Published in final edited form as:
Pigment Cell Melanoma Res. 2013 September ; 26(5): 677–684. doi:10.1111/pcmr.12117.

DNA Repair Variants, Indoor Tanning and Risk of Melanoma

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Summary

Although ultraviolet radiation (UV) exposure from indoor tanning has been linked to an increased risk of melanoma, the role of DNA repair genes in this process is unknown. We evaluated the association of 92 single nucleotide polymorphisms (SNPs) in 20 DNA repair genes with the risk of melanoma and indoor tanning among 929 melanoma patients and 817 controls from the Minnesota Skin Health Study. Significant associations with melanoma risk were identified for SNPs in ERCC4, ERCC6, RFC1, XPC, MGMT, and FBRSL1 genes; with a cut-off of $p<0.05$. ERCC6 and FBRSL1 gene variants and haplotypes interacted with indoor tanning. However, none of the 92 SNPs tested met the correction criteria for multiple comparisons. This study, based on an a priori interest in investigating the role of DNA repair capacity using variants in base excision and nucleotide excision repair, identified several genes that may play a role in resolving UV-induced DNA damage.

Keywords

single nucleotide polymorphisms; DNA repair; indoor tanning; melanoma; gene-environment interaction; artificial UV

INTRODUCTION

Ultraviolet radiation (UV) exposure is an established risk factor for melanoma. Whether solar or artificial, UV is generally evaluated in terms of UVB (280–320 nm) and UVA wavelengths (320–400 nm), responsible for DNA damage in skin cells following exposure (Abdel-Malek et al., 2010). Recently, indoor tanning has become increasingly popular, particularly among young people (Hoerster et al., 2009); however, tanning bed users receive higher doses of UVA and UVB radiation than those who tan in the sun (Gerber et al., 2002; Hornung et al., 2003; Nilsen et al., 2012). Nilsen and colleagues (2011) measured UV lamp output from 194 tanning salons in Norway and found that UVB irradiance was up to 3.7 times higher than the Oslo summer sun and the UVA irradiance was 3 to 26 times higher. These potentially higher UVB and UVA exposures are cause for concern. Although the industry is subject to FDA (Food and Drug Administration), FTC (Federal Trade Commission) and FCC (Federal Communications Commission) regulations and in some cases state regulations, they are often not enforced and there are no penalties for noncompliance. These findings are important since UV wavelength relates to differences in

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cellular responses to DNA damage; UVA predominantly generates reactive oxygen species and other free radicals however UVA and UVB exposure both induce cyclobutane pyrimidine dimers (CPDs) and 6,4 photoproducts (Kappes et al., 2006; Abdel-Malek et al., 2010).

Two major DNA repair mechanisms mitigate UV-induced damage and compromised DNA repair capacity has been associated with the development of melanoma. In general, UVB damage is repaired by nucleotide excision repair (NER) while UVA damage is repaired by base excision repair (BER); however, DNA repair is ubiquitous and each pathway fulfills multiple functions (Abdel-Malek et al., 2010).

Compromised DNA repair capacity has been associated with the development of melanoma (Li et al., 2006). In their review of melanoma susceptibility and prognostic genes and genetic variants, Ward et al. (2012) identified several studies that investigated single nucleotide polymorphisms (SNPs) found in genes involved in the NER pathway. In these studies, SNPs within DNA repair genes appeared to affect melanoma risk. In particular, Povey et al. (2007) found an increased risk of developing melanoma in individuals with variants in ERCC1 and ERCC4 genes in a case-control study conducted in Scotland. Melanoma risk was noted in carriers of variants within XRCC3 (Figl et al., 2010); a similar association was found with SNPs within APEX1 (Li et al., 2006). Although few studies have reported an association with melanoma risk and polymorphisms in BER genes, a small Central-Southern Italian study found melanoma to be strongly correlated with two SNPs in XRCC1 (OR= 4.7 p=0.02; OR= 3.3 p=0.045) (Santonocito et al., 2012). Taken together these studies and others (see review by Ward et al., 2012), demonstrate differences in DNA repair gene variants among melanoma patients compared to healthy controls and potentially point to important cellular signaling pathways involved in the etiology of this disease. None of the previous studies, however, have examined the effect of UV exposure from artificial sources such as tanning beds in relationship to DNA repair variants and melanoma risk.

Our *a priori* hypothesis that variants in DNA repair genes would interact synergistically with artificial UV exposures to modify the risk of developing cutaneous malignant melanoma was evaluated using germline DNA from the Minnesota Skin Health Study, a large population-based case-control study of indoor tanning exposure and melanoma (Lazovich et al. 2010).

RESULTS

Individual characteristics

Among the 1746 genotyped individuals, 1659 (95%) remained after exclusion of non-white subjects (n=46) and those missing phenotypic indices (n=7), including 893 melanoma cases (96.1% of cases with samples) and 766 unaffected individuals (93.7% of cases with samples). Age and gender distributions were similar in the cases and controls due to the frequency-matched design (Lazovich et al., 2010) (Table 1). As shown in Table 1, the phenotypic index, number of lifetime sunburns, and ever use of indoor tanning were significantly associated with an increased risk of melanoma.

SNP genotype analysis

We analyzed 21 SNPs in the BER pathway, 38 SNPs in the NER pathway, and 24 SNPs within the “direct reversal” DNA repair gene MGMT. In addition, 9 SNPs in the novel FBRSL1 gene were included; the function of FBRSL1 is not yet understood but was originally included in this analysis as an NER gene. Overall, we investigated 92 SNPs in 20 genes. For each SNP, the allele frequency by case-control status and the p-values for Hardy Weinberg Equilibrium (HWE) in the control population are shown in Supplementary Table

1. Nine polymorphisms were significantly associated with the risk for melanoma (p-value <0.05) (Table 2). Associations with melanoma risk were identified for ERCC4 (rs9302507), ERCC6 (rs4253190, rs4838518 and rs4253121), RFC1 (rs2066782), XPC (rs2733537, rs3731143), MGMT (rs4750766), and FBRSL1 (rs4883571) polymorphisms. The association with the lowest p-value was with ERCC4 (rs9302507, p=0.0059), which was inversely associated with the risk of melanoma (adjusted OR= 0.77; 95%CI: 0.63, 0.93). Notably, none of these were in the BER pathway. However, none of the 92 SNPs tested met the correction criteria for multiple tests using either Bonferroni, permutation p-value, or False Discovery Rate cutoffs.

Haplotype Analysis

Associations of haplotypes with melanoma risk were assessed by comparing carriers of the haplotype to non-carriers in cases and controls. For seven genes, we tested the association of each haplotype within the 15 identified haplotype blocks with melanoma risk (Supplementary Table 1). Four haplotypes were significantly associated with melanoma risk (Table 3). The ERCC6 GGA haplotype was inversely associated with risk of melanoma (adjusted OR= 0.84; 95%CI: 0.71, 0.99; p=0.038). Other associations with risk for melanoma were identified with the MGMT Block17 haplotype GA (adjusted OR= 1.23; 95%CI: 1.05, 1.44; p=0.009), and the XPC haplotype AGCAG (adjusted OR= 1.37; 95%CI: 1.01, 1.87; p=0.045).

Interaction analysis among SNP genotypes or haplotypes, indoor tanning and melanoma risk

We assessed the interactive effect of each individual SNP and indoor tanning exposure on the risk of melanoma (Supplementary Table 2). Interactions with indoor tanning were observed for two SNPs (Table 4a): FBRSL1 rs4883557 (p-value for interaction =0.006) and ERCC6 rs10745261 (p-value for interaction =0.025). Among individuals who had ever used a tanning bed, these two polymorphisms, were associated with increased risk of melanoma (Table 4a). The attributable proportion (AP) of the combined effect that is due to interaction for each SNP was significant when rs4883557 minor homozygotes were compared to major homozygotes (OR=0.54; 95% CI: 0.26, 0.81) and when rs10745261 combined heterozygotes and minor homozygotes were compared to major homozygotes (OR=0.39; 95% CI: 0.13, 0.65), representing biological interaction with indoor tanning on the additive scale.

The interactive effect between each haplotype and indoor tanning exposure on the risk of melanoma was investigated (Supplementary Table 2). Two haplotypes were shown to interact with indoor tanning on melanoma risk (Table 4b): ERCC6 haplotype AAG (p for interaction = 0.036) and MGMT haplotype GAG (p for interaction = 0.042).

DISCUSSION

This study evaluated the associations of 92 SNPs in 20 DNA repair genes with risk of melanoma and their interaction with tanning bed use. We identified SNPs and haplotypes associated with melanoma in four NER genes (ERCC4, ERCC6, XPC, and RFC1) and two additional genes FBRSL1 and MGMT but found no associations with SNPs in BER genes (complete list of genes analyzed -Supplemental Table 1). Whether the main source of UV-induced DNA damage arises from UVA or UVB exposure is debatable. Our findings support the notion that NER plays a greater role in resolving UV-induced damage than BER as suggested by others (e.g., Novarina et al., 2011; Povey et al., 2007). Due to the preponderance of UVA from tanning devices (Nilsen et al, 2012) and the suggestion that UVA is generally repaired by BER (Abdel-Malek et al., 2010), it is possible that unmeasured SNPs within BER genes may still modify melanoma risk.

As previously hypothesized, two SNPs and two haplotypes on four genes interacted with indoor tanning and were associated with melanoma risk. These were located on ERCC6 (SNP rs10745261 and haplotype rs10745261-rs6537537-rs4253121), FBRSL1 (rs4883557) and MGMT (haplotype rs4751118-rs3793903-rs7897057). The FBRSL1 SNP demonstrated both an additive interaction and a multiplicative interaction with melanoma risk among tanners. When subjects were stratified by indoor tanning exposure, the interaction analysis with FBRSL1 rs4883557 revealed that those individuals carrying the minor allele and who had ever tanned had the greatest risk of developing melanoma. This interaction was also supported by the increase in odds ratio of the multiplicative interaction as the number of minor alleles increase. Further, the joint effect of this gene/environment interaction, FBRSL1 rs4883557 and indoor tanning exposure, significantly predicted risk for melanoma based on the AP values. This type of statistical test is most appropriate for inferring biological interactions, thus strengthening the biological relevance of our analyses (Kalilani and Atashili, 2006). We had included nine SNPs located within Polε, but following genotyping, these SNPs were designated to FBRSL1. While the function of FBRSL1 is unknown, Gao et al., 2012 has suggested that FBRSL1 is associated with histone post-translational modification and chromatin compaction. These findings are interesting and warrant further validation in other cohorts to determine whether they represent involvement of a novel gene in melanoma.

A number of SNPs in four NER genes (ERCC6, ERCC4, XPC, RFC1) were associated with overall melanoma risk, regardless of indoor tanning status. We identified three SNPs (rs4253190, rs4838518 and rs4253121) and one haplotype (rs10745261-rs6537537-rs4253121) in ERCC6, involved in the UV-induced DNA damage response, that were associated with overall melanoma risk. Within the XPC gene, involved in detecting DNA damage and recruiting necessary accessory proteins (Kraemer KH and DiGiovanna JJ, 2012; Lagerwerf et al., 2011), two SNPs (rs2733537 and rs3731143) and one haplotype (rs2228001-rs2279017-rs3731143-rs3731093-rs3731068) were associated with melanoma risk. Interestingly, Blankenburg et al (2005) identified two SNPs; rs2228001 (genotype CC; OR 1.82) and rs2279017 (genotype AA; OR 1.83) within XPC that were associated with an increased risk of melanoma; whereas our haplotype results indicated that a person with at least one copy of the haplotype ACGAC, where the first two alleles represent rs2228001 and rs2279017, have an increased risk of developing melanoma (OR 1.37). The analysis by Blankenburg et al was at the single SNP level indicative of the SNPs having functional significance in DNA repair capacity. Our analysis involved these variants in a haplotype block which encompasses predictive power of multiple SNPs, potentially including predictive power of other SNPs that are in linkage disequilibrium with those included in the haplotype.

SNPs involved in other pathways were also associated with overall melanoma risk. In MGMT, which repairs alkylated guanines (Jiang et al., 2012), rs4750766 along with its haplotype rs12917-rs4750766 modified melanoma risk similar to previous findings by Gu and colleagues (2009). Additionally, a SNP (rs4883571) in FBRSL1was associated with melanoma risk.

Notably, several SNPs with no previous evaluation in melanoma were associated with melanoma risk: rs9302507 in ERCC4, involved in endonuclease formation; rs2066782 in RFC1, which interacts with Polδ and Polε during template elongation (Ogi et al., 2010); and rs4883571 in FBRSL1. Although this is the first report of ERCC4 and RFC1 and melanoma, they have been linked to other cancers (e.g., Chang et al., 2009, Wheless et al., 2012, Zheng et al., 2010; Vijayakrishnan and Houlston, 2010).

There were limitations in this study. First, more than 70% of the SNPs evaluated were located within intronic regions of their respective genes and their functional significance is not yet known. However, these intronic SNPs are tagging SNPs and selected because they allow for identification of a chromosomal region that may have functional importance. These SNPs, or other SNPs in high linkage disequilibrium, may have a functional effect on the genes and may modify their role in DNA repair following high levels of UV exposure such as those found with indoor tanning. Second, in some cases we were unable to analyze complete haplotype blocks in genomic regions where tagging SNPs were closer than 60 base pairs due to limitations in the genotyping platform. Third, although there are more than 120 genes known to be involved in DNA repair; this study included only 20 genes. Finally, our data did not meet the correction criteria for multiple comparisons (Bonferroni, permutation, and FDR). However, this is an exploratory study based on our *a priori* hypothesis that genetic variants in DNA repair pathways would synergistically interact with tanning bed exposure to modify risk of melanoma and as such sheds light on this important interaction. It should be pointed out that our findings confirmed other reports in the literature and highlight the need for additional studies.

Strengths of this study include the identification of interactions with exposure to indoor tanning devices in risk for melanoma, the identification of novel DNA repair genes involved in melanoma risk (ERCC4, RFC1 and FBRSL1), and the population-based study design with extensive exposure information. As melanoma incidence rates continue to rise, particularly among younger individuals who tend to use tanning devices more frequently (Hornung et al., 2003; Lazovich et al., 2010), this study addresses a gap in the literature. Additional studies are warranted to validate these findings in other populations. Functional studies are needed to determine the relevance of the genes, SNPs and haplotypes identified to melanoma risk among individuals who participate in indoor tanning.

METHODS

Study population

The Skin Health Study has been previously described (Lazovich et al., 2010); briefly, this population-based case-control study was conducted in Minnesota among persons diagnosed between 2004 and 2007 aged 25–59 with invasive cutaneous melanoma (cases) and healthy controls. Cases were ascertained by the Minnesota state cancer registry. This study was approved by the institutional review board and state cancer registry. Persons without melanoma (controls) frequency matched (1:1) to cases on age and gender, were randomly selected from the state drivers' license list (including persons with state identification cards). Altogether, 1167 cases and 1101 controls (84.6% and 69.2% of eligible, respectively) completed a self-administered questionnaire and telephone interview. Among these subjects, 1753 submitted DNA samples for genotyping; 893 cases and 766 controls.

Exposure measurement—Demographic, phenotypic and exposure information were assessed by self-administered and telephone-administered questionnaire. The questionnaire was adapted by Lazovich et al. (2010) using the Genes, Environment and Melanoma (GEM) Study questionnaire developed by Kricker et al. (2007) and further developed for detailed information on tanning bed exposures.

The phenotypic index was defined using hair color, eye color and ability to tan. This index ranges from 1 to 5 and is represented by the sum of the three scores: hair color (1=black/dark brown; 2=light brown/blond; 3=red), eye color (0=black/brown; 1=hazel/green/gray/blue), and ability to tan (0=easily tan; 1=poorly tan) (Kanetsky et al., 2006). The phenotypic index has been shown to be associated with increased melanoma risk (Kanetsky et al., 2006).

Selection of SNPs

The *a priori* hypothesis of this study was that genetic variants in the DNA repair pathways would interact synergistically with tanning bed exposure to modify risk of melanoma. Therefore, SNPs from dbSNP and reports from the literature were selected to represent NER, BER and repair reversal pathways. We selected 154 SNPs from 28 DNA repair genes based on two criteria. First, we identified functionally relevant SNPs from the literature that were located in genes associated with DNA repair. These SNPs were reported to occur at a frequency of >5% in Caucasian populations. Second, using Haploview 4.1, we identified tagging SNPs within haplotype blocks using the Tagger feature in this software. This ensured coverage for genes involved in DNA repair regardless of their reported frequency. Later, we confirmed haplotypes that were statistically significant using Haploview, version 4.2 (Barrett et al., 2005).

Genotyping platform

DNA from buccal cells was collected using SCOPE mouthwash and mailed directly to the University of New Mexico Molecular Epidemiology Laboratory where it was extracted using Qiagen kits following the manufacturer's instructions. DNA was evaluated and quantitated using nanodrop and SNPs were genotyped on the Illumina BeadExpress Golden Gate platform by the University of Utah Genotyping Core.

Quality control

To eliminate potential confounding by race/ethnicity, 46 non-white subjects were removed from the analysis. Seven additional subjects who were missing phenotypic indices were also excluded. SNPs (n=6) and samples (n=34) with low call rates (<95%) were excluded in order to minimize error due to genotyping uncertainty and sample quality. After quality control, the study sample consisted of 1659 eligible individuals who had both genotype and phenotype data; this included 929 cases and 817 controls (79.6% of the total 1167 and 74.2% of the total 1101, respectively who completed data collection). The overall genotyping call rate in the final analysis set was 98.8%. Seven monomorphic SNPs and 47 SNPs with Minor Allele Frequency (MAF) <0.05 in the remaining sample were also excluded from our analysis, resulting in a total of 92 SNPs in 20 DNA repair genes. The quality control process was performed with PLINK 1.07 (Purcell et al., 2007).

Hardy-Weinberg Equilibrium

An exact test for HWE (Wigginton et al., 2005) was performed in the control group for each of the genotyped SNPs. Extreme deviation from HWE can be an indication of population stratification, or even massive genotyping errors (Wigginton et al., 2005; Teo et al., 2007). The genotype distributions for four SNPs showed deviation from the HWE after Bonferroni correction for multiple tests, thus these SNPs were removed from the analysis. Testing for HWE was performed with PLINK 1.07 (Purcell et al., 2007)

Statistical Analysis

Single SNP association analysis—Multiple logistic regression models were used to investigate the association between each SNP and melanoma risk. An additive genotype model was used, except when the minor homozygote count was small (<10 for either the case or control group), the minor homozygotes were combined with the heterozygotes, and the genotypes were subsequently coded as 0,1. The odds ratios (OR), 95% confidence intervals (CI) and *p*-values for the regression coefficients were calculated. The ORs were adjusted for age, gender and phenotypic index in the single SNP, haplotype and interaction analyses. We treated age as a continuous variable in the adjustments, as this adjustment would remove any bias induced by the 5-year age group matching, as well as remove any

residual confounding within each of the initial 5-year age groups (Rothman et al., 2008). We also performed a sensitivity analysis by adjusting for the categorical five-year age group variable, and found the result to be similar.

Haplotype inference and association analysis—Haplotype block structures were determined for seven genes that contained at least one SNP that was either significantly associated with melanoma risk or significantly interacted with indoor tanning (Supplemental Table 2). We first determined the haplotype blocks for gene regions of interest using the Haploview software algorithm created by the Broad Institute (Barrett et al., 2005) based on the reported CEU population. Within each haplotype block, we reconstructed the haplotypes in terms of probabilities from the SNP genotype input data using the PHASE algorithm, which is a Bayesian method in which the prior was chosen to approximate the coalescent (Stephens and Donnelly, 2003). We assessed the association between melanoma risk and the haplotype by performing haplotype trend regressions (HTR) for each haplotype block. Using a regression framework relating inferred individual haplotype probabilities to the melanoma outcome, the HTR method effectively took into account the haplotype phase uncertainty and reduced bias (Zaykin et al., 2002). For each block only haplotypes with frequency of >0.01 were included in the haplotype association analysis.

Interaction between individual SNPs or haplotypes and indoor tanning

Multiplicative interactions were assessed using multiple logistic regression including the product terms of indoor tanning status and individual SNPs or inferred haplotype probabilities. The *p*-values for the interactions on the multiplicative scale for all the genotyped SNPs and haplotype blocks were calculated through likelihood ratio tests comparing the full model, including the interaction term, to the reduced model without the interaction term. Stratified analyses were conducted to investigate the modification of the ORs for SNPs and haplotypes by indoor tanning. Significant interactions on the multiplicative scale were also assessed on the additive scale (Rothman 1986). For SNPs significantly interacting with indoor tanning on the multiplicative scale, we also assessed their interaction with indoor tanning on the additive scale. We report the attributable proportion (AP) along with the 95% CIs as the measure and magnitude of biological interaction. Association and interaction analyses were conducted using the glm (generalized linear model) function in statistical package R (http://www.r-project.org/).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this research was provided by the American Cancer Society (RSGPB-04-083-01-CCE) and the National Cancer Institute (5R01CA106807) to DAL and (K05 CA131675) to MB.

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Significance

Rising melanoma incidence rates in younger populations, who use tanning devices more frequently, makes our analysis of the interaction between DNA variants, indoor tanning, and this type of cancer timely. Our findings identify an interaction between indoor tanning exposure and genetic variants in nucleotide excision repair genes in the etiology of melanoma. In addition we identified potential molecular targets that may inform future studies of melanoma etiology.

Table 1

Demographic comparison of eligible cases and controls in the Skin Health Study with available DNA samples.

Characteristic	Cases n (%)	Controls n (%)	Crude OR (95% CI)
Total [+]	893 (100)	766 (100)	
Age [*](y)			
25 – 29	55 (6.2)	45 (5.9)	1.07 (0.70 – 1.63)
30 – 29	140 (15.7)	126 (16.5)	0.97 (0.74 – 1.29)
40 – 49	316 (35.4)	260 (33.9)	1.07 (0.86 – 1.33)
50 – 59	382 (42.8)	335 (43.7)	1.00
Sex [*]			
Male	360 (40.3)	322 (42.0)	0.93 (0.77 – 1.13)
Female	533 (59.7)	444 (58.0)	1.00
Income			
<$60,000	265 (29.7)	242 (31.6)	0.91 (0.74 – 1.12)
$60,000+	617 (69.1)	511 (66.7)	1.00
Missing	11 (1.2)	13 (1.7)	
Completed college			
No	454 (50.8)	408 (53.4)	0.90 (0.74 – 1.10)
Yes	439 (49.2)	356 (46.6)	1.00
Eye color			
Gray/blue	395 (44.2)	329 (43.0)	1.15 (0.89 – 1.48)
Green	140 (15.7)	102 (13.3)	1.31 (0.94 – 1.83)
Hazel	181 (20.3)	166 (21.7)	1.04 (0.77 – 1.40)
Brown	177 (19.8)	169 (22.1)	1.00
Natural hair color			
Red	94 (10.5)	27 (3.5)	**4.17 (2.62 – 6.64)**
Blonde	266 (29.8)	162 (21.2)	1.97 (1.51 – 2.57)
Light brown	316 (35.4)	317 (41.4)	1.19 (0.94 – 1.52)
Dark brown/black	217 (24.3)	260 (33.9)	1.00
Skin color			
Very fair	157 (17.6)	93 (12.1)	**3.38 (1.32 – 8.67)**
Fair	646 (72.3)	530 (69.2)	2.44 (0.98 – 6.08)
Light olive	83 (9.3)	129 (16.8)	1.29 (0.50 – 3.32)
Dark olive, brown, black	7 (0.8)	14 (1.8)	1.00
Family History in first and second relatives			
Yes	173 (19.4)	156 (20.4)	0.92 (0.72 – 1.17)
No	714 (80.0)	593 (77.4)	1.00
Missing	6 (0.7)	17 (2.2)	
Ever Use of Indoor Tanning			

Characteristic	Cases n (%)	Controls n (%)	Crude OR (95% CI)
Yes	563 (63.0)	392 (51.2)	1.63 (1.34–1.98)
No	330 (37.0)	374 (48.8)	1.00
Lifetime number of painful sunburns			
None	20 (2.2)	35 (4.6)	1.00
1 – 2	121 (13.5)	150 (19.6)	1.41 (0.78 – 2.57)
3 – 5	176 (19.7)	141 (18.4)	**2.18 (1.21 – 3.95)**
>5	574 (64.3)	440 (57.4)	**2.28 (1.30 – 4.01)**
Missing	2 (0.2)	0 (0.0)	
Phenotype Index			
1**	65 (7.3)	76 (9.9)	1.00
2	162 (18.1)	206 (26.9)	0.92 (0.62 – 1.34)
3	401 (44.9)	331 (43.2)	1.42 (0.99 – 2.03)
4	213 (23.9)	139 (18.2)	**1.79 (1.21 – 2.66)**
5	52 (5.8)	14 (1.8)	**4.34 (2.21 – 8.54)**
BMI			
< 18.5	9 (1.0)	8 (1.1)	0.86 (0.33 – 2.25)
18.5 – 24.9	362 (40.6)	276 (36.1)	1.00
25.0 – 29.9	318 (35.7)	289 (37.8)	0.84 (0.67 – 1.05)
30.0 +	203 (22.8)	191 (25.0)	0.81 (0.63 – 1.04)

*Controls were frequency-matched to cases in a 1:1 ratio on age and gender

**Phenotypic index defined as hair color, eye color and ability to tan (Kanetsky et al., 2006); Reference index = black/brown hair, black/brown eyes, easily tan

+Samples were limited to those that have been genotyped, and passed the quality control process

Table 2

Significant associations of the DNA repair genes with melanoma and their genotype counts.

Pathway	Gene	SNP	Minor Allele	Major Allele	MAF	P for HWE in Controls	OR (95% CI)*	*p* value	Adjusted** OR (95% CI)	*p* value***	Permutation *P* value	FDR Corrected *P* value
NER												
	ERCC4	rs9302507	A	G	0.16	0.71	0.76 (0.63,0.92)	0.005	0.77 (0.63,0.93)	0.006	0.006	0.3266
	ERCC6	rs4253190	A	G	0.44	>0.99	1.22 (1.06,1.40)	0.005	1.19 (1.04,1.37)	0.012	0.012	0.3772
	ERCC6	rs4838518	A	G	0.49	0.94	0.85 (0.74,0.97)	0.017	0.86 (0.75,0.99)	0.033	0.037	0.4366
	ERCC6	rs4253121	A	G	0.22	0.54	0.83 (0.71,0.98)	0.032	0.84 (0.71,0.99)	0.042	0.049	0.4366
	XPC	rs2733537	G	A	0.33	0.28	1.16 (1.00,1.35)	0.043	1.17 (1.01,1.36)	0.035	0.035	0.4366
	XPC	rs3731143+	G	A	0.06	0.72	1.33 (0.98,1.80)	0.072	1.38 (1.01,1.88)	0.044	0.029	0.4366
	RFC1	rs2066782+	G	A	0.13	0.11	0.76 (0.61,0.95)	0.017	0.76 (0.61,0.95)	0.017	0.017	0.3841
Other												
	FBRSL1	rs4883571	A	G	0.38	0.41	0.87 (0.75,1.00)	0.050	0.87 (0.75,1.00)	0.049	0.069	0.4366
	MGMT	rs4750766	A	G	0.34	0.09	1.23 (1.06,1.44)	0.007	1.24 (1.06,1.44)	0.007	0.009	0.3266

*ORs were calculated based on an additive genetic model, unless otherwise indicated

**Adjusted for age, gender and phenotypic index

+A dominant genetic model was used to calculate the ORs, because there were not enough minor homozygous samples

****p*-values are NOT adjusted for multiple comparisons

Table 3

Significant associations of the haplotypes with melanoma disease status

Haplotype Blocks	Frequencies in the population	OR (95% CI)*	*p*-value*
ERCC6 (rs10745261,rs6537537,rs4253121)			
GGA	**0.21**	**0.84 (0.71,0.99)**	**0.038**
MGMT (rs12917,rs4750766)			
GA	**0.34**	**1.23 (1.05,1.44)**	**0.009**
XPC (rs2228001,rs2279017,rs3731143,rs3731093,rs3731068)			
ACGAC	**0.06**	**1.37 (1.01,1.87)**	**0.045**

*adjusted for age, gender and phenotypic index

Table 4a

Interaction analysis between SNP genotypes and indoor tanning

SNP Gene	Genotype	Indoor Tanning Exposure	Controls (n)	Cases (n)	OR* (95% CI)	Additive Interaction AP* (95% CI)	*p*-value for Multiplicative Interaction*
rs4883557 FBRSL1							
	Major Homozygotes	Never	130	135	1.00		
		Ever	169	219	1.37 (0.97, 1.93)		
	Heterozygotes	Never	188	157	1.00	0.24 (−0.07, 0.55)	
		Ever	178	252	1.98 (1.43, 2.73)		
	Minor Homozygotes	Never	56	38	1.00	0.54 (0.26, 0.81)	0.006
		Ever	45	92	2.55 (1.42, 4.60)		
rs10745261 ERCC6							
	Major Homozygotes	Never	309	282	1.00		
		Ever	327	446	1.58 (1.25, 2.01)		
	Heterozygotes or Minor Homozygotes	Never	65	48	1.00	0.39 (0.13, 0.65)	0.025
		Ever	65	117	3.42 (1.96, 5.97)		

*Adjusted for age, gender and phenotypic index

Table 4b

Interaction Analysis between haplotypes and indoor tanning

		Stratified OR* (95% CI)		
Gene	**Haplotype**	**Never Indoor Tan**	**Ever Indoor Tan**	***p*-value for Interaction***
ERCC6 (rs10745261,rs6537537,rs4253121)				
	AAG	**0.81 (0.55,1.19)**	**1.35 (0.99,1.84)**	**0.035**
	GAG	1.08 (0.83,1.4)	0.92 (0.73,1.16)	0.375
	GGG	1.13 (0.92,1.38)	1.03 (0.86,1.24)	0.507
	GGA	0.85 (0.66,1.1)	0.86 (0.69,1.08)	0.909
MGMT (rs4751118,rs3793903,rs7897057)				
	ACG	0.87 (0.7,1.07)	0.98 (0.82,1.19)	0.325
	AAA	1.2 (0.57,2.56)	1.41 (0.68,2.92)	0.771
	GCA	0.98 (0.44,2.24)	1.23 (0.69,2.22)	0.648
	GAA	1.01 (0.82,1.26)	1.07 (0.88,1.3)	0.719
	GAG	**1.28 (0.94,1.76)**	**0.85 (0.65,1.12)**	**0.042**

*Adjusted for age, gender and phenotypic index