Published in final edited form as:

Melanoma Res. 2016 April ; 26(2): 181–187. doi:10.1097/CMR.0000000000000106.

4-Nitroquinoline-1-oxide-induced mutagen sensitivity and risk of cutaneous melanoma: a case–control analysis

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Abstract

Mutagen sensitivity assay, which measures the enhanced cellular response to DNA damage induced *in vitro* by mutagens/carcinogens, has been used in the study of cancer susceptibility. 4-Nitroquinoline-1-oxide (4-NQO), an ultraviolet (UV) radiation-mimetic chemical, can produce chromosomal breaks in mammalian cells and induce cancer. Given the potential role of 4-NQO as the experimental mutagen substituting for UV as the etiological carcinogen of cutaneous melanoma (CM), we tested the hypothesis that cellular sensitivity to 4-NQO is associated with the risk of developing CM in a case–control study of 133 patients with primary CM and 176 cancer-free controls. Short-term blood cultures were treated with 4-NQO at a final concentration of 10 $\mu\text{mol/l}$ for 24 h and scored chromatid breaks in 50 well-spread metaphases. Multivariable logistic regression was used to calculate odds ratios and 95% confidence intervals. We found that the log-transformed frequency of chromatid breaks was significantly higher in 133 patients than in 176 controls ($P = 0.004$) and was associated with an increased risk for CM (adjusted odds ratio = 1.78, 95% confidence interval: 1.12–2.84) after adjustment for age and sex. Moreover, as the chromatid break values increased, the risk for CM increased in a dose-dependent manner ($P_{\text{trend}} = 0.003$). Further analysis explored a multiplicative interaction between the sensitivity to 4-NQO and a

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^{*}In memory of the 10th death anniversary of Dr T.C. Hsu, Professor in the Department of Cancer Biology, who died in 2003.

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Conflicts of interest

There are no conflicts of interest.

family history of skin cancer ($P_{\text{interaction}} = 0.004$) on the risk of CM. Therefore, our findings suggest that sensitivity to 4-NQO may be a risk factor for the risk of CM, which is more sensitive than UV-induced chromosome breaks.

Keywords

chromosome aberrations; cutaneous melanoma; cytogenetics; epidemiology; 4-nitroquinoline-1-oxide

Introduction

Cutaneous melanoma (CM), the most serious form of skin tumor, is a growing health problem in the USA [1]. Although molecular mechanisms on how CM arises from melanocytes have not been well established, it is generally agreed that ultraviolet (UV) radiation-induced DNA damage and subsequent mutations are initiating steps for the onset of CM [2,3]. The DNA in melanocytes is presumed to be the target, as seen in individuals with DNA repair deficiency, such as those with xeroderma pigmentosum (XP), who have a more than 1000-fold increased risk of sunlight-induced CM [4]. Hence, UV exposure and the related sensitivity may play important roles in the development of CM. Because exposure to UV light and the incidence rate of CM continue to increase in the USA, searching for genetic susceptibility biomarkers will help identify at-risk individuals for cancer prevention aiming at reducing the burden of CM in the general population.

Dr T.C. Hsu, who was the first to determine the accurate haploid chromosome number of Homosapiens and characterized the human karyotype and published a historic paper entitled ‘Mammalian chromosomes *in vitro* – the karyotype of Man’ in 1952, had also developed the mutagen sensitivity assay 20 years ago for epidemiological studies [5]. This assay measures the number of mutagen-induced chromatid breaks per cell (b/c) in cultured primary peripheral blood lymphocytes with bleomycin, a radiomimetic chemical, as a test mutagen. Dr Hsu and his coworkers successfully used this assay to investigate genetic susceptibilities to tobacco-related cancers [5–8]. Similarly, he had later used 4-nitroquinoline-1-oxide (4-NQO) to perform the mutagen sensitivity assay. 4-NQO is a water-soluble quinoline derivative and a UV-mimetic chemical [9] that can cause bulky DNA adducts and chromosomal aberrations in exposed cells [10]; therefore, he used it as a test mutagen to determine UV sensitivity in lymphoblastoid lines and primary cultures of peripheral blood samples [11,12]. Dr Hsu reported that lymphoblastoid cells from a XP patient were most sensitive to 4-NQO, followed by cells from two melanoma patients and apparently normal individuals [11]. He also reported that the frequency of chromatid breaks caused by 4-NQO exposure *in vitro* was significantly higher in 62 melanoma patients than that in 103 normal individuals [12].

Inspired by Dr Hsu’s passion in melanoma research, we collaborated with him to carry out this study on 133 CM patients and 176 cancer-free healthy controls, with adjustment for demographic and exposure factors, to further evaluate the sensitivity to 4-NQO as a biomarker for genetic susceptibility to melanoma in the general population.

Materials and methods

Study population

The study included patients with CM, who were registered at The University of Texas MD Anderson Cancer Center from April 1994 to June 1999. There were no restrictions on age, sex, or ethnicity. Controls were self-reportedly cancer-free individuals recruited from among genetically unrelated visitors who were accompanying cancer patients to clinics or for a cancer screening program at the MD Anderson Cancer Center during the same time period, and were frequency matched to CM patients included in this analysis in terms of age (± 5 years) and sex. The exclusion criteria included previous chemotherapy or radiotherapy, any metastasis, any history of cancer other than CM for case participants, and any blood transfusion in last 6 months for all participants. Informed consent was obtained from all participants, and a standardized, self-administered questionnaire was used to collect data on demographic information and risk factors, such as natural hair color, eye color, skin color, history of sunlight exposure (including freckling in the sun as a child, tanning ability, and number of sunburns), medical history, and family history of first-degree relatives with any cancer. These variables were qualitative. For example, skin color was self-reported on a scale from 1 (very fair) to 10 (dark brown) and categorized into 'Dark brown (4)' and 'Fair (3)' groups; poor tanning ability was defined as 'always burn easily with a painful burn and blistering, followed by peeling, with little or no tan' or 'usually burn easily with a painful burn, without blistering, lasting for at least 2 days followed by peeling, with minimal tanning'. All participants donated 20 ml of blood after diagnosis for case patients and after recruitment for control participants. The study protocol was approved by the institutional review board of MD Anderson.

Mutagen sensitivity assay

The mutagen sensitivity was expressed as the number of 4-NQO-induced chromatid b/c after cells were treated for 24 h [11–13]. Briefly, short-term cultures of 1 ml of fresh whole blood were established in 9 ml of RPMI 1640 medium supplemented with 20 v/v% fetal bovine serum and phytohemagglutinin (Remel, Lenexa, Kansas, USA) at a final concentration of 112.5 $\mu\text{g/ml}$ to stimulate T-lymphocyte growth. After 48 h of culture, cells were treated with 4-NQO at a final concentration of 10 $\mu\text{mol/l}$ and were allowed to grow for another 23 h before being treated with colcemid (Gibco BRL, Carlsbad, California, USA) at 0.06 $\mu\text{g/ml}$ to induce mitotic arrest 1 h before harvesting, which was a routine procedure based on Dr Hsu's earlier report on 4-NQO's genotoxicity with mitotic index and b/c rate of the in-vitro treated lymphocytes [11]. We used conventional chromosome harvesting procedures that Dr Hsu had established by treating cells for 15 min (min) with a 60 mmol/l hypotonic KCl solution and fixing three times for 5 min each with freshly prepared methanol: acetic acid (3:1 v/v), after which air-dried slides were prepared as described previously [5]. Slides were then stained with 4% Giemsa (Biomedical Specialties, Santa Monica, California, USA) for 7 min. All slides were evaluated for chromosomal aberrations by the late Dr Hsu, who was blinded to the case-control status of the participants. The number of simple chromatid breaks was scored from 50 well-spread metaphases per blood sample.

Statistical analysis

The number of chromatid b/c was analyzed as a continuous variable and Student's *t*-test was used to compare the difference in the mean numbers of chromatid b/c between groups. Because chromatid breaks were not normally distributed, we also used Student's *t* tests for log-transformed data. We used the median and quartile of chromatid b/c in the control group as cutoff values to calculate odds ratios (ORs). Pearson correlation analysis was used to explore relationships between sun exposure variables. Univariate and multivariate logistic regression analyses with adjustment for age and sex were carried out to calculate crude and adjusted ORs and 95% confidence intervals (CIs) for associations between 4-NQO-induced chromatid b/c and the risk of CM. Some participants did not provide information on certain variables (such as hair color, eye color, skin color, tanning ability, number of sunburns, and freckling, dysplastic nevi, and family history of skin cancer), and these variables were treated as missing data in the analysis. We also explored interactions between known risk factors and the sensitivity to 4-NQO. To assess evidence for multiplicative interactions, we modeled interaction terms between variables using standard unconditional logistic regression. When multiplicative interactions were not found, we performed additive interaction tests using Stata 8.2 software (StataCorp LP, College Station, Texas, USA) by implementing the bootstrapping method to identify subgroups of individuals who may be at a particularly high risk of developing CM. All statistical tests were two sided, and *P* less than 0.05 was considered statistically significant. We analyzed all data, except for additive models, using SAS software (version 9.2; SAS Institute, Cary, North Carolina, USA) [13,14].

Results

Characteristics of the study participants and risk factors

Because we only recruited a few minorities, this analysis focused only on non-Hispanic whites. As a result, the final analysis included 133 CM patients and 176 cancer-free controls. Because of frequency matching of age (± 5 years) and sex, there were no statistical differences in the frequency distribution of age and sex between cases and controls (Table 1). However, there were significant differences in the frequency distributions of most of known risk factors, such as hair color, eye color, skin color, tanning ability, number of sunburns, freckling, dysplastic nevi, and family history of skin cancer. Using a multivariate logistic regression analysis with adjustment for age and sex, we assessed adjusted ORs and 95% CIs to estimate the risk of CM associated with these risk factors. It was apparent that these risk factors, except for eye color and family history of skin cancer, were associated with a significantly increased risk of CM in this analysis (Table 1). Most of these known risk factors were statistically correlated with each other as expected, similar to what was reported previously for this study population [13]. For example, in 176 control participants, number of sunburns was highly correlated with hair color ($r = 0.191$, $P = 0.012$), eye color ($r = 0.212$, $P = 0.005$), skin color ($r = 0.227$, $P = 0.003$), tanning ability ($r = 0.326$, $P < 0.001$), and freckling in the sun ($r = 0.357$, $P < 0.001$). The results suggested that these known risk factors are important correlates that play a role in sporadic CM development.

4-NQO-induced chromatid breaks

The number of simple chromatid breaks was scored from 50 well-spread metaphase cells from each participant and expressed as chromatid b/c as Lee *et al.* [15] showed that the statistical efficiency of reading 50 and 100 metaphase spreads was similar. Because the mean spontaneous b/c value derived from 50 metaphases of untreated cells was 0.02 [16,17], which was less than 10% of that of 4-NQO-treated cells (0.25 in controls) [13], we used 4-NQO-induced b/c values only for statistical comparisons, as recommended by Hsu *et al.* [18]. As the frequency distribution of 4-NQO-induced b/c was not normal, we used log-transformed values to evaluate differences between cases and controls as well as their associations with the risk of CM in this analysis. Overall, 4-NQO-induced b/c values were statistically higher in CM cases than in controls ($P = 0.004$; Table 2). After stratification by age, sex, and selected risk factors listed in Table 1, log-transformed mean values of 4-NQO-induced b/c were statistically significantly higher among CM cases than in controls in most of subgroups ($P < 0.05$), except for the young age group of less than 50 years, male sex, blond or red hair color, blue eye color, fair skin color, good tanning ability, with freckling in the sun as a child, and with dysplastic nevi ($P > 0.05$). The difference in the log-transformed mean values of 4-NQO-induced b/c between cases and controls was borderline significant for the age group of 50–60 years ($P = 0.051$) and poor tanning ability after prolonged sun exposure ($P = 0.057$). However, we did not find significant differences among/between strata within cases or controls, except for sex in cases with a borderline significantly higher NQO-induced b/c values in men than women ($P = 0.053$, not listed in Table 2). We further evaluated whether the sensitivity to 4-NQO was associated with tumor characteristics including tumor stage, Clark levels, tumor site, and tumor thickness, but we did not find statistically significant differences in 4-NQO-induced b/c values among subgroups in patients (data not shown).

Association between 4-NQO-induced b/c values and the risk of CM

We then carried out univariate and multivariate logistic regression analyses to calculate ORs and 95% CIs without or with adjustment for age and sex. As shown in Table 3, log-transformed 4-NQO-induced b/c values were fitted in logistic regression models either as continuous or as categorical variables. We found that a unit increment in log-transformed b/c values was associated with more than a 40% increased risk of CM (crude and adjusted ORs = 1.44 and 1.41; 95% CI: 1.12–1.85 and 1.10–1.82, respectively). Using the median b/c value of controls as the cutoff point, sensitive to 4-NQO with high log-transformed b/c values were associated with an almost two-fold increased risk of CM (crude OR and 95% CI: 1.83, 1.15–2.91; adjusted OR and 95% CI: 1.78, 1.12–2.84). When quartiles of 4-NQO-induced b/c values of controls were used as cutoff points to evaluate the trend of effects, we found that as the b/c values increased, the risk of CM increased in a dose-dependent manner. Using the lowest quartile of the b/c values as the reference, the crude ORs (95% CIs) for higher 4-NQO-induced b/c values in the 50th, 75th, and 100th were 2.13 (1.02–4.48), 2.49 (1.21–5.13), and 3.28 (1.60–6.74), respectively. The ORs adjusted for age and sex were similar to the crude ORs = 2.01 (0.95–4.25), 2.34 (1.13–4.87), and 3.09 (1.50–6.38), respectively. P values for trend tests were 0.002 and 0.003 (Table 3).

Multivariate logistic regression analysis

Next, we included all selected risk factors including age, sex, hair color, eye color, skin color, tanning ability, number of sunburns, and freckling, dysplastic nevi, and family history of skin cancer in a multivariate logistic regression model for those who had provided complete information in a smaller data set (114 cases and 126 controls; Table 4). We found that hair color, tanning ability, lifetime sunburn with blistering, dysplastic nevi, and high sensitivity to 4-NQO, expressed as frequencies of b/c values using the median in controls as the cutoff point, remained statistically significantly associated with the risk of CM (Table 4). Usually, ORs were attenuated because of the residual effects of age, sex, and confounding from other risk factors, and overadjustment for the correlation among risk factors (OR = 2.26; 95% CI: 1.28–3.99 for the sensitivity to 4-NQO in the model without nonsignificant risk factors from univariate logistic regression presented in Table 1); therefore, these data suggest that in-vitro sensitivity to 4-NQO was an independent risk factor for CM in this study population.

Interactions between 4-NQO-induced b/c values and selected risk factors

Finally, we explored possible interactions between the 4-NQO sensitivity, expressed as log-transformed 4-NQO-induced b/c values, and each of the selected risk factors in multivariate logistic regression models. Hypotheses of multiplicative interactions were tested when we included the interaction (or cross-product) terms (i.e. dichotomized b/c values using the median value in controls as the cutoff point $\times$ each dichotomized risk factor) in the multivariate logistic regression models that included age, sex, main effect of the 4-NQO sensitivity, and the corresponding selected risk factor. A departure from the multiplicative model was followed by the test for the departure from an additive model, if no evidence was found for a multiplicative interaction, with adjustment for age and sex. In this relatively small study, we did not find strong evidence of multiplicative interactions between 4-NQO-induced b/c values and other selected risk factors, except for that between 4-NQO-induced b/c values and a family history of skin cancer ($P_{\text{interaction}} = 0.004$; Table 5). The tanning ability and lifetime sunburn showed a borderline significant additive interaction with the sensitivity to 4-NQO in the risk of CM ($P = 0.051$ and 0.050 , respectively; Table 5). Trend tests were all statistically significant ($P < 0.01$) for all categories (Table 5). This implied that if a patient was sensitive to 4-NQO and had a family history of skin cancers, or poor tanning ability, or had sunburns, he/she would have a three- to five-fold increased risk of developing CM compared with those who were not sensitive to 4-NQO and had no family history of skin cancers, or good tanning ability, or without lifetime sunburns. However, it may generate a false-positive finding because of the limited sample size, particularly for the subgroup with no family history and low sensitivity to 4-NQO.

Discussion

In this case-control study, we found that compared with cancer-free controls, CM patients had significantly higher frequencies of 4-NQO-induced b/c that were associated with an almost two-fold increased risk of CM and that the 4-NQO-induced mutagen sensitivity was an independent risk factor for CM in a dose-response manner in this study population after

adjustment for other confounders. Therefore, this 4-NQO mutagen sensitivity assay may be a marker for genetic susceptibility to CM.

Mutagen sensitivity assays performed in primary lymphocytes as a surrogate tissue with different testing mutagens have been used successfully as susceptibility markers for risk assessment of different cancer types, such as BPDE (benzo[a]pyrene diol epoxide) for smoking-related cancers, gamma-radiation for glioma and breast cancer, or UV for skin cancer [7,8,16,19–25]. Among these, 4-NQO was tested as a UV-mimetic agent by Dr Hsu in XP and melanoma patients 20 years ago [11, 12]. Even though 4-NQO mimicked UV light in mutagenesis and sensitivity in bacteria and human XP cells [9, 26,27], they were different in causing cytotoxicity and DNA repair response [28,29]. It is reported that the UV-induced DNA lesions are mainly repaired by transcription-coupled repair, whereas the lesions induced by 4-NQO are repaired by global genome repair [30,31], both being parts of the nucleotide excision repair pathway as we discussed previously [13]. Furthermore, it is well known that UV not only causes different kinds of photoproducts that may require different repair pathways, such as base and nucleotide excision repair pathways, but it can also cause immunosuppression that plays an important role in the skin carcinogenesis [32]. We had recently assessed the association of 4-NQO-induced mutagen sensitivity and the risk of nonmelanoma skin cancer in 110 basal cell carcinoma and 81 squamous cell carcinoma and reported that high b/c values were associated with a more than two-fold increased risk for both basal cell carcinoma (OR = 2.69, 95% CI: 1.59–4.54) and squamous cell carcinoma (OR = 2.46, 95% CI: 1.28–4.71) after adjustment for age and sex using the median 4-NQO-induced b/c value of controls as the cutoff point [13]. However, we did not find a significant correlation between 4-NQO-induced and UV-induced chromosome breaks in 157 cancer-free controls [13,14] as well as 110 CM patients with both data available in the present analysis, suggesting different mechanisms in the formation of chromatid breaks induced by these two agents.

Several lines of evidence provided support our finding that CM patients may be sensitive to 4-NQO. One early study showed that nonmalignant fibroblasts from CM were abnormally hypersensitive to in-vitro 4-NQO-induced mutations [33]; later studies found that lymphocytes from CM patients were also sensitive to in-vitro exposure to 4-NQO compared with those cells from normal participants and head and neck patients [11,12]. Although mechanisms underlying 4-NQO induce chromosomal breaks are still poorly understood, in-vivo activation of 4-NQO to 4-hydroxyaminoquinoline 1-oxide can cause GC to AT transitions and G to pyrimidine transversions, and two main guanine adducts at positions C8 and N2 with different ratios in double-strand and single-strand damaged DNA may cause subsequent chromosome instability [33–35].

In summary, we found that the frequency of 4-NQO-induced b/c was significantly higher in CM cases than in controls. This higher frequency was associated with an almost two-fold increased risk of CM and a dose–response relationship was found between the quartile of mutagen sensitivity and risk for CM, independent of other known risk factors. These findings suggest that sensitivity to 4-NQO may be a risk factor for CM, which is apparently more sensitive than UV-induced chromosome breaks [14], but the present study had a limited sample size.

Because of inherent selection bias of patients in a hospital-based case–control study, such as bias from self-reported cancer-free status and recall bias on exposures and skin lesions in addition to the small sample size, the findings need further validation by other independent, larger, or prospective studies. Once validated in larger studies, this assay may be used for construction of a risk model to identify a group of patients at high risk of developing skin cancer to target for prevention.

Acknowledgments

This study was supported in part by the National Institutes of Health grant R01 CA100264 (to Q. W.), the National Institute of Environmental Health Sciences grant R01 ES11740 (to Q. W.), MD Anderson Cancer Center SPORE in Melanoma P50 CA093459, Cancer Center Support Grant CCSG P30 CA016672, Myriam and Jim Mulva Foundation, and AIM Foundation. The authors thank Margaret Lung for assistance in recruiting study participants and Yijue Zhao for the laboratory assistance.

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Table 1

Distribution of selected known risk factors and logistic regression analysis

Selected variables ^a	<i>n</i> (%)		<i>P</i> value ^b	Adjusted OR (95% CI) ^c
	Cases (<i>N</i> = 133)	Controls (<i>N</i> = 176)		
Age (years)			0.126	
<50	61 (45.9)	61 (34.7)		
50–60	31 (23.3)	46 (26.1)		
>60	41 (30.8)	69 (39.2)		
Sex			0.448	
Male	67 (50.4)	81 (46.0)		
Female	66 (49.6)	95 (54.0)		
Hair color			0.003	
Black or brown	83 (64.3)	137 (79.7)		1.00 (Ref)
Blond or red	46 (35.7)	35 (20.3)		2.31 (1.36–3.93)
Eye color			0.703	
Not blue	89 (66.9)	120 (69.0)		1.00 (Ref)
Blue	44 (33.1)	54 (31.0)		1.19 (0.73–1.96)
Skin color			0.002	
Dark brown	59 (44.4)	107 (61.9)		1.00 (Ref)
Fair	74 (55.6)	66 (38.1)		2.00 (1.25–3.20)
Tanning ability after prolonged sun exposure			< 0.0001	
Good (high)	62 (46.6)	130 (74.7)		1.00 (Ref)
Poor (low)	71 (53.4)	44 (25.3)		3.22 (1.98–5.23)
Lifetime sunburns with blistering			< 0.0001	
0	27 (20.5)	83 (48.0)		1.00 (Ref)
1	105 (79.5)	90 (52.0)		3.33 (1.97–5.62)
Freckling in the sun as a child			0.009	
No	61 (46.2)	106 (61.3)		1.00 (Ref)
Yes	71 (53.8)	67 (38.7)		1.76 (1.08–2.88)
Dysplastic nevi			0.027	
No	109 (87.9)	141 (95.3)		1.00 (Ref)
Yes	15 (12.1)	7 (4.7)		2.67 (1.03–6.88)
Family history of skin cancer			0.481	
No	99 (79.2)	132 (82.5)		1.00 (Ref)
Yes	26 (20.8)	28 (17.5)		1.16 (0.63–2.14)

CI, confidence interval; OR, odds ratio.

^aThe total number of participants in each subgroup may be less than the total number of participants because some participants did not provide information.

^b χ^2 tests.

^cORs and 95% CIs were adjusted for age and sex.

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Table 2

Comparison of differences in natural log-transformed b/c values induced by 4-NQO between melanoma patients and cancer-free controls

Selected variables ^a	Cases		Controls		P value ^b
	N	Log-transformed b/c (mean±SD)	N	Log-transformed b/c (mean±SD)	
All	133	-1.48 ± 0.89	176	-1.79 ± 0.96	0.004
Age (years)					
< 50	61	-1.54 ± 0.97	61	-1.66 ± 0.98	0.493
50-60	31	-1.32 ± 0.80	46	-1.73 ± 0.95	0.051
> 60	41	-1.51 ± 0.83	69	-1.94 ± 0.93	0.017
Sex					
Male	67	-1.63 ± 0.83	81	-1.73 ± 0.97	0.483
Female	66	-1.33 ± 0.92	95	-1.83 ± 0.95	0.001
Hair color					
Black or brown	83	-1.51 ± 0.87	137	-1.83 ± 0.96	0.015
Blond or red	46	-1.45 ± 0.91	35	-1.69 ± 0.96	0.253
Eye color					
Not blue	89	-1.38 ± 0.81	120	-1.72 ± 0.93	0.006
Blue	44	-1.68 ± 1.01	54	-1.96 ± 1.03	0.189
Skin color					
Dark brown	59	-1.50 ± 0.81	107	-1.89 ± 0.97	0.011
Fair	74	-1.46 ± 0.95	66	-1.64 ± 0.95	0.260
Tanning ability after prolonged sun exposure					
Good (high)	62	-1.55 ± 0.81	130	-1.78 ± 0.97	0.095
Poor (low)	71	-1.42 ± 0.96	44	-1.77 ± 0.92	0.057
Lifetime sunburns with blistering					
0	27	-1.37 ± 0.81	83	-1.77 ± 0.95	0.048
1	105	-1.52 ± 0.91	90	-1.78 ± 0.96	0.047
Freckling in the sun as a child					
No	61	-1.53 ± 0.82	106	-1.85 ± 0.97	0.029
Yes	71	-1.43 ± 0.95	67	-1.71 ± 0.94	0.086

Selected variables ^a	Cases		Controls		P value ^b
	N	Log-transformed b/c (mean±SD)	N	Log-transformed b/c (mean±SD)	
Dysplastic nevi					
No	109	-1.44 ± 0.87	141	-1.75 ± 0.96	0.009
Yes	15	-1.72 ± 1.08	7	-1.55 ± 0.59	0.696
Family history of skin cancer					
No	99	-1.54 ± 0.91	132	-1.81 ± 0.97	0.036
Yes	26	-1.18 ± 0.67	28	-1.93 ± 0.94	0.002

b/c, breaks per cell; 4-NQO, 4-nitroquinoline-1-oxide.

^aThe total number of participants in each subgroup may be less than the total number of participants because some participants did not provide the information.

^bTwo-sided Student's *t* tests for the difference in b/c between cases and controls.

Table 3

Logistic regression analysis of log-transformed b/c values

Chromatid breaks/cell ^a	n (%)		P value ^b	Crude OR (95% CI)	Adjusted OR (95% CI) ^c
	Cases	Controls			
Log-transformed unit increment	133 (100)	176 (100)		1.44 (1.12–1.85)	1.41 (1.10–1.82)
By median			0.010		
0.16	47 (35.3)	88 (50.0)		1.00 (Ref)	1.00 (Ref)
> 0.16	86 (64.7)	88 (50.0)		1.83 (1.15–2.91)	1.78 (1.12–2.84)
By quartile			0.011		
0.08	15 (11.3)	44 (25.0)		1.00 (Ref)	1.00 (Ref)
0.09–0.16	32 (24.1)	44 (25.0)		2.13 (1.02–4.48)	2.01 (0.95–4.25)
0.17–0.35	39 (29.3)	46 (26.1)		2.49 (1.21–5.13)	2.34 (1.13–4.87)
>0.35	47 (35.3)	42 (23.9)		3.28 (1.60–6.74)	3.09 (1.50–6.38)
Trend test ^d				$P_{\text{trend}} = 0.002$	$P_{\text{trend}} = 0.003$

b/c, breaks per cell; CI, confidence interval; OR, odds ratio.

^aMedian or quartile chromatid breaks/cell values of controls.^b χ^2 tests.^cAdjusted for age and sex.

Table 4

Multivariate logistic regression analysis of associations between the frequency of 4-NQO-induced chromosome breaks and melanoma risk^a

Variables	OR (95% CI)	P value
Age (years)	0.99 (0.97–1.01)	0.208
Sex (male, female)	1.52 (0.83–2.77)	0.176
Hair color (black or brown, blond or red)	1.99 (1.01–3.94)	0.047
Eye color (not blue, blue)	0.94 (0.47–1.85)	0.847
Skin color (dark brown, fair)	1.07 (0.57–2.02)	0.830
Tanning ability after prolonged sun exposure (good, poor)	2.30 (1.21–4.38)	0.012
Lifetime sunburns with blistering (0, 1)	2.33 (1.16–4.69)	0.018
Freckling in the sun as a child (no, yes)	0.83 (0.43–1.60)	0.584
Dysplastic nevi (no, yes)	3.76 (1.19–11.90)	0.025
Family history of skin cancer (no, yes)	0.98 (0.47–2.02)	0.948
Sensitivity to 4-NQO, b/c (≤ 0.16, > 0.16) ^b	2.17 (1.21–3.89)	0.009

b/c, breaks per cell; CI, confidence interval; 4-NQO, 4-nitroquinoline-1-oxide; OR, odds ratio.

^aParticipants included in the model were 114 cases and 126 controls with all listed variables available.

^bThe median value in controls was used as the cutoff point.

Multivariate logistic regression analysis of interactions between frequencies of 4-NQO-induced chromosome breaks and sun exposure variables for the risk of melanoma

Table 5

Variables ^a	b/c ^b	Number of cases/controls ^c	P value ^d	OR (95% CI) ^e	P for interaction ^f
Hair color			0.001		0.594/0.404
Black or brown	Low	28/71		1.00 (Ref)	
Blond or red	Low	18/17		2.75(1.23–6.15)	
Black or brown	High	55/66		2.03 (1.15–3.60)	
Blond or red	High	28/18		4.17 (1.97–8.83)	
Trend test ^e				$P_{\text{Trend}} = 0.0004$	
Eye color			0.039		0.473/0.635
Not blue	Low	25/56		1.00 (Ref)	
Blue	Low	22/32		1.62(0.78–3.36)	
Not blue	High	64/64		2.16(1.20–3.91)	
Blue	High	22/22		2.42(1.12–5.24)	
Trend test ^e				$P_{\text{Trend}} = 0.006$	
Skin color			0.001		0.570/0.435
Dark brown	Low	21/58		1.00 (Ref)	
Fair	Low	26/30		2.30(1.10–4.79)	
Dark brown	High	38/49		2.05(1.06–3.98)	
Fair	High	48/36		3.57 (1.82–6.98)	
Trend test ^e				$P_{\text{Trend}} = 0.0005$	
Tanning ability			<0.001		0.486/0.051
Good	Low	24/64		1.00 (Ref)	
Poor	Low	23/23		2.61 (1.23–5.52)	
Good	High	38/66		1.53(0.82–2.85)	
Poor	High	48/21		5.68(2.82–11.45)	
Trend test ^e				$P_{\text{Trend}} < 0.001$	
Lifetime sunburns			<0.001		0.545/0.050
0	Low	11/41		1.00 (Ref)	
1	Low	36/46		2.74(1.23–6.12)	

Variables ^a	b/c ^b	Number of cases/controls ^c	P value ^d	OR (95% CI) ^e	P for interaction ^f
0	High	16/42		1.41 (0.58–3.42)	
1	High	69/44		5.37 (2.48–11.63)	
Trend test ^e				$P_{\text{trend}} < 0.001$	
Freckling as a child			0.003		0.510/0.101
No	Low	22/52		1.00 (Ref)	
Yes	Low	25/36		1.50(0.72–3.13)	
No	High	39/54		1.60(0.83–3.07)	
Yes	High	46/31		3.30 (1.63–6.68)	
Trend test ^e				$P_{\text{trend}} = 0.001$	
Dysplastic nevi			0.014		0.639/0.549
No	Low	37/69		1.00 (Ref)	
Yes	Low	6/3		3.51 (0.81–15.13)	
No	High	72/72		1.79(1.06–3.02)	
Yes	High	9/4		3.99(1.13–14.02)	
Trend test ^e				$P_{\text{trend}} = 0.009$	
Family history of skin cancer			0.001	0.004	
No	Low	40/65		1.00 (Ref)	
Yes	Low	4/18		0.30 (0.09–0.99)	
No	High	59/67		1.35(0.79–2.30)	
Yes	High	22/10		3.38(1.43–7.97)	
Trend test ^e				$P_{\text{trend}} = 0.009$	

b/c, breaks per cell; CI, confidence interval; 4-NQO, 4-nitroquinoline-1-oxide; OR, odds ratio.

^a Selected variables were categorized as indicated in the table.

^b b/c, breaks per cell, was expressed as dichotomized 4-NQO-induced chromosome breaks (0.16, > 0.16).

^c Participants were less than the total numbers of participants of this study because some did not provide the information.

^d χ^2 tests for frequency distributions of cases and controls.

^e Adjustment for age and sex in logistic regression models.

^f The interaction between each of selected risk factors and 4-NQO-induced b/c values was first tested for the departure from a multiplicative model, followed by the test for the departure from an additive model, if no evidence was found for a multiplicative interaction, with adjustment for age, sex, and their main effects.

P values were two sided.

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