrared (NIR) spectroscopy (50 mg). Hair samples for HPLC determinations and ultraviolet spectroscopy were washed twice with acetone, finely shredded, and left for at least 24 hours in an environment of 20 ± 2°C and relative humidity of 65 ± 2 percent. For HPLC determination, hair samples were then suspended in sodium hydroxide and 30 percent hydrogen peroxide (0.1 ml) and allowed to stand at 20 ± 2°C with stirring for 24 hours. The residual hydrogen peroxide was decomposed by addition of 0.2 ml of 5 percent sodium

metabisulfite, and then the mixture was acidified to pH 4 with 6 mM phosphoric acid and centrifuged at 12,000 rpm for 15 minutes. The supernatant was filtered and then injected for HPLC analysis. Oxidation with hydrogen peroxide in an alkaline medium, following the method of Napolitano et al. (10), proved to be more efficient compared with the previous method (9). Indeed, oxidation with hydrogen peroxide in an alkaline environment (13) leads to a higher PTCA yield, greater stability, good linearity of the calibration curve, and major simplicity in the analytical procedure.

For ultraviolet spectroscopy, acetone-washed and -conditioned hair samples (4 mg each) were subsequently dissolved in 1.0 ml of a Soluene 350 (PerkinElmer Life and Analytical Sciences, Inc., Waltham, Massachusetts):water mixture (9:1); then, they were maintained for 3 hours in vortex-mixed, capped tubes and heated in water at 80°C. After cooling and subsequent centrifugation, supernatants were analyzed for absorbance in the ultraviolet spectra (model V-520 spectrophotometer; Hitachi, Ltd., Tokyo, Japan). The absorbance at 500 nm per 1 mg of hair (A500) is considered to measure the total amount of eumelanin and phaeomelanin, while the ratio between absorbance at 650 nm (A650) and A500 represents the relative amount of eumelanin (14).

NIR spectroscopy is faster than other spectrophotometric techniques, as it does not require destructive analytical procedures and preparation of the sample before the analysis. In theory, NIR determination can be used to measure melanin in hair in situ with an optical-fiber NIR probe. For the measurements, 50 mg of hair were pressed so as to form a disc for the instrument’s measuring window. Calibration was performed against HPLC determination (15). The NIR spectrophotometer used was a PerkinElmer Fourier transform (FT)-NIR System model Spectrum IdentiCheck. Each analytical spectrum is the result of the recording software combination of 32 scans. Single NIR spectra were acquired for each sample.

Statistical analysis

We conducted statistical analysis in four steps as follows.

**Step 1: choice of a cutoff point.** Because the amount of melanin, measured with different methods, is on a continuous scale, we identified a cutoff point that maximized sensitivity and specificity in labeling caseness. That was accomplished with a logistic link modeling probability of being labeled as a case. We also pursued the analysis, taking into consideration a more flexible use of measurement scale of melanin amount, using an ordinal or continuous scale.

**Step 2: model selection.** Because the case set for the present analysis was randomly chosen from the main study, we had the opportunity of performing the statistical analyses in a Bayesian framework, using informative prior knowledge on confounding variables from the whole set’s results. In order to check the robustness of models to the variation of a limited range of priors and their errors, we also conducted an informal sensitivity analysis (16) using four sets of different priors: 1) all data from the main study; 2) subjects other than those selected for this study; 3) priors from the above-cited meta-analysis studies; or 4) noninformative priors. The binomial outcome (number of discordant pairs) was modeled with a logit link and normal distribution of estimated parameters with means and variances from the informative priors obtained in the main study. A set of variables had already been selected within the main study: pigmentation characteristics, nevi, freckles, sunburns, and intermittent sun exposure. Among these, we identified the significant ones through a stepwise procedure evaluating at each run the model fit through the deviance information criterion, the Bayesian information criterion, and the conditional predictive ordinate for monitoring outliers, influential points, and estimated parameters gradient (17). The models and their diagnostics were implemented using WinBUGS software (18).

**Step 3: analysis of melanin’s effect.** The selected model was finally used for evaluating the strength and significance of association with melanin determinations (PTCA-HPLC, PTCA-NIR, and A650/A500 absorbance ratio). Results are presented showing crude and adjusted odds ratios, adjusting by the variables which were kept in the selected model. The relationship between the melanin amount and other risk factors was scrutinized through log-linear (for binary outcomes) and linear (for continuous outcomes) models and checked against an asymptotic covariance matrix of parameter estimates from logit models.

**Step 4: NIR determinations.** Because NIR measurements were calibrated against HPLC measurements, considered to be a more precise type of melanin determination, it was possible to compute the sensitivity and specificity of the NIR method. Therefore, it was also possible to correct the results for measurement error according to the Bayesian approach proposed by Prescott and Garthwaite (19) for matched case-control studies, using results from the calibration study (15) as a validation substudy.

**RESULTS**

We analyzed 98 pairs of cases and controls since two cases and one control did not have a sufficient amount of pigmented hair. Reproducibility of PTCA-HPLC determinations showed an intraclass correlation coefficient of 0.99 for the workups and 0.98 for the injections (20). A limited amount, not statistically significant, of time drift was detected for the dark-hair reference sample measured over the whole duration of determination work (about 3 months), and results were accordingly corrected.

**Choice of a cutoff point**

Sensitivity and specificity in respect to caseness were maximized at values ranging between 75 and 100 ng/mg of PTCA, corresponding to a crude odds ratio of 3.7 for a cutoff of 85 ng/mg. In the analysis, we also used PTCA in its original continuous scale and set PTCA as an ordinal variable with six exposure categories defined around class 75–100 ng/mg and with class ≥150 as the reference category.

**Model selection**

In the framework of a Bayesian analysis, informative priors of confounding variables were gathered in different
ways as indicated in Materials and Methods for testing models’ robustness. Informative priors from the main study and those from the meta-analyses led to close results. Table 1 reports those from the whole unmatched set of cases and controls. Priors for PTCA parameters were kept neutral with a low precision, reflecting our indeterminacy in this first analysis of such factors, although a nonnull, positive association would have been expected from theoretical considerations. Two Markov chain Monte Carlo sequences were generated through sampling from posterior distribution with initial values set to null and to prior-centered values. For reaching stability and normality of estimated parameters, 10,000 updates with a burn-in of 1,000 were sufficient. In spite of substantial normality of parameters’ posterior distributions, we presented their median values and 2.5–97.5 percent credible regions. For each studied factor, table 1 reports the priors from the whole data set, the number of discordant pairs, and crude and adjusted (for all other factors in the table) odds ratios. Pale eye color, light hair color, freckles, and number of nevi emerged as significant and independent risk factors. Subsequently, we investigated whether successive deletion and insertion of each factor acted as a confounder for the remaining ones, with a stepwise procedure: This exercise confirmed those four risk factors. Then, we checked the effect of PTCA level controlled by the selected factors. The effect of freckles was no longer significant, while the posterior distributions of the hair color parameters were, as expected, highly correlated (sampled correlations ranging between 0.10 and 0.43) to PTCA either measured at the cutoff level of 85 ng/mg, categorized in six classes, or entered with its original continuous values.

### Analysis of melanin’s effect

The highest effect of PTCA was for categories ranging between 50 and 100 ng/mg, with narrower bound for values ranging between 75 and 100 ng/mg (table 2). Controlling for other phenotypic characteristics led to an approximate 50 percent reduction of the odds ratios. When the PTCA level was dichotomized at 85 ng/mg, its effect increased, still maintaining a slight separate effect from hair color assessed by the interviewers (table 2). Moreover, deviance information criterion statistics showed an improvement, dropping from 116.135 of the previous model to 104.212 using dichotomized values at the 85-ng/mg cutoff. Introducing the PTCA original continuous scale was still significant (table 2), although the deviance information criterion increased to 108.324, with the same number of estimated parameters.

### Table 1. Priors (standard error), number of discordant pairs, and crude and adjusted odds ratios by risk factors, Helios 2 Study, Europe and South America, 2001–2002

<table>
<thead>
<tr>
<th>Factor</th>
<th>Priors* (standard error)</th>
<th>Case+/control (no. of pairs)</th>
<th>Case-/control (no. of pairs)</th>
<th>Crude odds ratio</th>
<th>95% credible interval</th>
<th>Adjusted odds ratio †</th>
<th>95% credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>Referent</td>
<td>7</td>
<td>24</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Brown</td>
<td>0.95 (0.24)</td>
<td>25</td>
<td>18</td>
<td>3.1</td>
<td>1.29, 7.26</td>
<td>3.1</td>
<td>1.63, 4.20</td>
</tr>
<tr>
<td>Blonde</td>
<td>0.99 (0.32)</td>
<td>14</td>
<td>8</td>
<td>5.0</td>
<td>1.54, 16.14</td>
<td>2.7</td>
<td>1.43, 5.03</td>
</tr>
<tr>
<td>Light blonde/red</td>
<td>0.64 (0.38)</td>
<td>11</td>
<td>7</td>
<td>4.9</td>
<td>1.40, 17.17</td>
<td>1.9</td>
<td>0.87, 4.00</td>
</tr>
<tr>
<td>Eye color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale vs. dark</td>
<td>1.16 (0.32)</td>
<td>18</td>
<td>7</td>
<td>2.6</td>
<td>1.07, 6.16</td>
<td>3.1</td>
<td>1.23, 8.39</td>
</tr>
<tr>
<td>Tendency to sunburn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunburn/blisters vs. never</td>
<td>0.18 (0.20)</td>
<td>18</td>
<td>7</td>
<td>2.6</td>
<td>1.07, 6.16</td>
<td>1.4</td>
<td>0.51, 3.81</td>
</tr>
<tr>
<td>No. of nevi (chart)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Some/several vs. none</td>
<td>1.38 (0.28)</td>
<td>31</td>
<td>5</td>
<td>6.2</td>
<td>2.41, 15.94</td>
<td>6.9</td>
<td>2.86, 18.78</td>
</tr>
<tr>
<td>Freckles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Some/several vs. none</td>
<td>0.83 (0.23)</td>
<td>27</td>
<td>12</td>
<td>2.3</td>
<td>1.14, 4.44</td>
<td>2.3</td>
<td>1.03, 5.43</td>
</tr>
<tr>
<td>Sunburns lifelong</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Some/several vs. none</td>
<td>0.16 (0.12)</td>
<td>15</td>
<td>7</td>
<td>2.1</td>
<td>0.87, 5.25</td>
<td>1.1</td>
<td>0.40, 3.00</td>
</tr>
<tr>
<td>Sunburns in childhood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes vs. never</td>
<td>0.16 (0.22)</td>
<td>21</td>
<td>13</td>
<td>1.6</td>
<td>0.81, 3.23</td>
<td>1.2</td>
<td>0.50, 2.67</td>
</tr>
<tr>
<td>Holidays at the seaside (no. of weighted hours in a lifetime) &gt;7,000 hours vs. ≤7,000</td>
<td>0.74 (0.32)</td>
<td>4</td>
<td>1</td>
<td>4.0</td>
<td>0.45, 35.8</td>
<td>2.0</td>
<td>0.44, 10.10</td>
</tr>
</tbody>
</table>

* Priors are the multiple logistic coefficients (odds ratios) and their standard errors estimated from the whole data set of the main Helios 2 study (R. Zanetti et al. Br J Cancer 2006;94:743–51 (11)). † Adjusted by all the other variables in the table.
The values of the absorbance ratio at $A_{650}$ nm and $A_{500}$ nm, representing the relative amount of eumelanin over the total amount of melanin including pheomelanin, underwent the same analytical procedure, first identifying a cutoff point and then analyzing different measurement scales (table 3). The cutoff point that maximized the risk of cutaneous melanoma was for subjects with a ratio lower than 0.35, which, however, was not statistically significant (table 3). In addition, analyses with quartile categories (table 3) or a continuous scale (table 3) failed to detect any class with a significant association.

### NIR determinations

NIR spectroscopy (a less precise but faster and cheaper type of measurement of eumelanin) was evaluated at a cutoff point of 85 ng/mg, corresponding to the amount of PTCA in the hair previously identified with PTCA-HPLC. A crude odds ratio of 2 (not statistically significant) dropped to no effect when controlled by four risk group strata created from observed combinations of eye color, number of nevi, and hair color, properly collapsed in broader groups for ensuring a minimum number of observations for each stratum. The effect of a slight misclassification (overall sensitivity: 75 percent in cases and 81 percent in controls; overall specificity: 98 percent in cases and 96 percent in controls) was not negligible and amplified by simple controlling for confounding. Indeed, measurement error was not homogeneous across strata, which required a proper multivariable correction. The latter was accomplished by computing corrected stratum-specific frequencies, considering a sensitivity ranging from 50 percent to 100 percent in cases and from 67

### Table 2. Risk of cutaneous melanoma for different levels and measurement scales of PTCA* (eumelanin) in hair, Helios 2 Study, Europe and South America, 2001–2002

<table>
<thead>
<tr>
<th>Category of PTCA (ng/mg)</th>
<th>Case+ / control− (no. of pairs)</th>
<th>Crude odds ratio</th>
<th>95% credible interval</th>
<th>Adjusted odds ratio†</th>
<th>95% credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥150</td>
<td>6</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>125–149</td>
<td>16</td>
<td>2.5</td>
<td>0.91, 7.73</td>
<td>1.7</td>
<td>0.57, 6.07</td>
</tr>
<tr>
<td>100–124</td>
<td>18</td>
<td>2.6</td>
<td>0.94, 7.82</td>
<td>1.4</td>
<td>0.58, 5.25</td>
</tr>
<tr>
<td>75–99</td>
<td>26</td>
<td>5.6</td>
<td>1.97, 18.63</td>
<td>3.2</td>
<td>1.44, 13.20</td>
</tr>
<tr>
<td>50–74</td>
<td>13</td>
<td>5.6</td>
<td>1.57, 23.89</td>
<td>3.4</td>
<td>1.09, 19.71</td>
</tr>
<tr>
<td>&lt;50</td>
<td>5</td>
<td>6.2</td>
<td>1.12, 38.78</td>
<td>2.6</td>
<td>0.50, 30.20</td>
</tr>
<tr>
<td>&lt;85 ng/mg</td>
<td>26</td>
<td>3.7</td>
<td>1.61, 8.56</td>
<td>4.4</td>
<td>1.52, 14.54</td>
</tr>
<tr>
<td>Average risk per each 25-ng/mg decrease</td>
<td>63‡</td>
<td>1.38</td>
<td>1.129, 1.701</td>
<td>1.29</td>
<td>1.095, 1.689</td>
</tr>
</tbody>
</table>

* PTCA, 2,3,5-pyrroletricarboxylic acid.
† Odds ratio adjusted by hair color, eye color, and number of nevi.
‡ Number of pairs where PTCA levels were lower in cases.
§ Number of pairs where PTCA levels were lower in controls.

### Table 3. Risk of cutaneous melanoma for different levels and measurement scales of $A_{650}/A_{500}$ absorbance ratio (eumelanin vs. total melanin) in hair, Helios 2 Study, Europe and South America, 2001–2002

<table>
<thead>
<tr>
<th>Category of $A_{650}/A_{500}$ absorbance ratio</th>
<th>Case+ / control− (no. of pairs)</th>
<th>Crude odds ratio</th>
<th>95% credible interval</th>
<th>Adjusted odds ratio*</th>
<th>95% credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.35</td>
<td>21</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>0.32–0.34</td>
<td>18</td>
<td>1.6</td>
<td>0.72, 3.99</td>
<td>2.0</td>
<td>0.74, 6.04</td>
</tr>
<tr>
<td>0.29–0.31</td>
<td>15</td>
<td>1.2</td>
<td>0.50, 2.81</td>
<td>1.2</td>
<td>0.44, 3.48</td>
</tr>
<tr>
<td>0.23–0.28</td>
<td>10</td>
<td>1.6</td>
<td>0.56, 5.04</td>
<td>1.9</td>
<td>0.48, 7.63</td>
</tr>
<tr>
<td>&lt;0.23</td>
<td>8</td>
<td>1.5</td>
<td>0.46, 5.10</td>
<td>0.6</td>
<td>0.12, 2.70</td>
</tr>
<tr>
<td>&lt;0.35</td>
<td>20</td>
<td>1.4</td>
<td>0.72, 2.83</td>
<td>1.5</td>
<td>0.66, 3.58</td>
</tr>
<tr>
<td>Average risk per each 5-unit decrease of absorbance ratio</td>
<td>51†</td>
<td>1.13</td>
<td>0.686, 2.856</td>
<td>1.09</td>
<td>0.657, 3.274</td>
</tr>
</tbody>
</table>

* Odds ratio adjusted by hair color, eye color, and number of nevi.
† Number of pairs where the absorbance ratio was lower in cases.
‡ Number of pairs where the absorbance ratio was lower in controls.
percent to 100 percent in controls, while the specificity was 100 percent in cases and from 95 percent to 100 percent in controls. After correction, the odds ratio increased to 3.4 (crude) and 2.3 (adjusted), with a 95 percent credible interval that excluded unity.

**DISCUSSION**

Melanin has been extensively studied in tissues and in hair (8–10, 21), either in studies lacking an epidemiologic design or within a case-control setting, which nevertheless lacked direct measurement (22). The originality of the present study consists in a design appropriate to analyze the relation between melanoma risk and amount of melanin that was measured directly. The pathogenic mechanism underlying the association, postulated from experimental studies in vitro or in animals, implies a role of melanin as a protective shield against ultraviolet radiation (21) and a possible role as oxygen-derived radicals scavenging (23). Biochemical studies have shown that, under the heading of “melanin,” there are at least two major classes of molecules: eumelanin and pheomelanin, the latter suspected to be the principal responsible for the lack of protection or, even worse, to be the direct agent responsible for cell damage. The well-known association between skin cancer risk and red hair is paralleled by the high ratio of pheomelanin to eumelanin in affected persons (24). In addition, pheomelanin is a photosensitizer that in some conditions generates active oxygen derivatives upon ultraviolet irradiation (25). However, chemically processing such highly stable macromolecules implies analytical difficulties that hindered the possibility of measuring directly the relative amount of each component.

In this study, we directly measured the 2.3.5-pyruroetraoxylic acid obtained through HPLC that was a quantitative eumelanin metabolite (9), while the pheomelanin amount was indirectly measured as the ratio between ultraviolet absorbance at 650-nm and 500-nm wavelengths. In addition, a new, faster, and less expensive measure than HPLC, based on NIR spectroscopy, was proposed for measuring the amount of PTCA in human hair directly, without subsequent degradation and extraction (10).

In theory, such metabolites, or indirect measures of them, could identify subjects at high risk of skin tumors more efficiently than traditional subjective measures of skin characteristics’ risk factors. A previous study by Dwyer et al. (22) measured melanin indirectly through skin reflectance, compared with extreme exposure categories, and estimated a statistically significant odds ratio of 6.3 in men and 2.0 in women. A limitation of that study is that adjustment of the odds ratio was limited to age and sun exposure.

Recent case-control studies concentrated on MC1R polymorphisms, the genetic control locus of melanin synthesis (26, 27). Kennedy et al. (26) reported odds ratios of 3.6 and 2.7 for subjects with two and one variants, respectively. The most common polymorphisms with an increased risk for melanoma were compound heterozygotes and homozygotes for the Val60Leu, Val92Met, Arg142His, Arg151Cys, Arg160Trp, Arg163Gln, and His260Pro variants, with odds ratios of approximately 4. The highest risk (odds ratio = 16.1 in compound heterozygotes) was for the MC1R gene variant Asp84Glu. The odds ratio was adjusted by skin type but not for the presence of nevi. The study by Dwyer et al. (27) investigated five MC1R variants (Val60Leu, Asp84Glu, Arg151Cys, Arg160Trp, Asp294His), together with indirect melanin measurements (skin reflectance) (22). They found that variant carriers had a significant lower mean concentration of melanin, compared with noncarriers, and a nonsignificant risk increase for any variant carriers among subjects with darker skin (i.e., with a melanin concentration above the median value). Among the latter, the variant corresponding to the highest (statistically significant) risk increase was Arg160Trp (odds ratio = 4.38). They concluded that the melanin was the major contributor to melanoma risk, and the advantage of adding information on the MC1R genotype was only modest (1–3 percent).

In epidemiologic studies, assuming that risk is not linked to a single and easily identifiable genetic change, the advantage of working with melanin is that it is an indicator of multiple, possibly independent, effects of different genetic controlling factors placed at the end of a complex process. Indeed, several genes control melanin synthesis and function such as, for example, those regulating tyrosine oxidation and dopachrome tautomerization, those involved in melanosome transport, and those modulating the signals that activate synthesis. With this perspective, the amount of PTCA fulfills this purpose. Our results show that it is the relative lack of eumelanin (here measured by PTCA) that matters for developing a melanoma. Other types of melanin (pheomelanin) seem to play a less important role or, at least, cannot be measured with sufficient precision. It is also possible that their prevalence is too low for giving rise to an observable elevation of risk. A fourfold risk increase, after adjustment for skin pigmentation and number of nevi, with the disappearance of an effect of intermittent sun exposure, stresses the fact that the amount of eumelanin, or, better, the amount of its degradation product, PTCA, is a strong and independent risk indicator for melanoma and maybe for other skin cancers. Surely, HPLC determination and degradation procedures kept under controlled conditions granted less biased results than other indirect measurement techniques. A less expensive alternative measurement of PTCA could be obtained through near-infrared spectroscopy, which, however, requires a calibration phase. In brief, the measurement reliability of results pointed to PTCA as an efficient measurement of risk, while it is open to discussion and demonstration of whether pheomelanin and its degradation products play a role.

Incorporating PTCA determination in epidemiologic studies is therefore recommended, although it should be integrated with more traditional epidemiologic measures, notably the amount of nevi and skin pigmentation characteristics. It also opens the opportunity for studying the role of melanin in skin tumor etiology at a population level.

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