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A functional SNP in the MDM2 promoter, pigmentary phenotypes, and risk of skin cancer

Hongmei Nan^{1,2,3,*}, Abrar A. Qureshi^{3,4}, David J. Hunter^{1,2,3}, and Jiali Han^{1,3}

¹ Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, MA

² Department of Epidemiology, Harvard School of Public Health, Boston, MA

³ Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

⁴ Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Abstract

The MDM2 oncoprotein is a key negative regulator of the tumor suppressor p53. A functional MDM2 single nucleotide polymorphism (SNP309) in the promoter region increases the affinity of transcription activator Sp1 for the MDM2 gene promoter, resulting in higher expression of MDM2 and thus inhibition of p53 transcriptional activity. UV-induced p53 activation promotes cutaneous transient pigmentation, and the common p53 Arg72Pro polymorphism alters the protein's transcriptional activity. We evaluated the effect of the MDM2 SNP309 and its interaction with the p53 Arg72Pro polymorphism on pigmentary phenotypes and skin cancer risk in a nested case-control study within the Nurses' Health Study (NHS) among 219 melanoma cases, 286 squamous cell carcinoma (SCC) cases, 300 basal cell carcinoma (BCC) cases, and 873 controls, and among controls from other studies. We found that the G allele of the MDM2 SNP309 was inversely associated with the presence/absence of moles on the arm among 3207 women pooled from controls of three nested case-control studies within the NHS. Compared with the MDM2 SNP309 T/T genotype, adjusted odds ratios (ORs) of having moles on the arms for T/G and G/G genotypes were 0.92 (95% confidence interval (CI), 0.78–1.08) and 0.68 (95% CI, 0.53–0.87), respectively (P , trend, 0.005). We observed suggestive evidence of the association between the carriage of the MDM2 SNP309 G allele and childhood tanning tendency (adjusted OR, 1.30; 95% CI, 1.01–1.68). No significant associations were found between the MDM2 SNP309 and any of the three types of skin cancer. For SCC, the trend of increased risk across the three genotypes of MDM2 was stronger among p53 Pro carriers (p , trend, 0.05) than p53 Arg/Arg wildtype group (p , trend, 0.99; p , interaction, 0.07). These results provide evidence for the potential involvement of MDM2 SNP309 in pigmentary traits.

Keywords

MDM2; p53; pigmentary phenotypes; skin cancer

Introduction

Skin cancer is the most common form of cancer in the United States, with approximately 1 million new cases per year [1]. Ultraviolet (UV) radiation is a well-established skin cancer risk factor and can cause DNA damage [2,3]. The tanning response to UV irradiation and

*Correspondence to: Hongmei Nan, Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115, USA. Telephone: +617-432-5896. E-mail: hnan@hsph.harvard.edu.

development of dark pigmentation is an important host protective factor against sun exposure-induced skin cancer [4,5].

The human homologue of mouse double minute 2 (MDM2) acts as a major regulator of the tumor suppressor p53 by targeting its destruction. The MDM2 protein binds to p53 directly, resulting in ubiquitination and subsequent degradation of p53; i.e., their physical interaction inhibits the transcriptional activity of p53 by regulating its location, stability, and activity [6, 7].

Recently, Cui et al. demonstrated that UV-induced p53 activation promotes cutaneous transient pigmentation such as the tanning response by increasing the transcriptional activation of pro-opiomelanocortin (POMC) in the skin [8]. POMC is the precursor of α -MSH, which activates the melanocortin 1 receptor (MC1R) and induces melanin products [9,10]. One functional polymorphism of the p53 gene is located at codon 72, which encodes the amino acids proline (Pro) and arginine (Arg) [11]. It has been shown that, compared with Arg allele, the Pro allele has higher transcriptional activity and lower capacity to induce apoptosis [12,13]. We also found that the Pro allele is associated with the tanning response [14].

A functional MDM2 single nucleotide polymorphism (called SNP309) is the T/G substitution at the 309 nucleotide position in the promoter region. It was shown that the G allele of the SNP309 increases the affinity of the transcriptional activator Sp1 for the MDM2 gene promoter, resulting in higher expression of MDM2 than that produced by the T allele, and subsequent inactivation of the p53 pathway [15].

We evaluated the effect of MDM2 SNP309 and its interaction with the p53 codon 72 polymorphism on pigmentary phenotypes (including childhood tanning tendency, childhood sunburn reaction, and the presence/absence of moles on the arm) and skin cancer risk in a nested case-control study within the Nurses' Health Study (NHS).

Materials and Methods

Study population

The NHS was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55, residing in 11 larger U.S. states, completed a self-administered questionnaire on their medical histories and baseline health-related exposures. Updated information has been obtained by questionnaires every 2 years. Between 1989 and 1990, blood samples were collected from 32,826 of the cohort members. The distribution of risk factors for skin cancer in the subcohort of those who donated blood samples was very similar to that in the overall cohort. Eligible cases in this study were women with incident skin cancer from the subcohort who had given a blood specimen, including SCC and BCC cases with a diagnosis any time after blood collection up to June 1, 1998 and melanoma cases up to June 1, 2000 who had no previously diagnosed skin cancer. A common control series 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 or two controls were matched to each case by year of birth (± 1 year). The nested case-control study consisted of 219 melanoma cases, 286 SCC cases, 300 BCC cases, and 873 matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women's Hospital, Boston, MA.

Exposure data

Information regarding skin cancer risk factors was obtained from the prospective biennial questionnaires and a retrospective supplementary questionnaire mailed to cases and controls in 2002. Questions on natural hair color at age 20 (black, dark brown, light brown, blonde, and

red), childhood and adolescent tanning tendency (practically none, light tan, average tan, and deep tan), and childhood sunburn reaction (practically none, some redness only, burn, painful burn, and painful burn with blisters) were asked in the 1982 prospective questionnaire; number of moles on the arms (larger than 3 mm diameter) in the 1986 prospective questionnaire; and ethnic group in the 1992 questionnaire. In the skin cancer nested case-control study, natural skin color and other sun exposure-related information was collected by the retrospective supplementary questionnaire. Estimation of past sunlight exposure for each subject was described previously [16].

Laboratory assays

The MDM2 SNP309 (rs2279744) was genotyped by restriction fragment length polymorphism (RFLP), while the p53 Arg72Pro polymorphism (rs1042522) was genotyped by the 5 nuclease assay (TaqMan®) in 384-well format, using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan® primer and probe were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case-control status, and 10% blinded quality control samples (duplicated samples) were inserted to validate genotyping procedures; concordance for the blinded quality control samples was 100%. Primers, probes, and conditions for genotyping assays are available upon request.

Statistical methods

In addition to the skin cancer nested case-control study, we had MDM2 SNP309 genotyping data for the participants in nested case-control studies of breast cancer and endometrial cancer as well as p53 codon 72 polymorphism genotyping data for women in the breast cancer nested case-control study in the NHS. To increase statistical power, we assessed the effect of the MDM2 SNP309 on pigmentary phenotypes (including childhood tanning tendency, childhood sunburn reaction, and the presence/absence of moles on the arm) among the 3207 women pooled from controls in three nested case-control studies of skin cancer, breast cancer, and endometrial cancer. Also, we used 2009 controls from two nested case-control studies (skin cancer and breast cancer) to evaluate the interaction between the MDM2 SNP309 and the p53 codon 72 polymorphism on pigmentary phenotypes.

We used a χ^2 test to assess whether the MDM2 genotype and the p53 genotype were in Hardy-Weinberg equilibrium among the controls. The genotype distributions of two SNPs were in Hardy-Weinberg equilibrium. We evaluated the association of the MDM2 SNP309 with pigmentary phenotypes among the controls. In the evaluation of pigmentary phenotypes as outcomes, the responses to a question about childhood tanning tendency were grouped into tan (light tan or average tan or deep tan) and non-tan (practically none); the responses to the question of childhood sunburn reaction were grouped into burn (some redness only, burn, painful burn, and painful burn with blisters) and non-burn (practically none); the responses to the question of number of moles on the arms were grouped into presence (the number of moles ≥ 1) and absence (none). A test for trend was performed across the three MDM2 genotypes. Unconditional logistic regression was used to calculate the association between the MDM2 SNP309 and pigmentary phenotypes as well as interactions between the MDM2 SNP309 and the p53 codon 72 polymorphisms on pigmentary phenotypes. Unconditional logistic regression was also employed to assess the interaction between the MDM2 SNP309 and the childhood tanning tendency (or childhood sunburn reaction, or natural hair color) on the presence/absence of moles on the arm. To summarize multiple constitutional phenotypic variables, we constructed a multivariate confounder score for skin cancer [17]. Briefly, we applied the logistic regression coefficients from a multivariate model, including age, race, natural skin color, natural hair color, childhood or adolescent tendency to burn, and the number of moles on arms, to each individual's values for the latter four variables and summed the values to

compute a susceptibility risk score in the logit scale. We used this score to define women with low and high constitutional susceptibility based on median among controls. We evaluated the association of the MDM2 SNP309 and its interaction with the p53 codon 72 polymorphism on the constitutional susceptibility score among controls of the skin cancer nested case-control study using unconditional logistic regression.

We evaluated the effects of the MDM2 SNP309 and its interaction with the p53 polymorphisms on skin cancer risk using unconditional regression models. We compared each type of skin cancer to the common control series in the skin cancer nested case-control study to increase statistical power.

In the interaction analysis, we used cross-classified categories of the MDM2 SNP309 and p53 polymorphisms (or childhood tanning tendency, childhood sunburn reaction, or natural hair color) compared to a common reference category. We modeled these predictors as ordinal variables to test the statistical significance of a single multiplicative interaction term.

Results

Descriptive Characteristics of Cases and Controls

A detailed description of the characteristics of cases and controls in the skin cancer nested case-control study has been provided elsewhere [18]. In brief, at the beginning of the follow-up of this nested case-control study, the nurses were between 43 and 68 years old (mean age 58.7). The mean age at diagnosis of incident melanoma cases was 63.4 years, and that of SCC cases and BCC cases was 64.7 and 64.0 years, respectively. A family history of skin cancer was a risk factor. Compared with controls, skin cancer cases had lighter pigmentation (skin color and hair color), more number of moles on the arms, higher cumulative sun exposure while wearing a bathing suit, and more lifetime severe sunburns that blistered.

Association between the MDM2 SNP309 and pigmentary phenotypes

We tested whether the MDM2 SNP309 was correlated with pigmentary phenotypes including childhood tanning tendency, childhood sunburn reaction, and the presence/absence of moles on the arm among controls from three nested case-control studies (Table 1). The G/G genotype was significantly inversely associated with the presence/absence of moles on the arm. This association was similar after adjusting for natural hair color, childhood tanning tendency, and childhood sunburn reaction. Compared with the T/T genotype, adjusted odds ratio (ORs) of the presence/absence of moles on the arm for T/G and G/G genotypes were 0.92 (95% confidence interval (CI), 0.78–1.08) and 0.68 (95% CI, 0.53–0.87), respectively (P , trend, 0.005). We did not observe a significant association between the MDM2 SNP309 and the constitutional susceptibility score among controls (P , trend, 0.23) (data not shown).

No significant trends were observed between the three genotypes of MDM2 SNP309 and childhood tanning tendency (P , trend, 0.15), childhood sunburn reaction (P , trend, 0.31) (Table 1), or natural hair color (p , trend, 0.51) (data not shown). However, we observed a marginally significant association between the carriage of the G allele and childhood tanning tendency (adjusted OR, 1.30; 95% CI, 1.01–1.68). This association was not found for childhood sunburn reaction (Table 1).

Interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on pigmentary phenotypes

We evaluated the interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on pigmentary phenotypes (including childhood tanning tendency, childhood sunburn reaction, and the presence/absence of moles on the arm) among controls from two

nested case-control studies. The tests for departure from multiplicative interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on childhood tanning tendency (p , interaction, 0.29), childhood sunburn reaction (p , interaction, 0.70), or the presence/absence of moles on the arm (p , interaction, 0.77) were not statistically significant (Table 2). There was no significant interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphisms on the constitutional susceptibility score among controls (p , interaction, 0.86) (data not shown).

We also explored the interaction between the MDM2 SNP309 and childhood tanning tendency, childhood sunburn reaction, and natural hair color on the presence/absence of moles on the arm among controls from three nested case-control studies, none of the tests for departure from multiplicative interaction between the MDM2 SNP309 and childhood tanning tendency (p , interaction, 0.37), childhood sunburn reaction (p , interaction, 0.62), and natural hair color (p , interaction, 0.78) on the presence/absence of moles on the arm showed statistical significance (data not shown).

Association between the MDM2 SNP309 and skin cancer risk

No significant associations were detected between the MDM2 SNP309 and any of the three types of skin cancer (Table 3).

No significant associations between the MDM2 SNP309 and age of diagnosis were found in the case of melanoma, SCC, and BCC. The p -value for ANOVA test was 0.90, 0.81, and 0.90, respectively. The mean age (years) at diagnosis of melanoma cases according to T/T, T/G, and G/G genotypes of MDM2 was 63.7, 63.2, and 63.4, respectively; that of SCC cases was 64.8, 64.8, and 64.0, respectively; and that of BCC cases was 64.0, 64.1, and 64.6, respectively.

Interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on skin cancer risk

For SCC, the trend of increased risk across the three genotypes of MDM2 was stronger among p53 Pro carriers (p , trend, 0.05) than p53 Arg/Arg wildtype group (p , trend, 0.99; p , interaction, 0.07) (Table 4). Similar interaction patterns were also observed for BCC and melanoma, however, the tests for departure from multiplicative interaction did not approach statistical significance (p -values for interaction for BCC and melanoma were 0.17 and 0.40, respectively).

We also found no significant interactions between the MDM2 SNP309 and pigmented phenotypes, sun exposure, and the number of lifetime severe sunburns on skin cancer risk (data not shown).

Discussion

In this study, we observed a significant inverse association of the MDM2 SNP309 G/G genotype with the presence/absence of moles on the arm. Few studies have attempted to assess the association of single nucleotide polymorphisms of candidate genes with development of nevi or freckles; and no statistically significant associations have been found [19,20]. Mole (nevus) known as the presumed precursor to melanoma occurs when melanocytes cluster together forming nests at the dermo-epidermal junction. Both benign and dysplastic nevi are characterized by disruption of the epidermal melanin transfer system where each melanocyte transfers melanin-containing melanosome to the suprabasal keratinocytes via dendrites, leading to an increased number of melanocytes [21]. Most moles appear in early childhood and during the first 20 years of a person's life. van Schanke et al. reported that excessive exposure to sunlight may play a role in the formation of acquired moles [22]. This leads to the hypothesis that the MDM2 SNP309 interacts with acquired or congenital pigmented phenotypes to affect the development of moles. Therefore, we further explored the interaction

between the MDM2 SNP309 and pigmentary phenotypes including childhood tanning tendency, childhood sunburn reaction, and natural hair color. However, we did not find any significant interactions. This result suggests that the effect of MDM2 SNP309 on development of moles may not be substantially influenced by other pigmentary phenotypes.

We found a significant correlation between the carriage of SNP309 G allele and childhood tanning tendency. However, no such a correlation was observed for childhood sunburn reaction. Sunburn is UV-induced apoptosis of keratinocytes, whereas tanning is UV-induced melanin synthesis and production in melanocytes [23,24]. This suggests that burning and tanning responses may be two different types of skin responses to UV involving independent molecular pathways. On the other hand, we cannot rule out the possibility that the effect on childhood tanning tendency could be due to chance. The issue of population stratification is important in assessment of the association of the SNP309 with childhood tanning tendency because light pigmented populations have less capacity to tan. In this study, we performed our analyses in Caucasian women only. The frequency of the G allele (35%) was similar with average frequency of G in Caucasians (37%), which was reported by Wilkening et al. based on several studies [25]. Furthermore, in our study, the similar genotype distribution among Southern Europeans and Scandinavians indicated that “sub-population” stratification is likely to be minimal.

In some previous studies, the MDM2 SNP309 was associated with the development of cancers such as hepatocellular carcinoma, endometrial cancer, or lung cancer [25–27], whereas other studies reported no significant associations between the SNP309 and cancer risk such as colorectal cancer, breast cancer, or ovarian cancer [25,28,29]. Wilkening et al. reported that the MDM2 SNP309 affected neither the risk nor the age at onset of BCC [30], which is consistent with our results. We observed no significant associations between the MDM2 SNP309 and either the age at skin cancer diagnosis or the risk of the three types of skin cancer.

Because the MDM2 oncoprotein is a key negative regulator of the tumor suppressor p53, MDM2 overexpression is considered one mechanism of p53 inactivation [6,31]. MDM2 downregulates p53 activity by binding it directly and forming the MDM2-p53 complex, which results in ubiquitination and proteosomal degradation of p53 through the E3 ubiquitin ligase activity of MDM2 [32,33]. The functional MDM2 SNP309 locus is located in the transcriptional activator Sp1-binding site in the MDM2 promoter region. It has been shown that the G allele of SNP309 increases the affinity of Sp1 to the MDM2 promoter, resulting in increased expression levels of MDM2 mRNA and protein and thus increased inhibition of p53 activity [15]. Cui et al. reported that p53 can partially control the transient tanning response [8]. There is a common functional p53 Arg/Pro polymorphism at codon 72 in exon 4 [34]. The Pro allele was positively associated with the transcriptional activity of p53 [13] and childhood tanning response [14]. In this regard, reduced p53 activity by the G allele of MDM2 SNP309 should attenuate the transient tanning response. However, we observed a positive association between the carriage of the MDM2 SNP309 G allele and childhood tanning tendency. We did not find any significant interactions between the MDM2 SNP309 and the p53 Arg72Pro polymorphisms on pigmentary phenotypes such as childhood tanning tendency, childhood sunburn reaction, or the presence/absence of moles on the arm. MDM2 is highly expressed in both the basal and suprabasal layers of the skin, where keratinocytes initiate differentiation [35,36]. Although the MDM2-p53 dependent pathway is well-characterized, some previous studies reported that MDM2 expression in keratinocytes and epithelia and its activity in cell cycle progression, differentiation, and transformation are independent of p53 [36–38]. A study using transgenic mice showed that MDM2 overexpression induced increased proliferation of the basal layer and thickening of the epidermis, resulting in a specific skin phenotype that appears to be p53-independent [35]. Hence, it is plausible that MDM2 is involved in distinct

molecular pathways to modify pigmentary phenotypes, such as mole counts and the development of skin cancer.

Exposure misclassification is always a concern in epidemiologic studies. The high education level and interest in health of cohort members allows high quality and valid information to be collected on self-administered forms. In our study, because most of constitutional host factor information was collected prior to diagnosis we will avoid the potential for differential misclassification, i.e. recall bias. Test-retest reliability of collecting phenotypic factors from questionnaires is moderate to substantial, including skin color, tanning/burning tendency, and sunburn history [39–41]. The majority of studies on nevus counts have shown substantial agreement between nevus self-counts and dermatologist-counts [42–44]. The Spearman correlation coefficient was 0.91 between nevus counts by patients and physicians, suggesting quite a good correlation between the two measurements [45]. These previous reports support the reliability and validity of self-reported skin characteristics. Nevertheless, we cannot exclude the potential misclassification because the information regarding mole counts was limited on the arms and only asked once in this study.

In conclusion, our study showed a significant association of the MDM2 SNP309 G/G genotype with the presence/absence of moles on the arm. We also observed suggestive evidence of the association between the MDM2 SNP309 and childhood tanning tendency. No associations were found between the MDM2 SNP309 and childhood sunburn reaction, or skin cancer risk. We found no significant interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on pigmentary phenotypes. These results provide evidence for the potential involvement of MDM2 SNP309 in pigmentary traits.

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Abbreviations

BCC	basal cell carcinoma
SCC	squamous cell carcinoma
CI	confidence interval
OR	odds ratio
UV	ultraviolet

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Table 1

Relationship between the MDM2 SNP309 and childhood tanning tendency, childhood sunburn reaction, and the presence/absence of moles on the arm

Childhood tanning tendency				
	Tan (%)	Non-tan (%)	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
T/T	1161 (43)	134 (49)	1.00	1.00
T/G	1209 (44)	105 (38)	1.33 (1.02–1.74)	1.35 (1.03–1.78)
G/G	348 (13)	35 (13)	1.15 (0.78–1.70)	1.14 (0.76–1.71)
<i>P</i> , trend			0.15	0.15
T/G or G/G	1557 (57)	140 (51)	1.28 (1.00–1.65)	1.30 (1.01–1.68)
Childhood sunburn reaction				
	Burn (%)	Non-burn (%)	Crude OR (95% CI)	Adjusted OR ^b (95% CI)
T/T	1080 (44)	233 (41)	1.00	1.00
T/G	1081 (44)	249 (44)	0.94 (0.77–1.14)	0.97 (0.80–1.19)
G/G	305 (12)	81 (14)	0.81 (0.61–1.08)	0.84 (0.63–1.12)
<i>P</i> , trend			0.17	0.31
T/G or G/G	1386 (56)	330 (59)	0.91 (0.75–1.09)	0.94 (0.78–1.14)
Moles on arms				
	Presence (%)	Absence (%)	Crude OR (95% CI)	Adjusted OR ^c (95% CI)
T/T	500 (45)	755 (42)	1.00	1.00
T/G	490 (44)	804 (44)	0.92 (0.79–1.08)	0.92 (0.78–1.08)
G/G	115 (10)	258 (14)	0.67 (0.53–0.86)	0.68 (0.53–0.87)
<i>P</i> , trend			0.004	0.005
T/G or G/G	605 (55)	1062 (58)	0.86 (0.74–1.00)	0.86 (0.74–1.00)

Based on the controls in three nested studies of skin cancer, breast cancer, and endometrial cancer within the NHS. OR>1 means increased likelihood of tanning, burning, or having moles on arms.

^aUnconditional logistic regression adjusted for natural hair color, childhood sunburn reaction, and moles on arms.

^bUnconditional logistic regression adjusted for natural hair color, childhood tanning tendency, and moles on arms.

^cUnconditional logistic regression adjusted for natural hair color, childhood tanning tendency, and childhood sunburn reaction. The percentages may not sum to 100 due to rounding.

Table 2
Interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on childhood tanning tendency, childhood sunburn reaction, and the presence/absence of moles on the arm

P53 Genotype	MDM2	T/T	Interaction on childhood tanning tendency		P, trend
			T/G	G/G	
Arg/Arg	Tan/Non-tan	389/51	439/29	143/17	
	Crude OR	1.00	1.99 (1.23–3.19)	1.10 (0.62–1.97)	0.19
	Adjusted OR ^a	1.00	1.99 (1.22–3.24)	0.89 (0.49–1.63)	0.38
Arg/Pro or Pro/Pro	Tan/Non-tan	340/37	338/17	88/7	
	Crude OR	1.21 (0.77–1.89)	2.61 (1.48–4.60)	1.65 (0.72–3.75)	0.07
	Adjusted OR ^a	1.12 (0.70–1.77)	2.45 (1.37–4.38)	1.68 (0.72–3.92)	0.05
<i>p</i> , interaction, 0.29					
Interaction on childhood sunburn reaction					
Arg/Arg	Burn/Non-burn	364/77	392/86	123/37	
	Crude OR	1.00	0.96 (0.69–1.35)	0.70 (0.45–1.09)	0.18
	Adjusted OR ^b	1.00	1.01 (0.72–1.43)	0.76 (0.48–1.19)	0.31
Arg/Pro or Pro/Pro	Burn/Non-burn	319/68	283/73	78/18	
	Crude OR	0.99 (0.69–1.42)	0.82 (0.58–1.17)	0.92 (0.52–1.62)	0.50
	Adjusted OR ^b	1.02 (0.71–1.47)	0.92 (0.64–1.32)	0.99 (0.55–1.76)	0.67
<i>p</i> , interaction, 0.70					
Interaction on moles on arms					
Arg/Arg	Presence/Absence	163/250	191/273	48/112	
	Crude OR	1.00	1.07 (0.82–1.41)	0.66 (0.44–0.97)	0.12
	Adjusted OR ^c	1.00	1.08 (0.82–1.41)	0.67 (0.45–0.99)	0.16
Arg/Pro or Pro/Pro	Presence/Absence	141/237	132/222	22/66	
	Crude OR	0.91 (0.69–1.22)	0.91 (0.68–1.22)	0.51 (0.30–0.86)	0.11
	Adjusted OR ^c	0.91 (0.69–1.22)	0.92 (0.68–1.23)	0.51 (0.30–0.86)	0.09
<i>p</i> , interaction, 0.77					

Based on the controls in two nested studies of skin cancer and breast cancer within the NHS. OR>1 means increased likelihood of tanning, burning, or having moles on arms. *p*-value for trend was calculated across the three MDM2 genotypes.

^aUnconditional logistic regression adjusted for natural hair color, childhood sunburn reaction, and moles on arms.
^bUnconditional logistic regression adjusted for natural hair color, childhood tanning tendency, and moles on arms.
^cUnconditional logistic regression adjusted for natural hair color, childhood tanning tendency, and childhood sunburn reaction.

Table 3

The MDM2 SNP309 and skin cancer risk

Genotype	Controls (%)	Melanoma			SCC			BCC		
		Cases (%)	Multivariate OR ^b		Cases (%)	Multivariate OR ^b		Cases (%)	Multivariate OR ^b	
			Multivariate OR ^a			Multivariate OR ^a			Multivariate OR ^a	
T/T	380 (45)	97 (45)	1.00	1.00	117 (42)	1.00	1.00	119 (41)	1.00	1.00
T/G	356 (42)	92 (42)	1.00 (0.72–1.37)	1.02 (0.73–1.43)	119 (42)	1.08 (0.81–1.45)	1.15 (0.85–1.56)	127 (44)	1.14 (0.85–1.52)	1.16 (0.86–1.56)
G/G	115 (14)	29 (13)	0.95 (0.59–1.51)	0.93 (0.57–1.52)	45 (16)	1.28 (0.85–1.91)	1.27 (0.84–1.94)	43 (15)	1.20 (0.80–1.81)	1.18 (0.77–1.81)
P for trend			0.85	0.87		0.25	0.22		0.30	0.33

^aUnconditional logistic regression adjusted for the age.

^bUnconditional logistic regression adjusted for the age, family history of skin cancer, the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), natural skin color, natural hair color, childhood tanning tendency, childhood sunburn reaction, and moles on arms. The percentages may not sum to 100 due to rounding.

Table 4
Interaction between the MDM2 SNP309 and the p53 Arg72Pro polymorphism on skin cancer risk

P53 Genotype	MDM2	T/T	Interaction on melanoma risk		P, trend
			T/G	G/G	
Arg/Arg	Case/Controls	46/203	48/211	15/72	
	Multivariate OR ^a	1.00	0.97 (0.62–1.53)	0.86 (0.45–1.65)	0.79
	Multivariate OR ^b	1.00	0.96 (0.60–1.53)	0.83 (0.42–1.62)	0.82
Arg/Pro or Pro/Pro	Case/Controls	46/169	42/136	13/42	
	Multivariate OR ^a	1.19 (0.75–1.88)	1.36 (0.84–2.18)	1.34 (0.66–2.70)	0.62
	Multivariate OR ^b	1.17 (0.73–1.89)	1.43 (0.88–2.34)	1.38 (0.66–2.87)	0.46
<i>p</i> , interaction, 0.40					
Interaction on SCC risk					
Arg/Arg	Case/Controls	67/203	61/211	22/72	
	Multivariate OR ^a	1.00	0.87 (0.59–1.30)	0.93 (0.53–1.62)	0.73
	Multivariate OR ^b	1.00	0.93 (0.62–1.41)	0.96 (0.54–1.70)	0.99
Arg/Pro or Pro/Pro	Case/Controls	47/169	57/136	22/42	
	Multivariate OR ^a	0.84 (0.55–1.29)	1.27 (0.84–1.92)	1.59 (0.88–2.86)	0.03
	Multivariate OR ^b	0.90 (0.58–1.40)	1.40 (0.91–2.14)	1.65 (0.89–3.05)	0.05
<i>p</i> , interaction, 0.07					
Interaction on BCC risk					
Arg/Arg	Case/Controls	67/203	69/211	22/72	
	Multivariate OR ^a	1.00	0.99 (0.67–1.47)	0.93 (0.54–1.62)	0.90
	Multivariate OR ^b	1.00	0.99 (0.66–1.48)	0.93 (0.52–1.65)	0.87
Arg/Pro or Pro/Pro	Case/Controls	52/169	58/136	21/42	
	Multivariate OR ^a	0.94 (0.62–1.42)	1.29 (0.85–1.95)	1.53 (0.85–2.78)	0.08
	Multivariate OR ^b	0.97 (0.63–1.50)	1.37 (0.89–2.10)	1.52 (0.82–2.82)	0.10
<i>p</i> , interaction, 0.17					

^aUnconditional logistic regression adjusted for the age.

^bUnconditional logistic regression adjusted for the age, family history of skin cancer, the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), natural skin color, natural hair color, childhood tanning tendency, childhood sunburn reaction, and moles on arms.