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A case-control study of the risk of cutaneous melanoma associated with three selenium exposure indicators

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Summary

Aims and background—A direct association between exposure to the metalloid selenium and risk of cutaneous melanoma has been suggested by some observational and experimental cohort studies, while other studies yielded inconsistent results. Since some of the inconsistencies may be due to exposure misclassification arising from the use of exposure indicators that do not adequately reflect body tissue selenium content or the levels of the biologically relevant species of this metalloid, we examined this issue using multiple indicators of exposure.

Methods—We analyzed the relation of selenium exposure with risk of cutaneous melanoma using two different biomarkers, plasma and toenail selenium concentration, and estimated dietary selenium intake in a community-based case-control series (54 cases, 56 controls) from an Italian community.

Results—In unmatched and matched logistic regression models as well as nonparametric generalized additive models, higher plasma selenium levels were strongly associated with excess disease risk. In contrast, toenail and dietary selenium exhibited little relation with melanoma risk. The pattern of correlation among indicators of exposure differed by disease status, with dietary intake associated with plasma selenium levels in patients but not in controls.

Conclusions—Our data show that different selenium exposure indicators can yield different inferences about melanoma risk. While the study was small, our results are consistent with a positive association between circulating levels of selenium and melanoma risk. Further investigation of the exposure classification performance of various selenium biomarkers and of metabolic patterns of the metalloid and of its speciation are needed to help elucidate the relation between selenium exposure and human health.

Keywords

melanoma; selenium; plasma; diet; toenails; case-control study; risk

Introduction

The relation of metalloid selenium to human health and particularly cancer is puzzling. While some observational studies have suggested a chemo-preventative effect of selenium, a recent large trial and a meta-analysis by the Cochrane Collaboration found that there is currently no convincing evidence that selenium supplements can prevent cancer in humans^{1,2}. Other studies have suggested the possibility of increased cancer risk^{1,2}. The possibility that exposure to selenium may increase the risk of melanoma was suggested in the 1990's by the observation of an excess risk of this cancer in a residential cohort consuming unusually high levels of the element in its inorganic hexavalent form, selenate, through drinking water³. Other prospective observational investigations examining the relation between indicators of selenium exposure and melanoma risk have yielded inconsistent results⁴⁻⁷. Studies of the relation between selenium and other cancers and chronic diseases have also variously found beneficial, null and adverse effects^{1,8-11}. More recently, an excess melanoma incidence in females, but not in males, was reported from an intervention trial with vitamins C and E, β -carotene, zinc and organic selenium in French adults¹²; no further increased incidence was observed after stopping supplementation¹³. A slight and imprecise increase of melanoma risk was observed in a U.S. trial supplementing with organic selenium as selenized yeast¹⁴, while no data on melanoma risk were reported in another, large trial administering selenium in the organic form of *L*-selenomethionine¹.

Some of the inconsistencies in the literature regarding the relation of selenium with melanoma risk may be due to exposure misclassification. In particular, null findings could be due to the use of exposure indicators that do not adequately reflect body tissue selenium content or the biologically active species of the metalloid. In the present study, we aimed to analyze the relation among three indicators of selenium exposure -- plasma selenium concentration, toenail selenium concentration and dietary intake -- and the relation of these indicators with the risk of cutaneous melanoma in a case-control series from an Italian community.

Methods

Study participants

We carried out the present study on a series of melanoma patients and controls originally enrolled in a community-based study of environmental and lifestyle risk factors of cutaneous melanoma, as reported in detail elsewhere¹⁵⁻¹⁸. Fifty-nine individuals residing in the province of Modena, northern Italy (population around 700,000) were recruited at the Department of Dermatology of Modena and Reggio Emilia University following the diagnosis of cutaneous melanoma from 1999 to 2002. Immediately after enrollment of each patient, which occurred at the beginning of clinical follow-up, we recruited one population control matched to the case on sex, age (± 5 years) and residence in the province, by approaching by phone potential participants identified from a general population database made available by the Local Health Unit of Reggio Emilia. All controls were recruited within three months of enrollment of the corresponding case, in most cases within one month. The participation rate was 72.0 % for cases, drawn from a consecutive series of patients, and 56.3% for referents.

After obtaining written informed consent from each participant, we carried out a dermatological examination and administered questionnaires to assess demographic characteristics, family history of melanoma, lifestyle factors including history of sunburns and other indicators of sun exposure, and a dietary assessment. Study participants also provided blood and toenail specimens.

Dietary intake of selenium

For the dietary assessment, we used a semiquantitative food frequency questionnaire specifically developed for northern Italy that including 248 questions on frequency and quantity of consumption of 188 items. The questionnaire, developed and validated by the EPIC (European Prospective Investigation on Cancer and Nutrition) project^{19,20}, carried 17 sets of illustrations of sample dishes having small, medium and large sizes, and took into consideration cooking procedures and alcohol and beverage consumption. These questionnaires were read by expert personnel using an optical reader at the Milan National Cancer Institute Nutritional Epidemiology Unit, and the overall selenium dietary intake was obtained by linking the foods consumption data to a nutritional database for selenium that was specifically developed by the authors of the present study using published data and *ad hoc* food analyses, using a methodology previously described in detail¹⁰.

Toenail selenium concentration

Right foot toenail clippings were obtained from the participants, cleaned, dried and analyzed using instrumental neutron activation analysis at the Helmholtz Center (former 'Hahn-Meitner Institute') in Berlin, Germany, as reported in detail elsewhere¹⁶.

Plasma selenium concentration

Fasting venous blood samples were collected in EDTA-containing plastic tubes, immediately centrifuged for 10 min at 3000 rpm and stored at -20°C until use in aliquots of 1 ml. Blood samples from five cases and three controls were unavailable for analysis, and therefore the present study is limited to 110 of the 118 original study participants. We determined selenium plasma concentrations using a direct electrothermal atomic absorption spectrometer (AAnalyst 600, Perkin-Elmer). We used Pd and $\text{Mg}(\text{NO}_3)_2$ matrix modifiers and a transverse-heated graphite atomizer (THGA) with integrated pyrolytic graphite-coated platform and longitudinal Zeeman-effect background correction. We prepared a standard calibration curve, ranging from 10 to 40 $\mu\text{g/l}$ of Se, adding to a pool of plasma different volumes of the stock standard solution of sodium selenite (Sigma Aldrich). Calibration blank was pool of plasma itself. In order to evaluate the performance of the procedure we used a certified reference material, BCR 638 (IRMM Institute of Reference Materials and Measurement), containing $104 \pm 7 \mu\text{g/l}$ of selenium. All plasma samples were prepared daily by dilution 1:5 v/v in double-distilled water. Volumes of 20 μl of all samples were injected into the graphite furnace with 5 μl of matrix modifier solution. The temperature programme, optimised for selenium determination in serum/plasma samples, provides pyrolysis and atomization temperatures at 1200°C and 1900°C , respectively. We used an electrode discharge lamp for selenium and the 'end-capped' graphite tubes for volatile elements such as selenium.

Data analysis

We compared the distributions of each of the three selenium measures for cases and controls using two-sample Student's t-test. The associations among the three measures were quantified using Spearman rank correlation coefficients; this rank-based measure of association was used rather than Pearson correlation coefficients to reduce the influence of several moderate outliers. We estimated the relative risk (RR) of cutaneous melanoma

associated with each indicator of selenium exposure by computing odds ratios in conditional and unconditional logistic regression models. Conditional logistic regression models used the case-control matching; unconditional logistic regression models controlled for age and gender. Each selenium exposure indicator was examined both as a categorical and a continuous predictor. We also fit multivariate models controlling for education (dichotomized as high school graduate or higher versus less than high school), phototype (dichotomized as Type I or II versus III or IV) and history of sunburns (dichotomized as any versus none). These analyses were conducted using Stata 11.1 (StataCorp, College Station, TX 2010).

We obtained nonparametric estimates of the relation between melanoma risk and selenium exposure indicators while controlling for potential confounders using a generalized additive model²¹. The estimates were obtained using a natural cubic smoothing spline, with interior and boundary knots at the unique values of selenium level. These analyses were conducted using the gam package in R version 2.9.2²².

Results

Demographic and clinical characteristics of the cases and controls are provided in Table 1. The subjects ranged in age from 25 to 79 years, and men and women were about equally represented. About half of the subjects had less than a high school education. As expected, cases tended to have lighter phototypes than controls, and also were more likely to report a history of sunburns or a family history of melanoma.

The distributions of plasma and toenail selenium concentration and dietary selenium intake in cases and controls are reported in Table 2. Plasma selenium levels showed a clear tendency towards higher levels in patients compared with referents, with differences more pronounced for females than for males and for the youngest subjects (data not shown). The distributions of toenail selenium concentration and dietary selenium intake were similar between cases and controls, when considering the sample overall and stratified by age and sex. In the study sample as a whole, dietary selenium was mainly due to intake of fish (28% of overall intake), meat (22.7%), cereals (14.9%) and dairy products (12.6%), while the remaining food groups were minor contributors.

In the overall sample, plasma selenium was weakly correlated with toenail concentration (Spearman rank correlation coefficient $r_s=0.22$, $P=0.022$) and other correlations were near zero (Table 3). In analysis by disease status, the estimated correlations between plasma and toenail selenium were similar in magnitude among cases and controls, and there was positive correlation between plasma levels and dietary intake among cases but not controls (Table 3).

In unmatched and matched logistic regression models using quartiles of plasma selenium in the overall sample as explanatory variables, higher selenium levels were strongly associated with excess disease risk (Table 4). The relative risk estimates remained similar when adjusting for potential confounders including education, phototype and sunburn history, and when restricting the analysis to subjects without a family history of melanoma (results not shown). Family history of melanoma could not be used as a predictor due to incomplete data and small numbers. Using plasma selenium concentration as a continuous variable, a 10 $\mu\text{g/l}$ increase was associated with a RR of 1.41 (95% CI 1.11–1.79, $P=0.005$) in a matched analysis and 1.43 (95% CI 1.15–1.78, $P=0.001$) in unmatched analysis, with control for age and sex only. Table 5 provides results of a multivariate analysis using plasma selenium as a continuous variable and controlling for age, sex, education, phototype and sunburn history. The RR estimate was similar after these adjustments (1.43, 95% CI 1.13 – 1.81, $P=0.003$).

Further restricting the analysis to the subset of subjects without a family history of melanoma also yielded a similar estimate (1.38, 95% CI 1.07 – 1.77, $P=0.012$).

Toenail and dietary selenium exhibited far less consistent evidence of a relation with melanoma risk. In analyses using quartiles of exposure indicators (Table 4), point estimates of RRs were below unity for the highest exposure categories but above or near unity for middle exposure categories; all confidence intervals included unity. The RR of the disease associated with a 0.1 $\mu\text{g/g}$ increase in toenail selenium concentration in an unconditional logistic model adjusting for sex and age was 0.87 (95% CI 0.60–1.27, $P=0.470$), while the corresponding estimate for a 10 $\mu\text{g/day}$ increase in dietary selenium was 0.99 (95% CI 0.83–1.19, $P=0.943$). Adjusting for education, phototype and history of sunburns in multivariate analysis did not appreciably change these estimates (Table 5).

Figure 1 displays nonparametric estimates of the relation between melanoma risk and each of the three selenium exposure indicators from a generalized additive model, controlling for age, sex, education, phototype and history of sunburn. The estimates support the appropriateness of modeling the exposure indicators as linear on the log odds scale, as was done in the Table 5 analyses. The nonparametric results suggest a strongly positive association with plasma selenium level. This estimated relation was similar after removal of two potentially influential observations, namely, the subjects with the lowest and highest plasma selenium values (figure not provided). Generalized additive models for toenail and dietary selenium suggested null associations

Discussion and Conclusion

In this study, we found that different selenium exposure indicators yielded different relations with risk of cutaneous melanoma. We observed a strong positive association between plasma selenium levels and risk of melanoma^{12,14,23}. We did not observe an association between melanoma risk and toenail selenium, as previously reported in this population¹⁶.

Though toenail selenium is expected to better reflect long-term intake of the metalloid compared with circulating levels²⁴, some evidence from laboratory studies indicates that the various chemical forms of selenium may be associated with different distributions in body tissues²⁵ notwithstanding comparable overall selenium intake, suggesting that differences in specific selenium compounds might explain the different behavior of plasma and toenail levels in predicting melanoma risk we observed in the present study. These results also suggest the importance of circulating levels of selenium in influencing disease risk compared with levels characterizing other body compartments, emphasizing the importance of a comprehensive approach when estimating selenium exposure and assessing its health effects².

We observed a low or null association of estimated dietary selenium intake with plasma selenium levels as well as with melanoma risk in the overall study sample, not an entirely unexpected finding due to what has been observed in some populations^{26–29} though not in others^{30–32}. The lack of concordance is generally explained by inaccuracies in dietary histories, variability of selenium levels in foodstuffs and absorption rates of the ingested metalloid during the digestive process, and the influence of additional sources of selenium exposure apart from dietary intake, such as smoking, occupational settings and environmental pollution^{24,33,34}. However, when we stratified the study sample according to disease status, dietary intake was associated with plasma selenium levels in patients, and not in controls. This difference related to disease status could be due to chance. However, it might indicate some difference in the chemical forms of ingested selenium between the two groups or in their metabolic pathways within the body, factors which may in turn be related to excess disease risk. The possibility also exists that a direct relation between selenium

exposure and melanoma risk is induced by different individual patterns in element detoxification due to genetic or non-genetic factors or by its specific chemical forms, which may exert different toxic effects even at equivalent amounts of overall selenium ingestion³⁵.

The magnitude of our risk estimates for plasma selenium were roughly in line with those reported in two intervention trials, despite the low number of melanoma cases on which the analysis was based in those studies^{12,14}. Interestingly, in the former trial, a slight increase in melanoma incidence was accompanied by an increased risk of basal cell carcinoma and, in particular, of squamous cell carcinoma, an observation which the investigators judged to be causally related to the antecedent selenium supplementation²³ and which suggests that skin may be a target organ of selenium toxicity.

The biological plausibility of the selenium-melanoma relation is an issue of major importance. While for other diseases such as diabetes and amyotrophic lateral sclerosis laboratory studies have provided strong support for an association with selenium, particularly in recent years^{11,36}, an association between cutaneous melanoma and selenium is less well established. In laboratory studies, selenium compounds have been shown to induce immunotoxicity³⁷, genotoxicity^{38–40} and oxidative stress^{40,41} at doses as low as those found in our study, and to affect Akt enzymatic activity⁴² and p53 expression^{43,44}, mechanisms which have been implicated in melanoma etiopathogenesis⁴⁵, although neither of these effects clearly confirms a specific activity of selenium in inducing melanoma onset or progression. Inorganic tetravalent selenium has also been shown to act as an environmental estrogen⁴⁶ and some epidemiologic data indicate that estrogens increases melanoma risk⁴⁷, but evidence on this point is limited and controversial^{48,49}. Selenium has also been shown to be carcinogenic in some studies^{50–53}, but none of these investigations included animal or *in vitro* models of cutaneous melanoma.

To adequately compare our findings to those yielded by laboratory studies, we should know which kind of selenium species are present in the blood of the study subjects, information that is unfortunately unavailable in our sample as well as more generally in almost all epidemiologic studies, to our knowledge². The laboratory methodologies generally used to determine selenium content of biological indicators or of diet are unable to identify the specific inorganic and organic compounds, though we know that nearly all selenium provided by diet, usually its major source, is in organic forms⁵⁴. This represents a major limitation since laboratory studies show that biological effects of the different selenium species strongly differ^{55–57}, even leading to entirely opposite effects⁵⁸. Application of more advanced analytical methodologies and selenium speciation in epidemiologic studies may be of major importance to elucidate the intriguing relation between the metalloid selenium and human health.

The relation we observed between selenium and melanoma risk occurred at overall levels of selenium exposure that have been considered to be in the ‘safe’ range, i.e., in a population having median values of intake around 60 µg/day and plasma levels near 95 µg/l. However, recent epidemiologic^{1,9–11,59} and laboratory studies^{37,39–41,60} have suggested or demonstrated that selenium toxicity occurs at levels lower than previously believed⁶¹.

Several limitations must be acknowledged. The first caveat is the small size of the study, consisting of a total of 110 subjects. However, despite the small sample size, we observed a strong exposure-risk effect across the distribution of plasma selenium concentrations represented in the sample, and the size of the effect was stable after adjustment for several potential risk factors. A non-experimental study such as the present one also has a serious risk of confounding, even after adjusting for or stratifying on known potential risk factors such as family history of disease, phototype and history of sunburns. We conducted limited

adjustments for sun-related variables because we were concerned about the potential for recall bias or inaccuracies for self-reported measures of sun exposure, such as sunburn history, time or days spent outdoor or at sea or travels to tropical countries; however, when we also included the two latter variables in the regression model results changed little, and this was also true when taking into account vitamin D intake, being a potential protective factor for melanoma at least in the Italian population⁶² (results not shown). Nevertheless, it is still possible that the relation we detected between selenium and melanoma is not due to a true effect of the metalloid, but rather to other risk factors not included in our analysis. We also note the null results of the only other population-based case-control study examining the relation between selenium and melanoma to date⁶³, though the individuals examined in that investigation, drawn from Oahu, Hawaii, were characterized by much higher selenium levels than those experienced by our subjects and likely had a different pattern of melanoma risk factors.

In conclusion, our data demonstrate that findings regarding the relation between selenium and melanoma risk can be highly dependent on the nature of the exposure indicator. Our finding of a positive association with plasma selenium call for further epidemiological evidence on this issue, such as an analysis of melanoma incidence among selenium-administered subjects from a recent large trial¹ and in individuals heavily exposed to selenium compounds from environmental or occupational sources. Advancements in analytical methodologies and their application in epidemiologic studies and studies of metabolic patterns are also needed to better understand the relation between selenium and melanoma, as well as other diseases. In the meantime and until new evidence on this issue is available, these findings add to the increasing epidemiologic evidence^{1,2,10,11,59,64} suggesting caution in increasing selenium exposure, particularly among subjects already at high risk of melanoma.

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Figure 1. Nonparametric estimates from generalized additive model of melanoma risk for each of three selenium exposure indicators, adjusted for age, gender, education, phototype and history of sunburn. Rugplots at the bottom of each plot provide the observed values of plasma exposure indicators.

Table 1

Characteristics of the study sample

	Cases		Controls	
	n	%	n	%
All subjects	54	100	56	100
Sex				
Male	26	48	25	45
Female	28	52	31	55
Age				
25–39 y	11	20	10	18
40–59 y	19	35	24	43
60–79 y	24	45	22	39
Educational attainment				
Elementary school or less	18	33	20	36
Middle school	9	17	8	14
High school	19	35	18	32
College or more	8	15	10	18
Phototype				
I	3	6	1	2
II	27	51	18	33
III	23	43	31	56
IV	0	0	5	9
History of sunburns				
None	22	42	37	66
One	26	50	15	27
Two or more	4	8	4	7
Family history of melanoma				
Yes	4	8	0	0
No	45	92	56	100

Some frequencies do not sum to the total number of cases or controls due to missing values

Table 2

Distribution of plasma and toenail selenium concentration and dietary selenium intake levels in cases and controls

	Minimum	25 th percentile	Median	75 th percentile	Maximum	P-value, cases vs. controls
Plasma selenium concentration (µg/l)						
Cases (n = 54)	53	88	99	116	143	
Controls (n = 56)	15	77	89	101	123	<0.001
Toenail selenium concentration (µg/g)						
Cases (n = 50)	0.40	0.56	0.64	0.70	0.89	
Controls (n = 55)	0.39	0.57	0.65	0.73	0.94	0.510
Dietary selenium intake (µg/day)						
Cases (n = 51)	21	46	54	68	159	
Controls (n = 56)	22	48	57	75	96	0.958

Table 3

Spearman rank correlation coefficients among plasma selenium levels, toenail selenium concentration and dietary selenium intake, with 95% confidence intervals

All subjects	Plasma concentration	Dietary intake
Dietary intake	0.01 (−0.18, 0.20) (n=107)	
Toenail concentration	0.22 (0.03, 0.40) (n=105)	0.08 (−0.11, 0.27) (n=102)
Cases (n = 54)	Plasma concentration	Dietary intake
Dietary intake	0.25 (−0.02, 0.49) (n=51)	
Toenail concentration	0.23 (−0.05, 0.48) (n=50)	0.09 (−0.20, 0.37) (n=47)
Controls (n = 56)	Plasma concentration	Dietary intake
Dietary intake	−0.15 (−0.39, 0.12) (n=56)	
Toenail concentration	0.27 (0.01, 0.50) (n=55)	0.10 (−0.18, 0.35) (n=55)

Table 4

Estimates of relative risk (RR) of melanoma with 95% confidence intervals (CI), by quartile of selenium exposure indicator

A: Plasma selenium (n = 110)

Quartile (cutpoints - $\mu\text{g/l}$)	Cases/controls	Matched analysis RR (95% CI)	P value	Unmatched analysis controlling for age and sex RR (95% CI)	P value
I (<81)	9/20	1.00	--	1.00	--
II (81 - <93)	11/15	2.13 (0.56 - 8.04)	0.266	2.30 (0.72 - 7.31)	0.157
III (93 - <105)	16/12	2.86 (0.79 - 10.32)	0.109	3.36 (1.04 - 10.92)	0.043
IV (105)	18/9	5.86 (1.53 - 22.31)	0.010	6.42 (1.94 - 21.24)	0.002

B: Toenail selenium concentration (n = 105)

Quartile (cutpoints - $\mu\text{g/g}$)	Cases/controls	Matched analysis RR (95% CI)	P value	Unmatched analysis controlling for age and sex RR (95% CI)	P value
I (<0.57)	15/15	1.00	--	1.00	--
II (0.57 - <0.65)	13/14	1.32 (0.43 - 4.07)	0.627	0.95 (0.33 - 2.69)	0.916
III (0.65 - <0.73)	11/12	1.41 (0.44 - 4.54)	0.566	0.90 (0.30 - 2.69)	0.850
IV (0.73)	11/14	0.72 (0.22 - 2.38)	0.586	0.77 (0.26 - 2.28)	0.633

C: Dietary selenium intake (n = 107)

Quartile (cutpoints - $\mu\text{g/day}$)	Cases/controls	Matched analysis RR (95% CI)	P value	Unmatched analysis controlling for age and sex RR (95% CI)	P value
I (<46)	12/14	1.00	--	1.00	--
II (46 - <56)	15/12	1.50 (0.46 - 4.86)	0.500	1.42 (0.48 - 4.22)	0.526
III (56 - <71)	15/13	1.28 (0.45 - 3.63)	0.648	1.35 (0.46 - 3.96)	0.583
IV (71)	9/17	0.64 (0.20 - 2.04)	0.454	0.59 (0.19 - 1.83)	0.357

Table 5

Estimates of relative risk (RR) for continuous measures from unconditional logistic regression analyses controlling for gender, age, education, phototype and history of sunburns, by selenium exposure indicator.

A: Plasma selenium (n = 107)			
Predictor	RR	(95% CI)	P value
Plasma selenium concentration, 10 µg/l increase	1.43	(1.13 – 1.81)	0.003
Male (reference: female)	0.67	(0.28 – 1.62)	0.374
Age, one year increase	1.01	(0.97 – 1.04)	0.691
Education, high school graduate or higher (reference: less than high school)	1.17	(0.41 – 3.34)	0.773
Phototype III or IV (reference: I or II)	0.41	(0.16 – 1.02)	0.054
History of one or more sunburns (reference: no history of sunburns)	1.69	(0.69 – 4.15)	0.253
B: Toenail selenium concentration (n = 102)			
Predictor	RR	(95% CI)	P value
Toenail selenium concentration, 0.1 µg/g increase	0.90	(0.60 – 1.33)	0.583
Male (reference: female)	0.78	(0.32 – 1.87)	0.573
Age, one year increase	1.00	(0.96 – 1.03)	0.822
Education, high school graduate or higher (reference: less than high school)	0.94	(0.33 – 2.67)	0.907
Phototype III or IV (reference: I or II)	0.51	(0.22 – 1.24)	0.138
History of one or more sunburns (reference: no history of sunburns)	2.12	(0.89 – 5.07)	0.091
C: Dietary selenium intake (n = 104)			
Predictor	RR	(95% CI)	P value
Dietary selenium intake, 10 µg/day increase	0.99	(0.82 – 1.19)	0.889
Male (reference: female)	0.65	(0.28 – 1.52)	0.321
Age, one year increase	1.00	(0.97 – 1.04)	0.895
Education, high school graduate or higher (reference: less than high school)	1.06	(0.38 – 2.97)	0.913
Phototype III or IV (reference: I or II)	0.49	(0.20 – 1.18)	0.111
History of one or more sunburns (reference: no history of sunburns)	2.15	(0.92 – 5.06)	0.078