Carcinogenesis, 2016, Vol. 37, No. 9, 897–903

doi:10.1093/carcin/bgw072
Advance Access publication July 5, 2016
Original Manuscript

ORIGINAL MANUSCRIPT

Pre-diagnostic leukocyte mitochondrial DNA copy number and skin cancer risk

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Abstract

No previous study has examined the association between mitochondrial DNA copy number (mtCN) and skin cancer risk prospectively. We examined the associations between peripheral blood leukocytes mtCN level and the risks of skin cancers in a case–control study nested within the Nurses' Health Study of non-Hispanic White women, including 272 melanoma cases and 293 controls, 508 squamous cell carcinoma (SCC) cases and 550 controls, and 515 basal cell carcinoma (BCC) cases and 536 controls. Relative mtCN in peripheral blood leukocytes was measured by quantitative PCR-based assay. Unconditional logistic regression models were used to examine the associations between mtCN and skin cancer risks. Compared with those with high mtCN, the risk for melanoma was 1.06 [95% confidence interval (CI) = 0.70–1.62] in the median group and 1.19 (95% CI = 0.78–1.81) for the low group. There was suggestive evidence that increased risk for melanoma was apparent among low constitutional susceptibility group [odds ratio $(OR)_{low\ versus\ high}$ = 1.80, 95% CI = 0.95–3.39, *P* for trend = 0.07, *P* for interaction = 0.06]. The increased risk of melanoma was also apparent among high cumulative UV exposure group ($OR_{low\ versus\ high}$ = 3.40, 95% CI = 1.46–7.92, *P* for trend = 0.004, *P* for interaction = 0.01). For non-melanoma skin cancers, compared with high-mtCN group, low-mtCN group had an increased risk for SCC (OR = 1.26, 95% CI = 0.93–1.71) and BCC (OR = 1.35; 95% CI = 1.00–1.82). Because some of the associations were marginally significant, the results only provided suggestive evidence. Further studies are warranted to replicate these findings and better understand the underlying mechanisms.

Introduction

Skin cancer is the most common neoplasm among Caucasians in the USA. There are three major types of skin cancer: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma. Among them, melanoma is the most fatal form, whereas BCC is the most common type, followed by SCC (1). The incidence of both non-melanoma and melanoma skin cancers has been increasing in fair-skinned populations around the world for decades (2–4). Exposure to ultraviolet (UV) light is accepted as a main trigger for cutaneous carcinogenesis. Wavelengths in the UVB range (280–320 nm) can directly cause DNA damage (5). If not repaired, DNA alterations tend to introduce mutations (6). In addition, UVB can indirectly induce DNA modification by generating reactive oxygen species (ROS) (7). The deleterious effects of UVA (320–400 nm) primarily involve the production of ROS that damage cellular targets. ROS produced by UV light exacerbate multistage carcinogenesis processes, such as tumor initiation, promotion and progression (8,9).

Received: March 20, 2016; **Revised:** June 12, 2016; **Accepted:** June 24, 2016

Abbreviations

BCC	basal cell carcinoma
CI	confidence interval
mtCN	mtDNA copy number
mtDNA	mitochondrial DNA
NHS	Nurses' Health Study
OR	odds ratio
ROS	reactive oxygen species
SCC	squamous cell carcinoma
UV	ultraviolet

Mitochondria are organelles in the cytoplasm of eukaryotic cells. Mitochondria possess their own genome, which is maternally inherited in humans and consists of a 16 569 bp circular double-stranded DNA molecule encoding 13 core polypeptide subunits of the respiratory chain apparatus (10). Mitochondrial DNA (mtDNA) is located close to the source of ROS production and is extremely susceptible to oxidative damage due to the absence of protective histones, the lack of introns and a scarcity of efficient DNA repair mechanisms. As a result, mtDNA acquires mutations at a much higher rate than nuclear DNA (10- to 200-fold) (11,12). Consequently, the mtDNA copy number (mtCN) may either increase or decrease under the combined effect of mitochondrial and nuclear DNA mutations. For instance, somatic point mutations close to the replication region in the D-loop of mtDNA have been significantly associated with reduced mtCN in hepatocellular carcinoma, invasive breast cancer and Ewing's sarcoma (13–15).

Mitochondria have been found to play a critical role in many cellular repair mechanisms in response to sunlight-induced carcinogenesis and toxicity via ROS (16,17). MtCN has also been assessed as a potential biomarker in previous cohort and case–control studies in relation to cancer risk and showed significant associations with the risk of various cancers (18–24). To our knowledge, however, no study has investigated the associations between mtCN and skin cancer risks prospectively. Recently, the PGC-1s/MITF pathway has received increased attention in the study of melanoma development. It has been shown that UV exposure can induce α-MSH secretion, which strongly induces PGC-1α expression and stabilizes both PGC-1α and PGC-1β proteins. The PGC-1s in turn activate the MITF promoter, a melanocytic lineage-specific transcription factor with an extensive role in malignant melanoma (25). As a key regulator for mitochondrial biogenesis, PGC-1α at high levels can also increase the expression of mitochondrial respiration complex and oxidative phosphorylation. Despite the biological evidence, population-based investigations of potential interactions between mtCN and PGC-1s/MITF-mediated melanomagenesis are lacking. In addition, lower levels of mtCN might also affect carcinogenesis through mechanisms such as oxidative phosphorylation deficiency and glycolysis enhancement (26–30). In this study, we examined the associations between peripheral blood leukocytes mtCN and both melanoma and non-melanoma skin cancer risk, as well as the potential effect modification by known host and environmental risk factors in a prospective case–control study nested within the Nurses' Health Study (NHS) of non-Hispanic White women.

Materials and methods

Study population and recruitment

The NHS began in 1976, when 121 700 registered nurses aged 30–50 years in 11 states in the USA completed a baseline questionnaire regarding risk factors for cancer and cardiovascular diseases. Participants completed self-administered, mailed follow-up questionnaires biennially with updated information on their lifestyle, medical history and diet. Between 1989 and 1990, blood samples were collected from 32 826 members of the NHS.

Skin cancer case ascertainment and validation

Eligible cases in this study consisted of women with incident skin cancer from the subcohort who gave a blood specimen, including melanoma, SCC and BCC cases with a diagnosis any time after blood collection up to 1 June 2006 with no previously diagnosed skin cancer. All available pathologically confirmed melanoma and SCC cases and >500 self-reported BCC cases randomly selected from self-reported BCC cases were included. The validity of self-report of BCC is high in this medically sophisticated population (90%) (31,32). All the SCC and BCC cases had no history of melanoma diagnosis. A common control series (case/control, 1:1) was randomly selected from participants who gave a blood sample and were free of diagnosed skin cancer up to and including the questionnaire cycle in which the case was diagnosed. One control was matched to each case by year of birth (±1 year) and self-reported race (Caucasian, Asian, Hispanic, African American and unknown). More than 95% of cases and controls were Caucasian. Only Caucasian women were included in our study. After deleting samples with missing laboratory data, 272 pathologically confirmed melanoma cases and 293 controls; 508 SCC cases and 550 controls; and 515 self-reported BCC cases and 536 controls from NHS were included.

Questionnaire data for skin cancer risk factors

The prospective biennial questionnaires and the retrospective supplementary questionnaire addressing skin cancer-related environmental and lifestyle risk factors were obtained. Questions on natural hair color and childhood and adolescent tendency to sunburn or tan were asked in the 1982 prospective questionnaire (ethnic group in the 1992 questionnaire). The retrospective supplementary questionnaire consisted of questions in three major areas: (i) pigmentation, constitutional and susceptibility factors; (ii) history of residence (states and towns), sun exposure habits and severe sunburns at different ages; and (iii) family history of skin cancer (father, mother and siblings). In addition, the 11 states of residence of cohort members at baseline were grouped into three regions: Northeast (Connecticut, Massachusetts, Maryland, New Jersey, New York and Pennsylvania), North Central (Michigan and Ohio) and West and South (California, Texas and Florida).

To estimate sunlight exposure for each subject, a UV database for 50 states in USA was developed. The database used reports from the Climatic Atlas of the United States, which reported mean daily solar radiation (in Langleys) at the earth's surface from weather stations around the country (33). The records of average annual solar radiation for January and July were extracted to represent winter and summer radiation, respectively. The mean solar radiation for each residence was derived from the UV values measured at the nearest weather station, and both summer and winter radiation indices were developed for each residence. A cumulative UV exposure score for each participant was developed using cumulative UV flux as described previously (34). In brief, participants' residences were used in combination with the information on their local UVB and UVA data collected by the Robertson–Berger meters to calculate the total amount of UV fluxes a participant was exposed to during each follow-up cycle. Cumulative UV flux for each participant was then calculated based on the updated residence information over the follow-up. We also calculated the sun exposure score in youth using sun exposure in high school and college as well as between 25 and 35 years of age. We classified both cumulative UV exposure and sun exposure in youth into three groups based on the scores among controls.

We created the constitutional susceptibility score, a multivariate confounder score, to summarize multiple risk factors for skin cancer. Briefly, we applied the logistic regression coefficients from a multivariate model, including family history of skin cancer, natural hair color, childhood or adolescent tendency to burn, number of moles on arms against different skin cancer risks, to each individual's values for these variables, and we summed the values to compute a susceptibility risk score in the logit scale. We used the median value of constitutional susceptibility score among controls to identify women with low and high constitutional susceptibility.

MtCN ascertainment and validation

For quantitative PCR-based assay of relative mtCN, total DNA was extracted from buffy coat fractions using the QIAmp (Qiagen, Chatsworth, CA) 96-spin blood protocol. DNA concentrations were determined via PicoGreen

quantitation using a Molecular Devices 96-well spectrophotometer. Relative mtCN was assessed using a quantitative PCR-based method in a high-throughput 384-well format with an Applied Biosystems 7900HT Real Time PCR system. DNA concentration was standardized to 5 ng/μl. About 10 ng of genomic DNA per reaction was added to a 384-well reaction plate and dried down. DNA was reconstituted in 10 μl of multiplex ND2 (single-copy mitochondrial gene) and AluYb8 (nuclear repeat element) PCR reaction mixture. A 20× multiplex reaction mixture consisted of 18 μM of each of the ND2-forward primer (5′-tgttggttatacccttcccgtacta-3′), ND2-reverse primer (5′-cctgcaaagatggtagagtagatga-3′), AluYb8-forward primer (5′-cttgcagtgagccgagatt-3′), AluYb8-reverse primer (5′-gagacggagtctcgctctgtc-3′) and 5 μM each of the 'actgcagtccgcagtccggcct' probe with VIC on the 5′ end and 'MGBNFQ' on the 3′ end, 'ccctggcccaaccc' with 6FAM on the 5′ end and 'MGBNFQ' on the 3′ end, plus 20× multiplex Taqman genotyping mastermix (Taqman). The multiplex reaction thermal cycling profile proceeded as follows: 95°C for 10 min, then 30 cycles of 95°C for 15 s and 60°C for 1 min. Triplicate reactions of multiplex reactions were performed on each sample on different plates.

The average slope of the standard curves for both reactions was between −3.5 ± 0.3. The R^2 coefficient of determination was 0.97 or higher for each reaction. The threshold cycle (Ct) value for each reaction represents the number of PCR cycles required to detect a signal over background fluorescence and is inversely proportional to the amount of DNA. The quantitative PCR-based assay determined the ratio of mitochondrial ND2 gene copy number to genomic single-copy gene copy number (N/S), a value proportional to the average number of mtCN. The N/S ratio (−dCt) for each sample was calculated by subtracting the average AluYb8 Ct value from the average ND2 Ct value. About 10 ng DNA standard curve point included on every 384-well plate was used as a calibrator DNA to help adjust for interassay variability. The relative N/S ratio (−ddCt) was calculated by subtracting the N/S ratio of the calibrator DNA from the N/S ratio of each sample.

Quality control procedures

In addition to the samples, each 384-well plate contained a 6-point standard curve from 0.625 to 20 ng using pooled buffy coat-derived DNA. The purpose of the standard curve is to assess and compensate for interplate variation in PCR efficiency. Ten percentage replicate quality control samples were included in the dataset to assess interplate and intraplate variability of Ct values. The average interplate coefficients of variation for the ND2 and AluYb8 Ct values were 0.40 and 0.79%, respectively, among the quality control samples. The average intraplate coefficients of variation for the ND2 and AluYb8 Ct values were 0.50 and 0.90%, respectively, among the quality control samples. Within-person stability over time showed a coefficient of variation of 7%, spearman correlation of 0.4 and intraclass correlation coefficient of 0.29.

Statistical analysis

The relative mtCN compared with the nuclear DNA content was presented as a ratio and standardized to account for the interassay variability. The chi-square test and student's *t*-test were used to compare the distribution of selected covariates between skin cancer cases and controls. We categorized participants into three mtCN groups (low, median, and high) based on the relative mtCN among controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression models. Tests for trend were performed by assigning the median value of each tertile of the mtCN among controls to both cases and controls in each category and modeling it as a continuous variable. Statistical significance of interaction was assessed by using the Wald test for cross-product terms of mtCN and cumulative UV exposure (or constitutional susceptibility score) in the logistic regression adjusted for risk factors. In all methods, 3 sets of covariate combinations were added to control for confounding: (i) matching factors: age; (ii) matching factors, constitutional susceptibility score, sun exposure in youth and cumulative UV exposure after enrolling in the NHS; and (iii) covariates in (ii) and smoking status (pack-years of smoking), alcohol drinking (non-drinker, drinker), history of diabetes and body mass index (kg/m^2).

All analyses in this study were two sided, adjusting for potential confounding factors and performed using SAS (Cary, NC).

Results

We included 272 melanoma cases and 293 controls; 508 SCC cases and 550 controls; and 515 BCC cases and 536 controls. The descriptive characteristics of the study population are provided in Table 1. As expected, compared with controls, cases of all three types were more likely to have a family history of melanoma, higher numbers of severe sunburns, less childhood tendency to tan, higher childhood tendency to burn, natural light hair color and more direct sun exposure in their youth. Melanoma cases tended to have more moles on arms, and SCC and BCC cases had higher cumulative UV exposure.

In the main effect analyses, compared with those with high mtCN, the risk for melanoma was 1.06 (95% CI = 0.70–1.62) in the median mtCN group and 1.19 (95% CI = 0.78–1.81) in the low-mtCN group. The risk for SCC was 1.26 (95% CI = 0.93–1.71) in the low-mtCN group compared with the high-mtCN group, and the risk for BCC was 1.35 (95% CI = 1.00–1.82) in the low-mtCN group compared with the high-mtCN group. Trend tests showed *P* values of 0.13 in SCC and 0.06 in BCC (Table 2), which were not significant after multiple comparisons. Results were similar after deleting cases occurred within 2 years of blood collection.

We further conducted stratified analysis based on host and environmental risk factors for skin cancers. First, we investigated the effect modification of mtCN on skin cancers by constitutional susceptibility score. The *P* value for the interactions between mtCN and the constitutional susceptibility score was 0.06 in melanoma, 0.42 in SCC and 0.61 in BCC. Among participants with low constitutional susceptibility score, mtCN was suggested to be inversely associated with risk of melanoma ($OR_{low\ versus\ high}$ = 1.80; 95% CI = 0.95–3.39; *P* for trend = 0.07. After deleting cases occurred within 2 years of blood collection $OR_{low\ versus\ high}$ = 1.95; 95% CI = 1.01–3.78; *P* for trend = 0.046) and SCC ($OR_{low\ versus\ high}$ = 1.54; 95% CI = 1.00–2.37; *P* for trend = 0.05. After deleting cases occurred within 2 years of blood collection $OR_{low\ versus\ high}$ = 1.77; 95% CI = 1.13–2.79; *P* for trend = 0.014) (Table 3), which were not significant after multiple comparisons. Second, we examined the effect of mtCN on skin cancer risks in different cumulative UV exposure levels. The *P* value for the interactions between mtCN and the cumulative UV exposure showed significance in melanoma compared with multiple comparison adjusted *P* value threshold (0.016) (*P* = 0.01) but not in SCC (*P* = 0.61) or BCC (*P* = 0.17). Among participants in the highest cumulative UV exposure category, those in the lowest mtCN category had a 3.4-fold higher melanoma risk than participants in the highest mtCN category (OR = 3.40; 95% CI = 1.46–7.92; *P* for trend = 0.004). After deleting cases occurred within 2 years of blood collection, the association remained significant (OR = 2.66; 95% CI = 1.09–6.46; *P* for trend = 0.03). No such association was detected in any other cumulative UV exposure category (Table 4). We examined associations between mtCN and skin cancer risks in strata of UV exposure in youth as well as smoking status. No significant interactions were identified (Supplementary Table 1 and 2, available at *Carcinogenesis* Online).

Discussion

Our results suggest that women with low mtCN are more likely to develop skin cancer. The increased melanoma risk associated with low mtCN is more apparent among women with low constitutional risk or high UV exposure history. Because some of the associations were marginally significant the results only provided suggestive evidence. To our knowledge, this is the first prospective study investigating the association between prediagnostic leukocyte mtCN and skin cancer risk.

UV radiation is a constant external stressor for human skin. UVB is considered to be harmful to skin mainly due to its DNA mutagenesis effect. However, UVB has also been shown to cause

Table 1. Characteristics of skin cancer cases and controls in the nested case–control study

	Melanoma		SCC		BCC	
Characteristics	Cases (n = 272)	Controls (n = 293)	Cases (n = 508)	Controls (n = 550)	Cases (n = 515)	Controls (n = 536)
Age at diagnosis, mean (SD)	64.1 (7.5)	—	70.5 (9.5)	—	72.3 (8.2)	—
Age at blood draw, mean (SD)	56.5 (7.0)	56.7 (7.0)	57.8 (7.0)	57.9 (7.0)	57.0 (6.6)	57.2 (6.6)
Family history of melanoma, n (%)	48 (17.7)	28 (9.6)	63 (12.4)	52 (9.5)	58 (11.3)	52 (9.7)
Lifetime severe sunburn, n (%)	59 (21.7)	44 (15.0)	87 (17.1)	70 (12.7)	87 (16.9)	72 (13.4)
≥3 moles on arms, n (%)	57 (21.0)	23 (7.9)	55 (10.8)	57 (10.4)	76 (14.8)	71 (13.3)
Childhood tendency to tan[a], n (%)	158 (58.1)	204 (69.6)	328 (64.6)	405 (73.6)	340 (66.0)	383 (71.5)
Childhood tendency to burn[b], n (%)	62 (22.8)	37 (12.6)	92 (18.1)	70 (12.7)	94 (18.3)	67 (12.5)
Natural red or blonde hair color, n (%)	58 (21.3)	39 (13.3)	94 (18.5)	73 (13.3)	94 (18.3)	71 (13.3)
Alcohol consumption, g/day (SD)	5.8 (8.0)	4.4 (7.6)	6.3 (10.0)	5.1 (9.7)	5.5 (9.6)	4.9 (8.6)
Diabetes, n (%)	9 (3.3)	8 (2.7)	11 (2.2)	21 (3.8)	16 (3.1)	13 (2.4)
Smoking status						
Never	116 (42.7)	154 (52.7)	232 (45.9)	260 (47.5)	241 (46.8)	246 (45.9)
Past	130 (47.8)	105 (36.0)	209 (41.3)	225 (41.1)	216 (41.9)	224 (41.8)
Current	26 (9.6)	33 (11.3)	65 (12.9)	63 (11.5)	58 (11.3)	66 (12.3)
BMI (kg/m^2)						
<25	155 (57.2)	154 (52.6)	311 (61.2)	293 (53.4)	294 (57.1)	302 (56.3)
25 ≤ BMI < 30	81 (29.9)	96 (32.8)	145 (28.5)	182 (33.2)	160 (31.1)	156 (29.1)
≥30	35 (12.9)	43 (14.7)	52 (10.2)	74 (13.5)	61 (11.8)	78 (14.6)
Direct sun exposure in youth[c]						
Low (<1 h/week)	8 (4.0)	16 (7.1)	21 (5.1)	26 (6.2)	14 (3.2)	24 (5.5)
Median (1–5 h/week)	78 (38.6)	105 (46.7)	178 (43.2)	180 (43.2)	172 (39.5)	186 (42.6)
High (≥5 h/week)	116 (57.4)	104 (46.2)	213 (51.7)	211 (50.6)	250 (57.3)	227 (52.0)
Cumulative UV exposure[d]						
Low	88 (32.4)	108 (36.9)	145 (28.5)	184 (33.5)	168 (32.6)	202 (37.7)
Median	100 (36.8)	94 (32.1)	146 (28.7)	182 (33.1)	192 (37.3)	188 (35.1)
High	84 (30.9)	91 (31.1)	217 (42.7)	184 (33.5)	155 (30.1)	146 (27.2)

BMI, body mass index; SD, standard deviation.

[a]Childhood tendency to tan: practically none or light tan.

[b]Childhood tendency to burn: painful burn or painful burn with blisters.

[c]Direct sun exposure in youth: average time spent in direct sunlight during summer months before the age of 35 years. Low: <1 h/week, median: 1–5 h/week, high: ≥5 h/week.

[d]Category of cumulative UV exposure after enrolling in the NHS.

guanine oxidation and the alteration of intracellular antioxidant enzymes (35). UVA can penetrate deeper into the skin and cause erythema, also known as sunburn (36). Its main harmful effect is the generation of ROS and reactive nitrogen species (37). Mitochondria have been found to play a critical role in many cellular repair mechanisms in response to sunlight-induced carcinogenesis and toxicity via ROS (16,17). Meanwhile, when sunlight exposure passes a certain energy threshold, mitochondria may initiate apoptosis and programed cell death (38–40). Cellular and molecular analyses have also been employed to address the role of blood mtCN in UV-induced oxidative stress defense mechanisms (41). In laboratory settings where sublethal doses of simulated sunlight irradiation were applied to HaCaT cells, a significant increase in mitochondrial mass was observed <5.0 J/cm^2 simulated sunlight irradiation treatment, whereas a significant decrease was observed at higher levels of simulated sunlight irradiation dose (≥5.0 J/cm^2).

We found that among participants in the highest cumulative UV exposure category, low levels of mtCN were associated with an increased risk of melanoma compared with participants with high levels of mtCN. Although the specific biological mechanisms by which lower levels of mtCN affect carcinogenesis are not fully understood, several pathways could plausibly link decreased mtCN and the development of malignant disease, including oxidative phosphorylation deficiency and glycolysis enhancement, mtDNA depletion-mediated apoptosis resistance and epithelial–mesenchymal transition and the activation of nuclear factor-kappaB/Rel factors (26–30).

The constitutional susceptibility score was first developed by Han *et al.* in 2005 to summarize the effects on each type of skin cancer of constitutional risk factors including natural skin color, natural hair color, child or adolescent tendency to burn and the number of palpably raised moles on arms. The risk for the highest tertile of the susceptibility score was ~3.5-fold for melanoma and 3-fold for SCC and BCC, compared with the lowest tertile (42). In our study, compared with high levels of mtCN, lower levels of mtCN were associated with higher risk of melanoma and SCC in trend tests. This effect was not due to 'phenotype-behavior' feedback, by which identifiable phenotypic phenomena make people aware of their susceptibility, prompting them to reduce sun exposure. Similar effect modification of constitutional susceptibility was observed in a study investigating the association between the *XPD* gene (which is involved in the nucleotide excision repair pathway that removes DNA photoproducts induced by UV radiation) and the risk of skin cancer.

Table 2. Associations between relative mitochondrial DNA copy number and skin cancer risks

Relative mtCN[a]	OR[a] (95% CI)	OR[b] (95% CI)	OR[c] (95% CI)
Melanoma (cases = 272, controls = 293)			
Low	1.18 (0.79–1.77)	1.15 (0.76–1.75)	1.19 (0.78–1.81)
Median	1.04 (0.69–1.56)	1.06 (0.70–1.61)	1.06 (0.70–1.62)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.43	0.50	0.43
SCC (cases = 508, controls = 550)			
Low	1.22 (0.91–1.64)	1.24 (0.92–1.68)	1.26 (0.93–1.71)
Median	1.18 (0.88–1.59)	1.20 (0.88–1.62)	1.22 (0.90–1.65)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.19	0.15	0.13
BCC (cases = 515, controls = 536)			
Low	1.25 (0.93–1.69)	1.29 (0.96–1.75)	1.35 (1.00–1.82)
Median	1.10 (0.81–1.48)	1.12 (0.83–1.51)	1.16 (0.85–1.57)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.14	0.10	0.06

[a]Unconditional logistic regression models adjusted for age at blood draw.
[b]Unconditional logistic regression models adjusted for age at blood draw, constitutional susceptibility score, direct sun exposure in youth and cumulative UV exposure.
[c]Unconditional logistic regression models adjusted for the age at blood draw, constitutional susceptibility score, direct sun exposure in youth, cumulative UV exposure, body mass index, diabetes, pack-years of smoking and alcohol consumption.

Table 3. Association between relative mitochondrial DNA copy number and skin cancer risk in constitutional susceptibility score strata[a]

	Low constitutional susceptibility score		High constitutional susceptibility score	
mtCN	OR (95% CI)[b]	OR (95% CI)[c]	OR (95% CI)[b]	OR (95% CI)[c]
Melanoma	(cases = 105, controls = 153)		(cases = 167, controls = 140)	
Low	1.68 (0.92–3.06)	1.80 (0.95–3.39)	0.81 (0.45–1.44)	0.82 (0.45–1.49)
Median	1.56 (0.86–2.83)	1.64 (0.89–3.04)	0.70 (0.39–1.27)	0.72 (0.39–1.31)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.09	0.07	0.47	0.52
SCC	(cases = 248, controls = 309)		(cases = 260, controls = 241)	
Low	1.50 (0.99–2.27)	1.54 (1.00–2.37)	1.00 (0.65–1.53)	1.04 (0.67–1.62)
Median	1.35 (0.89–2.05)	1.40 (0.90–2.16)	1.05 (0.68–1.62)	1.04 (0.67–1.61)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.06	0.05	0.99	0.87
BCC	(cases = 243, controls = 292)		(cases = 272, controls = 244)	
Low	1.33 (0.87–2.02)	1.37 (0.89–2.10)	1.23 (0.81–1.88)	1.37 (0.89–2.12)
Median	1.30 (0.85–2.00)	1.33 (0.86–2.06)	0.94 (0.62–1.44)	1.00 (0.65–1.54)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.18	0.14	0.40	0.19

[a]*P* value for interaction was 0.06 for melanoma, 0.42 for SCC and 0.61 for BCC in model 2.
[b]Unconditional logistic regression models adjusted for age at blood draw.
[c]Unconditional logistic regression models adjusted for the age at blood draw, direct sun exposure in youth, cumulative UV exposure, body mass index, diabetes, pack-years of smoking and alcohol consumption.

Table 4. Association between relative mitochondrial DNA copy number and skin cancer risk in cumulative UV exposure strata[a]

	Low cumulative UV exposure		Median cumulative UV exposure		High cumulative UV exposure	
mtCN	OR (95% CI)[b]	OR (95% CI)[c]	OR (95% CI)[b]	OR (95% CI)[c]	OR (95% CI)[b]	OR (95% CI)[c]
Melanoma	(cases = 88, controls = 108)		(cases = 100, controls = 94)		(cases = 84, controls = 91)	
Low	0.75 (0.38–1.50)	0.78 (0.37–1.63)	0.92 (0.46–1.85)	0.74 (0.35–1.55)	2.65 (1.23–5.70)	3.40 (1.46–7.92)
Median	0.59 (0.30–1.18)	0.60 (0.29–1.25)	1.44 (0.73–2.85)	1.59 (0.77–3.27)	1.57 (0.71–3.51)	1.69 (0.71–4.00)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.37	0.45	0.91	0.56	0.01	0.004
SCC	(cases = 145, controls = 184)		(cases = 146, controls = 182)		(cases = 217, controls = 184)	
Low	1.45 (0.86–2.45)	1.50 (0.86–2.62)	1.09 (0.63–1.90)	1.24 (0.70–2.20)	1.18 (0.73–1.92)	1.18 (0.71–1.93)
Median	0.85 (0.49–1.50)	0.95 (0.52–1.72)	1.46 (0.85–2.50)	1.68 (0.96–2.94)	1.26 (0.78–2.04)	1.20 (0.73–1.96)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.16	0.15	0.74	0.46	0.47	0.51
BCC	(cases = 168, controls = 202)		(cases = 192, controls = 188)		(cases = 155, controls = 146)	
Low	1.27 (0.77–2.09)	1.31 (0.79–2.18)	1.58 (0.96–2.59)	1.70 (1.02–2.82)	0.91 (0.52–1.59)	0.95 (0.53–1.70)
Median	1.22 (0.73–2.02)	1.27 (0.76–2.13)	1.09 (0.65–1.84)	1.13 (0.66–1.91)	0.98 (0.57–1.69)	1.01 (0.58–1.77)
High	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
P trend	0.34	0.28	0.08	0.04	0.75	0.88

[a]*P* value for interaction was 0.01 for melanoma, 0.61 for SCC and 0.17 for BCC in model 2.
[b]Unconditional logistic regression models adjusted for age at blood draw.
[c]Unconditional logistic regression models adjusted for the age at blood draw, direct sun exposure in youth, cumulative UV exposure, body mass index, diabetes, pack-years of smoking and alcohol consumption.

Inverse associations between the presence of the $Lys^{751}Gln$ and $Asp^{312}Asn$ polymorphisms and the risk of melanoma were observed among women with low score but not among those with high score (43). People with pale skin, red hair, freckles, and an inability to tan generally have higher constitutional susceptibility scores for skin cancers. These phenotypes are frequently due to polymorphisms in the *Melanocortin 1 receptor* (*MC1R*) gene (44,45). A UV-independent pathway to melanoma carcinogenesis exists in the red hair-fair skin background (46). Even though this pathway contributes to melanomagenesis through the mechanism of oxidative damage, whole-genome expression profiling of skin primary cultures indicated that a wide range of key physiological processes including cell differentiation, adhesion and cell cycle progression—which may be directly related to the etiology of skin cancers—were significantly different in MC1R-defective cell lines (47). It is plausible that an mtCN-mediated oxidative stress defense mechanism is one of the pathological differences in carcinogenesis between individuals with high and low constitutional susceptibility.

There are no data available describing a direct correlation between mtCN copy number in peripheral blood leukocytes and that in skin tissues. However, the strength of the correlation between mtCN in peripheral blood leukocytes and that in skin tissues will not affect our ability to test our hypothesis, although we recognize it as the limitation of our study because it does influence interpretation of the results.

Two case–control studies were previously conducted to investigate the association between peripheral blood mtCN and melanoma risk (48,49). In both studies, cases and controls were identified followed by blood collection. Moreover, information of risk factors was obtained retrospectively from questionnaires. In one study of 136 cutaneous malignant melanoma cases and 302 controls from 53 melanoma-prone families, mtCN levels were found to be lower among cases compared with controls across most categories including age at blood draw and sex, but the difference was not significant when comparing all melanoma cases to all controls (48). In the other study of 500 melanoma cases and 500 controls, increased mtCN was associated with increased melanoma risk (49). Because of the retrospective nature of the two studies, observed mtCN changes may be due to complex physiological processes after cancer development. In contrast, in our perspective study, blood samples were collected prior to the skin cancer diagnosis. In addition, the sensitivity analysis deleting cases developed within 2 years of the blood collection further ensured the avoidance of reverse causation.

In conclusion, our results suggest that lower levels of mtCN are associated with a higher risk of melanoma among individuals exposed to high levels of UV. MtCN has also been suggested to be inversely associated with melanoma risk among individuals with low constitutional susceptibility. Further studies are warranted to replicate these findings and better understand the underlying mechanisms.

Supplementary material

Supplementary Tables 1 and 2 can be found at http://carcin.oxfordjournals.org/

Funding

National Institutes of Health (UM1CA186107, P01CA87969 and R01CA49449).

Acknowledgements

We thank Drs S.Wu, J.Wong and T.Li for their statistical and programing support; the genomic epidemiology research laboratory for their work on determining the relative mitochondrial DNA copy number; the participants in the Nurses' Health Study for their dedication and commitment; and the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.
Conflict of Interest Statement: None declared.

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