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Genetic Variation in DNA Repair Pathway Genes and Melanoma Risk

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Abstract

Reduced DNA repair capacity has been proposed as a predisposing factor for melanoma. We comprehensively evaluated 1,463 genetic variants across 60 DNA repair-related pathway genes in relation to melanoma risk in a nested case-control study of 218 melanoma cases (20% on head and neck) and 218 matched controls within the Nurses' Health Study (NHS). We then genotyped the two variants with the smallest *P* value in two replication sets: 184 melanoma cases (28% on head and neck) and 184 matched controls in the Health Professionals Follow-Up Study (HPFS); and 183 melanoma cases (10% on head and neck) and 183 matched controls in the NHS. The SNP rs3219125 in the *PARP1* gene was significantly associated with melanoma risk in the discovery set (odds ratio (OR) 3.14; 95% confidence interval (CI) 1.70–5.80) and in the HPFS replication set (OR, 1.92; 95% CI, 1.05–3.54) but not in the NHS replication set (OR, 1.07; 95% CI, 0.58–1.97). In the joint analysis, the OR was 1.89 (95% CI, 1.34–2.68) for this polymorphism, and this increased risk was more pronounced among patients with lesions in head/neck (OR, 3.19; 95% CI, 1.77–5.73 for head/neck, and OR, 1.54; 95% CI, 1.03–2.30 for other sites, *P* value for heterogeneity test = 0.036). Our findings suggest the possible involvement of the *PARP1* variant in melanoma development, especially for sites with high sun exposure. Further work on fine-mapping and on the functional characterization of this and linked SNPs in this region is required.

Keywords

DNA Repair; Melanoma; Etiology; Polymorphism

1. Introduction

Skin cancer is the most common neoplasm in Caucasians in the United States, and melanoma, the most serious type of skin cancer, causes about 79% of skin cancer deaths [1]. Ultraviolet (UV) radiation is an established environmental carcinogen for skin cancer,

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including melanoma [2–4]. Because UV radiation is capable of causing a wide range of lesions in DNA, one important defense mechanism against melanoma is the ability to repair DNA damage induced by UV light. Reduced DNA repair capacity is a risk factor for melanoma and may contribute to susceptibility to UV-induced melanoma among the general population [5]. Melanoma was reported in 5% of patients with xeroderma pigmentosum [6]. Even though a variety of factors modulate the path from genotype to phenotype, there are substantial correlations between DNA repair gene variants and DNA repair capacity [7–10]. Thus, genetic variants in relevant DNA repair genes may confer genetic susceptibility to melanoma. However, very few of them have been evaluated previously for associations with melanoma risk.

We conducted a comprehensive and systematical evaluation of genetic variation across 60 DNA repair-related pathway genes in relation to melanoma risk in a nested case-control study of 218 prospectively ascertained cutaneous malignant melanoma cases and 218 matched controls within the Nurses' Health Study (NHS), and replicated in both NHS and the Health Professionals Follow-Up Study (HPFS), with a total of 367 melanoma cases and 367 matched controls. These pathways/genes included direct reversion repair (*MGMT*); base excision repair (*APE1*, *LIG3*, *NEIL1*, *NEIL2*, *OGG1*, *PARP1*, *XRCC1*); nucleotide excision repair (*XPA*, *ERCC3*, *XPC*, *ERCC2*, *ERCC4*, *ERCC5*, *ERCC1*, *LIG1*, *ERCC6*, *ERCC8*, *RPA1*, *RPA2*, *RPA3*); double-strand break repair via 1) homologous recombination (*RAD50*, *RAD51*, *RAD52*, *XRCC2*, *XRCC3*, *NBN*, *MRE11A*) or 2) non-homologous end-joining (*XRCC4*, *XRCC5*, *XRCC6*, *ARTEMIS*, *PRKDC*, *LIG4*); DNA polymerases, nucleases, and helicases (*POLB*, *POLD1*, *POLE*, *POLI*, *POLK*, *PCNA*, *FEN1*, *BLM*); DNA-cross-link repair (*FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*); mismatch repair (*MSH2*, *MSH3*, *MSH6*, *MLH1*, *MLH3*, *PMS1*, *PMS2*); and genes involved in DNA damage recognition and response (*ATM*, *ATR*, *CHEK1*, *CHEK2*, *TP53*) [11–13].

2. Materials and Methods

2.1. Study population

Our study population consisted of participants in the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS). The NHS was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55, residing in 11 larger US states, completed and returned the initial self-administered questionnaire on their medical histories and baseline health-related exposures. Updated information was obtained by questionnaires every 2 years, and blood samples were collected from 32,826 participants in the NHS cohort between May 1989 and September 1990. For the HPFS, in 1986, 51,529 men from all 50 US states in health professions (dentists, pharmacists, optometrists, osteopath physicians, podiatrists, and veterinarians) aged 40–75 years answered a detailed mailed questionnaire, forming the basis of the HPFS, and during 1993–1994, 18,159 study participants provided blood samples. All the cases and controls in our study were from sub-cohorts of the NHS and HPFS who had given a blood specimen. Details of the two cohorts have been described previously [14,15].

For the discovery set, eligible cases consisted of women in the NHS with pathologically confirmed incident melanoma, diagnosed any time after blood collection up to June 1, 2000 and with no previously diagnosed cancer. Cases for replication consisted of pathologically confirmed melanoma cases diagnosed after the baseline up to 2006 follow-up cycle (for both cohorts) who had no previously diagnosed cancer. One control per case was randomly selected from participants who were free of diagnosed melanoma up to and including the questionnaire cycle in which the case was diagnosed. Controls were matched to cases by age (± 1 year). Cases and their matched controls were selected in the same cohort. All subjects were US non-Hispanic Caucasians. Finally, we recruited 585 melanoma cases and 585

matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women's Hospital, Boston, MA.

2.2. Exposure data

We obtained information regarding melanoma risk factors from prospective biennial questionnaires. Information on natural hair color at age 20, mole count on the left arm, childhood and adolescent tanning tendency, number of lifetime severe sunburns, and family history of melanoma in first-degree relatives were collected in both the NHS and HPFS prospective questionnaires.

2.3. Single nucleotide polymorphism (SNP) selection

We selected a high density of 1463 common SNPs across the promoter regions, untranslated regions (UTRs), and coding and non-coding regions of 60 DNA repair genes. For each gene, we first included all coding SNPs, followed by the selection of additional tagging-SNPs by a well-accepted tagger program [16], which combines pairwise r^2 methods [17] with the potential efficiency of multi-marker approaches [18]. The detailed description on the tagging-SNP selection for these 60 DNA repair genes was presented previously elsewhere [11]. Briefly, genotype data were collected from seven population samples, including 20 CEPH trios (60 individuals in total), which are a subset of the 30 trios used in the HapMap and 70 White subjects from the Multiethnic Cohort (MEC) study [19]. In total, 3,072 SNPs have been genotyped across these 60 genes, including a high density of common SNPs ($n > 2,700$, minor allele frequency $\geq 5\%$) selected from the public dbSNP database and all known missense SNPs (> 300 , minor allele frequency $\geq 1\%$) identified through gene resequencing from the Environmental Genome Project (<http://egp.gs.washington.edu/>); the average spacing of common SNPs across each locus is 1.7 kb. In the selection of tag-SNPs for Caucasians ($r^2 > 0.8$), the 3,072 SNPs genotyped in-house in the 20 CEPH trios and the HapMap phase I data of the same 60 Caucasians were combined to achieve a much higher density of SNP markers. The patterns of linkage disequilibrium (LD) in these individuals should provide an accurate estimate of the patterns in our study population [20]. In brief, 91% of HapMap phase II SNPs are predicted by this panel with 80% or greater multi-allelic r^2 .

2.4. Genotyping Assay

In the discovery stage, high-throughput genotyping was performed using the Illumina high-multiplex BeadArray genotyping system at the MIT Broad Institute, Center for Genotyping and Analysis. The assay employs allele-specific extension methods and universal PCR amplification reactions conducted at 1,536 loci. DNA samples were processed through the highly multiplexed GoldenGate protocol using bar-coded microwell plates and robust automation systems. Eight pairs of blinded duplicate samples were included. Among the 1,536 SNPs, there are 1,463 SNPs in 60 DNA repair genes, as described above.

For the replication study, we genotyped the top two SNPs in discovery stage with the smallest P value for the association with melanoma risk, using the OpenArray™ SNP Genotyping System (BioTrove, Woburn, MA). Laboratory personnel were blinded to case-control status, and blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded samples was 100%. Primers, probes, and conditions for genotyping assays are available upon request.

2.5. Statistical Analysis

We used a χ^2 test to assess whether the genotypes were in Hardy-Weinberg equilibrium. Unconditional logistic regression was employed to calculate odds ratios (ORs) and 95%

confidence intervals (CIs). The test for screening the main effects of 1,067 SNPs was based on the additive model, treating genotype as an ordinal variable (wildtype coded as 0, heterozygote as 1, and homozygotes variant as 2). For the top two SNPs with the smallest P values, odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated in dominant models due to the relatively low minor allele frequency. The P value for heterogeneity according to body sites was tested by a χ^2 test in the cases only. All P values were two-sided.

For the correction of multiple testing in the discovery stage, the Bonferroni correction, which is the most commonly used method to adjust type I error, α , treats each single-SNP test as an independent test and is overly conservative for SNPs that are in LD, because it ignores the correlation among SNPs. To address this limitation, we calculated the effective number of independent SNPs, $M_{eff,i}$, for each candidate gene i , on the basis of the spectral decomposition (SpD) of matrices of pair-wise LD between SNPs [21,22]. M_{eff} provides a simple correction for multiple testing of non-independent SNPs in LD with each other. For each SNP for candidate gene i , the multiplicity-adjusted point-wise α (α_p) was then calculated as $\alpha/M_{eff,i}$.

3. Results

3.1. Characteristics of cases and controls

Basic characteristics of cases and controls in our study are presented in Table 1. The mean age at diagnosis of melanoma cases was 63.5 years in the discovery set, and 62.9 and 57.6 years in the HPFS and NHS replication sets, respectively. Melanoma cases were more likely to have a family history of melanoma and to have red or blonde hair color. Cases had more lifetime severe sunburns that blistered and had more moles on the arms, while the childhood tanning ability of cases was less than that of controls (Table 1).

3.2. The multiplexed GoldenGate SNParray characteristics

After excluding 98 non-Caucasian SNPs, the 70 (4.9%) SNPs that had a genotyping success rate <80% were excluded from further analysis. Eight pairs of blinded duplicate samples were included. One SNP that failed the concordance test was excluded (rs967591). Among these 1,367 SNPs, 1,304 SNPs in the DNA repair genes remained for further analysis. All these SNPs had a genotyping success rate >80%.

Among controls, 1,115 out of the 1,304 SNPs had the minor allele frequency >0.01 (the initial set of SNPs was chosen to include tag-SNPs for other ethnicities). The genotype distributions for the 1,115 SNPs were examined and tested for agreement with Hardy-Weinberg equilibrium (HWE). Among the controls, 48 loci (4.3%) had HWE χ^2 p -values < 0.01 and were excluded. Hence, the final analysis included 1,067 SNPs in the DNA repair genes.

3.3. Analysis of 1,067 SNPs

Among the 1,067 SNPs in the final analysis, 10 were associated with melanoma risk in the discovery set (Table 2), with P value ≤ 0.01 in the additive model. The two genes with the smallest P value in the additive model were *ATR* ($P = 0.00002$) and *PARP1* ($P = 0.0002$). The data on the main effect of 1,067 SNPs are provided in Supplementary Table 1.

We calculated the M_{eff} value by SNPSpD for each of the 60 candidate genes (Supplementary Table 2) to correct for multiple comparisons. As shown in Supplementary Table 2, for four genes (*PARP1*, *ATR*, *FANCA*, *MLH1*), the smallest P value for individual SNP was equal to or smaller than the significance threshold adjusted by M_{eff} value.

3.4. Replication of the top two SNPs in the HPFS and NHS replication sets

For the top two genes (*PARP1* and *ATR*) with individual *P* values less than the significance threshold adjusted by M_{eff} value in the discovery set, we selected the SNP with the smallest *P* value located in each gene and genotyped them in the replication sets. As shown in Table 3, because of the low minor allele frequency, we evaluated the associations of the genotypes with the risk of melanoma in a dominant model and found a significant association of the rs3219125 in *PARP1* in both the discovery set and the HPFS replication set (OR, 3.14; 95%CI, 1.70–5.80 in discovery set, and OR, 1.92; 95%CI, 1.05–3.54 in the HPFS replication set). Although we failed to replicate this association in the NHS replication set (OR, 1.07; 95%CI, 0.58–1.97), we detected a significant risk effect with OR of 1.89 (95%CI, 1.34–2.68) in the joint analysis. This association remained significant after adjusting for melanoma-related risk factors, including family history of melanoma, lifetime severe sunburns that blistered, hair color, childhood tanning ability, and moles on the arms (multivariate OR, 1.67; 95%CI, 1.16–2.42). For the *ATR* rs13091637, we failed to replicate our discovery result in either of the two replication sets, with OR of 0.94 (95% CI: 0.60–1.46) in the HPFS, and OR of 1.12 (95% CI: 0.72–1.73) in the NHS, and the combined OR was 1.38 (95% CI: 1.06–1.80).

3.5. Subgroup analysis of *PARP1* rs3219125 by body sites and interaction analysis

Given that DNA repair protects against UV-induced melanoma, the sun-exposed body sites are more prone to insufficiency in DNA repair capacity. In the subgroup analysis of body sites including head/neck, upper extremity, trunk, and thigh/leg/knee, we found that the risk association with the *PARP1* rs3219125 variant was most apparent in those with highly sun exposed lesions (OR, 3.19; 95% CI, 1.77–5.73 for head/neck melanoma, OR, 1.90; 95% CI, 1.01–3.57 for upper-extremity melanoma, OR, 1.63; 95% CI, 0.98–2.72 for trunk melanoma, and OR, 1.36; 95% CI, 0.76–2.40 for thigh/leg/knee melanoma, Supplementary Table 3). We found a significantly stronger association of this polymorphism with melanoma in head/neck than that in other sites combined (OR, 1.54; 95% CI, 1.03–2.30, *P* for heterogeneity test = 0.036).

We also evaluated the interactions between melanoma risk factors and the *PARP1* rs3219125 genotypes, and the results were not statistically significant (data not shown).

3.6. Linkage disequilibrium (LD) analysis in the *PARP1* gene region

Since the association of the rs3219125 in the intron region of *PARP1* with melanoma risk may also be mediated by linkage disequilibrium (LD) with other causal loci, we conducted pair-wise LD analysis in the gene region, but found no polymorphism in strong LD with rs3219125. The LD plot for *PARP1* is displayed in Supplementary Figure 1.

3.7. Association between *FANCA* and melanoma risk

The *FANCA* gene, the third in our discovery set, is located ~180 kb from the *MC1R* gene, a well-known critical pigmentation gene consistently associated with melanoma risk [23]. We evaluated the effect of the three red hair color alleles (Arg151Cys, Arg160Trp, and Asp294His) in the *MC1R* gene on the association between *FANCA* and melanoma risk. After adjusting for the three *MC1R* alleles, the *FANCA* variant was not associated with melanoma risk. This suggests that the signal we detected in the discovery stage was produced by the causal *MC1R* variants nearby.

4. Discussion

Despite evidence of the high-penetrance phenotype of nucleotide excision repair (NER) deficiency in xeroderma pigmentosum patients, relatively little is known about the

importance of common inherited variants in DNA repair pathways and their interactions with host factors in causing melanoma. There are some published data on select genetic polymorphisms in DNA repair genes and melanoma risk. However, previous studies have not given extensive consideration to multiple genes and polymorphisms in the pathways. To our knowledge this is the most comprehensive evaluation of common variation in these 60 candidate DNA-damage repair and response pathway genes in relation to melanoma risk. We comprehensively evaluated the association between the genetic variants in DNA repair genes and melanoma risk and conducted a replication study for the top two SNPs. Our data suggest the possible involvement of the *PARP1* rs3219125 in the melanoma development, especially among patients with lesions on sun-exposed body sites such as the head/neck.

Specific DNA repair pathways are responsible for the repair of different types of DNA damage [13]. (1) Base excision repair (BER) is responsible for a wide variety of non-bulky exogenous and endogenous oxidative DNA damage and single-strand breaks [24]. (2) NER is a versatile repair system that removes a wide variety of bulky, helix-distorting lesions and UV-induced DNA photoproducts. (3) Homologous recombination (HR) and non-homologous end-joining (NHEJ) are two distinct mechanisms in the repair of double-strand breaks (DSBs) in mammalian cells. DSBs can be induced by other exogenous agents and endogenous reactive oxygen species. UV-induced photoproducts cause blockage of DNA replication, which can lead to the formation of DSBs, chromosomal aberrations, and recombination during the course of replication arrest. (4) Mismatch repair (MMR) is responsible for the repair of base mismatches and insertion/deletion mismatches. Mutations in genes involved in MMR (*MSH2*, *MLH1*, *PMS1*, and *PMS2*) result in microsatellite instability (MSI) and replication errors. (5) The O⁶-methylguanine DNA methyltransferase (*MGMT*) is the gene involved in the direct reversal DNA repair that removes alkyl or methyl adducts from the O⁶ position of guanine. (6) Other candidates include Fanconi Anemia complementation groups; DNA polymerases, nucleases, and helicases; and genes involved in DNA damage recognition and response.

By analyzing the main effect of 1,463 genetic variants across 60 DNA repair-related pathway genes in the discovery stage of this study, we found that the genes with the smallest *P* value for additive models were *ATR* and *PARP1*. We failed to replicate the association of the *ATR* polymorphism and melanoma risk in the replication sets. The risk association with the *PARP1* rs3219125 variant was significant in both the discovery and the HPFS replication set, and it remained significant after adjusting for melanoma-related risk factors in the joint analysis.

To account for chance associations from multiple tests of individual SNPs with melanoma risk, we calculated the false positive report probability (FPRP) [25]. We set a FPRP threshold of 0.5 as suggested for an initial study of a relatively rare cancer, and selected two levels of prior probability as 0.01 and 0.001, which were expected as the range for a candidate gene [25]. With the prior probability of 0.01 and 0.001, the FPRP to detect an odds ratio of 1.89 as estimated in the joint analysis for rs3219125 in our study was 0.065 and 0.414, respectively, which suggested that our finding was noteworthy.

Given that DNA repair protects against UV-induced melanoma, the sun-exposed body sites are more prone to insufficiency in DNA repair capacity. In subgroup analysis, the risk effect of the *PARP1* rs3219125 variant was significantly more pronounced in patients with melanoma in head/neck than those in other sites (heterogeneity *P* = 0.036). This further suggested the possible involvement of this locus in the etiology of melanoma via its influence on DNA repair capacity. In addition, there were significantly fewer head/neck melanoma cases in the NHS replication set (*P* value < 0.001), which may explain the failure of the replication in this set.

It has been reported that poly(ADP-ribose) polymerase-1 (PARP-1) is a zinc-finger DNA-binding protein encoded by the ADP-ribosyltransferase (*ADPRT*) gene that modifies various nuclear proteins by poly(ADP-ribosyl)ation and functions as a key enzyme in DNA-damage signaling, BER, and recombination [26,27]. It specifically binds DNA single-strand breaks, recruits other factors to the site of damage, and serves as an energy source for ligation [28,29]. Previous studies reported lower PARP activity in breast and laryngeal cancer cases than in the normal controls [30,31]. The Val762Ala polymorphism, located in the most conserved region coding for the COOH-terminal catalytic domain, was associated with lower enzymatic activity [32] and altered risk of multiple cancers [32–35]. We found neither an association of Val762Ala with melanoma risk nor strong LD between this polymorphism and the rs3219125. It was noted that the rs3219125 located in the intron region next to the Val762Ala polymorphism (351 bp downstream) might have an effect in mRNA splicing. We examined the potential alteration of this variation on splicing and transcription factor binding ability by using in silico tools (BDGP: NNSPLICE 0.9 version; NetGene2 server; ASSP, Alternative Splice Site Predictor; AliBaba2.1 and TFSEARCH). However, there was no evidence showing this locus as a splicing site or transcription factor binding site with either of the two alleles. It is possible that the linked SNPs in the PARP1 gene or even in a nearby gene are casual variants for this signal. Further work on fine-mapping and on the functional characterization of this and linked SNPs in this region is required.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

OR	odds ratio
CI	confidence interval
BER	base excision repair
NER	nucleotide excision repair
DSB	double strand break repair
HR	homologous recombination
NHEJ	non-homologous end-joining
MMR	mismatch repair

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Table 1

Characteristics of melanoma cases and controls

Characteristic	Discovery Set (n = 436)		Replication Set 1, HPFS (n = 368)		Replication Set 2, NHS (n = 366)	
	Case (n = 218)	Control (n = 218)	Case (n=184)	Control (n=184)	Case (n=183)	Control (n=183)
Age at diagnosis (mean, years)	63.5		62.9		57.6	
Age at blood draw (mean, years)	57.5	57.5	60.8	60.8	56.5	56.5
Family history of melanoma (%)	15.7	5.5	11.4	2.7	17.5	11.6
Number of lifetime severe sunburns, 6+ (%)	16.0	7.2	52.0	35.4	18.4	12.9
Natural hair color at age 20, red/blonde (%)	22.5	12.0	20.5	13.4	24.7	18.5
Tanning ability, tan or deep tan (%)	56.7	71.8	56.3	81.3	57.6	64.8
Mole count, 3+ (%)	22.4	9.9	26.3	10.7	24.4	10.8
Body site (%)						
head/neck	20.4		28.1		9.5	
upper extremity	23.0		12.6		20.1	
trunk	29.1		44.9		33.5	
thigh/leg/knee	27.6		14.4		36.9	

Table 2

Main effects of 10 SNPs on melanoma risk in the additive model with *P* value ≤ 0.01

Gene	SNP	Wildtype	Heterozygote		Homozygous variant		OR (95%CI) ^a	P-trend
		case/control	case/control	case/control	case/control	case/control		
<i>ATR</i>	rs13091637	115/150	80/58		15/1		2.20 (1.53–3.16)	0.00002
<i>PAPPI</i>	rs3219125	169/189	43/16		1/0		3.13 (1.75–5.74)	0.0002
<i>FANCA</i>	rs9926296	45/66	108/100		63/39		1.51 (1.15–1.99)	0.003
<i>BLM</i>	rs8037430	117/88	80/94		14/29		0.64 (0.48–0.87)	0.004
<i>MLH1</i>	rs1540354	132/154	68/49		11/4		1.69 (1.16–2.47)	0.006
<i>FANCA</i>	rs2239360	92/117	94/77		26/16		1.48 (1.11–1.98)	0.007
<i>BLM</i>	rs7175811	100/75	94/106		16/31		0.67 (0.50–0.90)	0.008
<i>PMS2</i>	rs3779109	132/156	71/53		9/4		1.66 (1.13–2.43)	0.009
<i>XRCC4</i>	rs35268	162/141	47/63		4/10		0.59 (0.40–0.88)	0.009
<i>XPG</i>	rs4150355	67/89	113/105		32/19		1.48 (1.10–2.00)	0.01

^a Adjusted by age.

Table 3

Replication and combined results of rs3219125 and rs13091637 on melanoma risk

Genotype	Discovery Set (n = 436)			Replication Set 1, HPFS (n = 368)			Replication Set 2, NHS (n = 366)			Joint Analysis (n =1170)		
	Case (n = 218)	Control (n = 218)	OR ^a (95% CI)	Case (n=184)	Control (n = 184)	OR ^a (95% CI)	Case (n=183)	Control (n = 183)	OR ^a (95% CI)	OR ^b (95% CI)	Adjusted OR ^c (95% CI)	
<i>PARP1</i> rs3219125												
AA	169	189	1(ref)	147	163	1(ref)	158	157	1(ref)	1(ref)	1(ref)	
AG/GG	44	16	3.14(1.70–5.80)	33	19	1.92(1.05–3.54)	25	23	1.07(0.58–1.97)	1.89(1.34–2.68)	1.67(1.16–2.42)	
<i>ATR</i> rs13091637												
CC	114	150	1(ref)	119	117	1(ref)	119	123	1(ref)	1(ref)	1(ref)	
CT/TT	95	59	2.12(1.41–3.18)	58	60	0.94(0.60–1.46)	62	57	1.12(0.72–1.73)	1.34(1.05–1.72)	1.38 (1.06–1.80)	

^a Adjusted by age

^b Adjusted by age and gender

^c Adjusted by age, gender, hair color, childhood tanning ability, moles on the arms, number of lifetime severe sunburn, and family history of melanoma in first-degree relatives.