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## Sun Exposure, Vitamin D Receptor Polymorphisms *FokI* and *BsmI* and Risk of Multiple Primary Melanoma

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### Abstract

Sunlight exposure increases risk of melanoma. Sunlight also potentiates cutaneous synthesis of vitamin D, which can inhibit melanoma cell growth and promote apoptosis. Vitamin D effects are mediated through the vitamin D receptor (*VDR*). We hypothesized that genetic variation in *VDR* affects the relationship of sun exposure to risk of a further melanoma in people who have already had one.

We investigated the interaction between *VDR* polymorphisms and sun exposure in a population-based multinational study comparing 1138 patients with a multiple (second or subsequent) primary melanoma (cases) to 2151 patients with a first primary melanoma (controls); essentially a case-control study of melanoma in a population of melanoma survivors. Sun exposure was assessed using a questionnaire and interview, and was shown to be associated with multiple primary melanoma. *VDR* was genotyped at the *FokI* and *BsmI* loci and the main effects of variants at these loci and their interactions with sun exposure were analyzed.

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Only the *BsmI* variant was associated with multiple primary melanoma (OR = 1.27, 95% CI 0.99-1.62 for the homozygous variant genotype). Joint effects analyses showed highest ORs in the high exposure, homozygous variant *BsmI* genotype category for each sun exposure variable. Stratified analyses showed somewhat higher ORs for the homozygous *BsmI* variant genotype in people with high sun exposure than with low sun exposure. *P* values for interaction, however, were high.

These results suggest that risk of multiple primary melanoma is increased in people who have the *BsmI* variant of *VDR*.

## Keywords

melanoma; *FokI*; *BsmI*; sun exposure

## Introduction

Exposure to sunlight plays a role in the development of melanoma. The incidence of melanoma per unit of surface area is higher on sun-exposed than non sun-exposed skin [1] and melanoma may be induced in animal models following ultraviolet light (UV) exposure [2]. Intermittent sun exposure has been observed as a risk factor for melanoma in many [3-5] studies, and many are now evaluating genetic risk in combination with UV exposure, the major environmental factor.

Sunlight potentiates the synthesis of the steroid hormone vitamin D<sub>3</sub> from precursors in the skin. It does so by way of UVB-mediated conversion of 7-dehydrocholesterol and isomerization in basal epidermal keratinocytes to vitamin D<sub>3</sub>, with subsequent hydroxylation to the biologically active 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) [6]. There is evidence to suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> plays a role in the development and progression of melanoma [7-8]. 1,25(OH)<sub>2</sub>D<sub>3</sub> promotes melanocyte differentiation and apoptosis [9-10] and inhibits cell growth, adhesion, migration, metastases and angiogenesis in vitro and in vivo [11-15].

Polymorphic variants of the vitamin D receptor (*VDR*) gene have been associated with increased risk of melanoma in a number of recent hospital-based studies [16-20] and a nested-case control study within the Nurses Health Study [21]. However, while measures of sun sensitivity including tanning ability and sunburn history were included in some of these studies, none incorporated sun exposure. Given that the *VDR* may only be activated by vitamin D and that sunlight exposure is intimately linked with vitamin D<sub>3</sub> production, consideration of the interaction between them is essential to understanding the impact of these factors on melanoma risk. In North America and based on the typical western diet, sun exposure is the fundamental source of vitamin D [22].

In an international population-based study, GEM, we examined the association of *VDR* genotype with sun exposure and development of melanoma (i.e. the gene-environment interaction). The study design for GEM consists of individuals diagnosed with single primary melanoma as controls for individuals with multiple primary melanomas as cases. As such, the design is essentially a case-control study of melanoma conducted in the population of melanoma survivors. *FokI* and *BsmI* polymorphisms were selected for study; *BsmI* was chosen as a representative of the 3' untranslated cluster because of the high degree of linkage between these polymorphisms in Caucasians, requiring the study of only one variant to characterize the 3' region [23-25]. *FokI* was chosen as it had previously been associated with melanoma risk [16] and Alimirah and colleagues [26] report that *FokI* differentially modulates the effects of vitamin D.

## Methods

Study subjects were recruited from 8 population-based cancer registries in New Jersey, California and North Carolina (USA), British Columbia and Ontario (Canada), Torino (Italy) and Tasmania and New South Wales (Australia). Recruitment was done as part of the GEM (Genes and Environment in Melanoma) study, an international multi-center, population-based study of multiple (second or subsequent) primary melanoma (MPM) compared to single primary melanoma (SPM).

As part of this study, genetic information (DNA) from each patient and detailed information relating to patients' characteristics (e.g., age, sex, skin/hair/eye color, tanning ability, freckling as a child, number of nevi), family history of skin cancer, past sun exposure, and tumor histology were collected for all participating subjects with incident primary melanoma in 8 population centers in North America, Europe and Australia. Further details of the GEM study design are given elsewhere [27-28].

The GEM study protocol was approved by the Institutional Ethics Review Board at the GEM coordinating center, Memorial Sloan-Kettering Cancer Center in New York, and at each of the study centers. All participants provided written informed consent. Separate approval was obtained at each center for this study.

GEM controls were people diagnosed with a pathologically confirmed first invasive primary melanoma during the six-month period January 1, 2000-June 30, 2000 with the following exceptions: the whole of 2000 in California and North Carolina; from January 1, 2000 to August 31, 2000 in Ontario; and from June 1, 2000 to May 31, 2001 in Turin, Italy. GEM cases were people diagnosed with a pathologically confirmed second or higher order invasive or in situ melanoma during the period January 1, 2000 to August 31, 2003, except in Ontario where case ascertainment ended February 28, 2003, and the centers in British Columbia, California, New Jersey and Tasmania, which recruited GEM Cases additionally in 1998 and 1999.

For the purposes of this analysis, we examined the three major types of sun exposure from our analysis of the relationship between solar exposure and melanoma risk [28] (1) ambient erythemal ultraviolet (UV) radiation dose at age 10, chosen to represent early lifetime sun exposure, (2) sunny vacations, at a place sunnier than usual, as average annual hours of exposure per year over the lifetime from age 5 to diagnosis, and (3) beach and waterside exposure as average hours per year from age 15, over the lifetime. Each of these exposure types has been shown to be associated with melanoma risk in previous studies [29-33].

A complete description of data collection and estimation relating to sun exposure variables has been previously published [28]. In brief, erythemally weighted solar ultraviolet irradiance (UVE) was estimated in  $\text{KJ/m}^2$  for each place of residence, using satellite-derived data. An estimate of UVE was assigned to each year of age, using residence information for the decade years of age, and UVE exposure at age 10 was used for these analyses. Data regarding beach and waterside exposure was elicited from age 15 to the time of diagnosis if an activity was reported between the hours of 9 and 5 on at least 10 days in any year since leaving school. If study participants did participate in beach or waterside activities, they were asked the years started and stopped and the usual outdoor hours per day by season. The total lifetime hours of exposure in these activities were the sum of all reported daily exposure hours weighted by frequency and duration. Sunny vacations reported over the lifetime were calculated as hours per year in the same manner although they were calculated from age 5 to diagnosis

## VDR Genotyping

The Molecular Epidemiology Laboratory of the Memorial Sloan-Kettering Cancer Center typed the *VDR FokI* and *BsmI* polymorphisms. DNA was extracted from buccal cells using Puregene® kits (Gentra Systems, Inc., Minneapolis, MN) replacing glycogen with tRNA (10µg/µl) for the DNA precipitation step. All genotyping was done with PCR-based methods and included melting temperature analysis [34] coupled to the LightTyper instrument (Roche Applied Science, Indianapolis, USA) for the analysis of the *FokI* SNP and pyrosequencing [35] with the PSQ™ MA instrument (Biotage AB, Uppsala, Sweden) for the analysis of *BsmI* SNP.

The *VDR-FokI* specific fragments (267 bp) were amplified in a PCR reaction mix containing 10-100 ng DNA, 200 µM dNTPs, 0.4 µM forward primer (5'-CTGAGCCAGCTATGTAGGGC-3'), 2.0 µM reverse primer (5'-GGTCAAAGTCTCCAGGGTCA-3'), 0.2 µM fluorescein labeled probe (5'-CTTGCTGTTCTTACAGGGACGGAG-3'), 1.5 mM MgCl<sub>2</sub>, 1M betaine and 0.05U/µl of Taq Polymerase. The cycling conditions included a denaturation and Taq activation step at 95°C for 10 minutes followed by 5 cycles at 95°C-25 seconds, 64°C-20 seconds, 72°C-30 seconds, 5 cycles at 95°C-25 seconds, 60°C-20 seconds, 72°C-30 seconds, 40 cycles at 95°C-25 seconds, 56°C-20 seconds, 72°C-30 seconds, and a post cycling extension at 72°C for 5 minutes.

The *VDR-BsmI* specific fragments (209bp) were amplified in a PCR reaction mix containing 10-100 ng genomic DNA, 1x buffer II (10mM Tris-HCl, pH 8.3, 50mM KCl) (PE, Roche Molecular Systems Inc., Branchburg, NJ), 200 uM dNTP, 0.42 uM forward primer (5'-CCTCACTGCCCTTAGCTCTG-3') and reverse primer (5'Biotin-CCATCTCTCAGGCTCCAAAG-3'), 2.5mM MgCl<sub>2</sub>, 5% DMSO and 0.05U/ul Taq Polymerase. Cycling conditions included a denaturation step at 95°C for 5 minutes followed by 2 cycles at 95°C-20 seconds, 59°C-20 seconds, 72°C-25 seconds, 40 cycles at 95°C-20 seconds, 57°C-20 seconds, 72°C-25 seconds, and a final extension at 72°C for 5 minutes. For the pyrosequencing reaction, the sequencing primer (5'-CCACAGACAGGCC-3') was added to single stranded DNA.

The output of the genotyping assays consisted of melting profiles (*FokI* SNP) and pyrograms (*BsmI* SNP), and in addition to the automatic genotype calls obtained by the software, the laboratory members reviewed individual signals manually. All genotyping assays included known internal controls (homozygous wild type and variant, and heterozygous DNAs) and blanks (water). For quality control, 10% of samples were split, relabeled, and re-analyzed. All results were interpreted at least twice by two different laboratory members. Assays were considered acceptable when all the control and water samples tested showed the expected genotype or no signal, respectively; there was 100% agreement in the genotyping calls between two independent laboratory members; and there was 100% concordance for the random selected samples tested in two independent assays. Quality control for data entry included an additional review of at least 20% of genotype calls.

Direct sequencing was performed using an independent PCR reaction to confirm the genotype of laboratory control samples, in randomly selected cases, or when a new SNP was identified by different sequence pattern in the pyrogram, or different melting profile in the LightTyper. Amplified samples were resolved on agarose gels, and specific bands were excised and purified according to the manufacturer's recommendations (Qiagen Inc., Valencia, USA). The purified DNA was sequenced in the Sequencing Facility Core of Memorial Sloan-Kettering Cancer Center on an ABI377 instrument (PE-Applied Biosystems).

## Statistical Analysis

Univariate and multivariate statistical analyses were undertaken using SAS Statistical Packages Version 9.2 (SAS Institute, Cary, NC). Homogeneity of and trend in odds ratios across strata were tested using StatXact Version 8.0.

Age at diagnosis was defined as age at first melanoma diagnosis for subjects with single primary melanoma and age at most recent diagnosis for subjects with multiple primary melanoma. A multiplicative age-sex interaction term was included in all models to control for potential confounding effects in recognition of the fact that the age incidence curves for melanoma are markedly different for males versus females.

Sun exposure variables were dichotomized using cut points based on the exposure distribution in all subjects combined. Phenotypic and demographic covariates were similarly categorized based on their distributions.

Study center was included as a covariate in all models to account for unmeasured differences among populations; control for study center has been demonstrated to adequately control for ancestry in these data (R. Millikan, personal communication). Conventional methods of analysis for case-control studies were followed. Individuals who developed a second primary melanoma during the ascertainment period were treated as both cases and controls. Descriptive statistics stratified by case status were calculated for all *VDR* genotypes, sun exposure variables, and phenotypic and demographic characteristics. Chi-square testing was performed to ensure that genotype frequencies did not differ greatly from those expected under Hardy-Weinberg equilibrium (HWE).

Unconditional logistic regression was used to estimate odds ratios for multiple primary melanoma and each *VDR* genotypic and sun exposure covariate separately, controlling for age, sex, a sex-age interaction and center. Ability to tan was included as a measure of phenotypic susceptibility, and family history was included because of the large difference between cases and controls in its prevalence. All three genotypes were incorporated into the model together, with homozygous wildtype genotypes chosen as the referent (FF for *FokI* and bb for *BsmI*). To test for the relationship between the *VDR* gene variants and sun exposure, separate models for each of the genetic variants (*FokI*, *BsmI*) and each of the sun exposure measures were constructed. Main effects, stratified and joint effects analyses were conducted. Interactions were identified using a likelihood ratio test at the alpha 0.1 level. Significance tests were two-sided and a Pvalue of less than 0.05 was considered significant.

## Results

A total of 3,289 eligible patients participated in the 8 study centers: 1,138 patients with multiple primary melanoma (52% of those ascertained) and 2,151 patients with single primary melanoma (53% of those ascertained). Only patients with complete information for all variables required for a model were used in each analysis, with resulting slight differences in sample sizes between models. There were similar rates of successful genotyping between cases and controls for both polymorphisms tested: *FokI* 95.4% for SPM, (2006 participants) and 95.1% for MPM (1058); *BsmI* 96.6% for SPM (2030) and 94.5% for MPM (1051). The largest proportions of participants came from New South Wales (53.4% of MPMs and 33.7% of SPMs). Patients with MPM were older than those with SPM (mean age (SD) 65 years (13.2) and 55.6 years (15.9), respectively). Those with MPM were more likely to be male (66.6% versus 51.8%) and have a family history of melanoma (21.9% versus 12.6%) (Table 1). Both *FokI* and *BsmI* were in Hardy-Weinberg equilibrium (Table 2).



There were no significant adjusted associations between *FokI* and risk of MPM, but there was an increased risk for the homozygous variant form of *BsmI* (the BB genotype, odds ratio (OR) 1.27, 95% confidence interval (CI) 0.99-1.62; P for trend 0.11) (Table 2). As might reasonably be expected, the association with the BB genotype was a little stronger when family history was excluded from the model – OR 1.29, 95% CI 1.02-1.63.

Interactions among the three sun exposure variables and *VDR* genotypes were tested separately for each polymorphism. No significant interactions on a multiplicative scale were noted between these sun exposure variables and either *VDR* polymorphism (Table 3). Assessment of the joint effects of genotype and sun exposure on risk of multiple primary melanoma showed that the highest ORs were observed in participants with high sun exposure and BB (the homozygous *BsmI* variant genotype), particularly with the recreational sun exposure measures: waterside recreation (OR for high exposure and BB genotype 1.92, 95% CI 1.30, 2.85) and sunny holidays (1.82, 95% CI 1.22, 2.73) (Table 3). These ORs and the other joint effects ORs were roughly consistent with multiplication of the sun exposure effects by the effects of the B allele. This pattern is not at all evident in the joint effects of sun exposure and *FokI* genotype (Table 3), where only the effects of sun exposure are evident, as would be expected from the lack of any material main effect of *FokI* (Table 2).

For each of the three sun exposure variables, stratum-specific joint effects analyses of the effect of *BsmI* genotype that controlled for age, sex, age-sex interaction, center, family history of melanoma and ability to tan gave similar results to those of the *BsmI* main effect analysis (Table 2), except that there was a slight tendency for the association of the BB genotype to be stronger in the higher sun exposure category than the lower one for each sun exposure variable. Thus for low and high UVE at age 10, the ORs for BB were 1.25 (95% CI 0.88, 1.78) and 1.26 (95% CI 0.92, 1.73) respectively; those for “no” and “any” waterside recreation were 1.19 (95% CI 0.86, 1.65) and 1.42 (95% CI 1.02, 1.98); and those for low and high periods spent in vacations to sunnier places were 1.23 (95% CI 0.84, 1.60) and 1.51 (95% CI 1.04, 2.19). High *P* values for interaction, however, indicate that these small differences could easily be due to chance.

## Discussion

We observed a weak positive association of the homozygous variant *BsmI*, but not the *FokI*, genotype with MPM in this study. In joint effects analyses, risk of MPM tended to increase with increasing sun exposure and increasing number of *BsmI* variant alleles, but not *FokI* variant alleles. The highest ORs were in those with highest sun exposure and BB genotype. The *P* values for the *BsmI* and sun exposure interactions were high indicating lack of interaction on a multiplicative scale; although in stratified analyses there was a greater increase in MPM risk with the homozygous variant *BsmI* genotype in the high sun exposure categories than the low sun exposure categories.

Our results for the main effect of *BsmI* differ from previous studies [18,21,36-37] which were generally null, with one exception [20] which had a small sample size of 101 cases and 101 controls (OR 0.35, 95% CI 0.15, 0.78). Randerson-Moor and colleagues [36] reported an odds ratio of 1.03 (95% CI 0.73, 1.47) for the *BsmI* polymorphism and risk of melanoma among 1026 cases and 402 controls. Li and colleagues [18] reported an association of 0.92 (95% CI 0.71, 1.19) for the association between *BsmI* and melanoma among 805 cases and 841 controls. Han and colleagues [21] reported an OR of 0.88 (95% CI 0.57, 1.36) for risk for melanoma; Gapska and colleagues [37] reported an OR of 0.97 (95% CI 0.7, 1.3) and a second study by Randerson-Moor and colleagues [36] reported an association of 0.69 (95% CI 0.45, 1.06). At the same time, *FokI* in the same studies was not statistically significant,

with the exception of Randerson-Moor and colleagues [36] (OR 1.88, 95% CI 1.25, 2.81). Importantly, no previous study has examined the interaction or joint effects of the *BsmI* variant genotype, or of any other *VDR* variant, with those of any sun exposure variables.

Our study employed a novel design where patients with multiple primary melanoma were compared with patients with single primary melanoma in a manner akin to comparison of cases to controls in a traditional case-control study. The fact that both cases and controls had melanoma in this study probably resulted in less difference in recall accuracy between cases and controls, and therefore a lower probability of bias, than might occur in a classical case-control study of similar design. This study design also has greater power to estimate relative risks for melanoma associated with rare genetic characteristics because the prevalence of these genetic characteristics is likely to be higher in MPM and SPM, respectively, than in the traditional case-control study where single primary cases are compared to population-based controls [38]. Our findings with this design also indicate that it is a reasonable proxy for a traditional case-control design; risk estimates for sun exposure [28] and phenotypic markers [27] obtained for patients with multiple primary melanoma relative to patients with single primary melanoma appear similar to risk estimates for these characteristics obtained for patients with single primary melanoma relative to controls in a recent meta-analysis [3]. The risk estimates found in the present study may therefore be cautiously generalized to risk of a first primary melanoma in previously unaffected people.

That our data are consistent with multiplicative joint effects of the *BsmI* variant genotypes and sun exposure associated with melanoma risk suggests that reduced *VDR* activity and sun exposure are acting independently and multiplicatively to increase melanoma risk. If true, this would be consistent with vitamin D produced by sun exposure reducing the risk of melanoma caused by sun exposure. There is, indeed, evidence that vitamin D produced in the skin acts by way of local autocrine or paracrine effects to protect keratinocytes from UV carcinogenesis [39]. There is as yet though limited direct evidence for any protective effect of vitamin D against melanoma development. Dietary studies have shown inconsistent effects for the role of dietary vitamin D and supplements [40-42]. In addition, three studies evaluating the association of serum vitamin D with melanoma risk have not observed a protective effect for serum vitamin D [36, 43-44]. The possibility of an effect of diet (including supplements) and/or sun exposure in combination with *VDR* polymorphisms on melanoma risk, thus, merits further investigations.

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**Table 1**

Distribution of demographic and host characteristics by multiple primary (MPM) and single primary melanoma (SPM) status in GEM

| Variables                             | SPM (control)<br>n (%) or mean (SD) | MPM (case)<br>n (%) or mean (SD) |
|---------------------------------------|-------------------------------------|----------------------------------|
| <b>Total Sample Size</b>              | N = 2151                            | N = 1138                         |
| <b>Study Centre</b>                   |                                     |                                  |
| British Columbia                      | 118 (5.5)                           | 39 (3.4)                         |
| California                            | 219 (10.2)                          | 89 (7.8)                         |
| New Jersey                            | 167(7.8)                            | 159 (14.0)                       |
| New South Wales                       | 725 (33.7)                          | 608 (53.4)                       |
| North Carolina                        | 285 (13.3)                          | 29 (2.6)                         |
| Ontario                               | 428 (19.9)                          | 134 (11.8)                       |
| Tasmania                              | 81 (3.8)                            | 63 (5.5)                         |
| Italy                                 | 128 (6.0)                           | 17 (1.5)                         |
| <b>Age (continuous)</b>               | 55.6 (15.9)                         | 65.2(13.2)                       |
| <b>Sex</b>                            |                                     |                                  |
| Male                                  | 1115 (51.8)                         | 758(66.6)                        |
| Female                                | 1036 (48.2)                         | 380(33.4)                        |
| <b>Freckles on face</b>               |                                     |                                  |
| None                                  | 957 (44.5)                          | 463(40.7)                        |
| Few                                   | 889 (41.3)                          | 514(45.2)                        |
| Many                                  | 296 (13.8)                          | 156 (13.7)                       |
| Missing                               | 9 (0.4)                             | 5 (0.4)                          |
| <b>Family History of Melanoma</b>     |                                     |                                  |
| No                                    | 1836 85.4)                          | 861 (75.7)                       |
| Yes                                   | 270 (12.6)                          | 249 (21.9)                       |
| Missing                               | 45 (2.1)                            | 28 (2.5)                         |
| <b>Skin colour</b>                    |                                     |                                  |
| Fair (Type 1or 2)                     | 1858 (86.4)                         | 1048 (92.1)                      |
| Dark (Types 3-6)                      | 292(13.6)                           | 88 ( 7.7)                        |
|                                       | 1 (0.5)                             | 2 (0.2)                          |
| <b>Eye colour</b>                     |                                     |                                  |
| Dark Eyes (brown, black)              | 409 (19.0)                          | 189 (16.6)                       |
| Light Eyes (blue, grey, green, hazel) | 1728 (80.3)                         | 947 (83.2)                       |
| Missing                               | 14 (0.7)                            | 2 (0.2)                          |
| <b>Hair colour</b>                    |                                     |                                  |
| Dark brown/black                      | 687 (31.9)                          | 297(26.1)                        |
| Light brown/blonde                    | 1261 (58.6)                         | 709 (62.3)                       |
| Red                                   | 186 (8.7)                           | 129 (11.3)                       |
| Missing                               | 17 (0.8)                            | 3 (0.3)                          |
| <b>Ability to tan</b>                 |                                     |                                  |

| Variables                               | SPM (control)      | MPM (case)         |
|---|--------------------|--------------------|
|   | n (%) or mean (SD) | n (%) or mean (SD) |
| Deep tan                                | 363 (16.9)         | 128 (11.3)         |
| Moderate tan                            | 867 (40.3)         | 456 (40.1)         |
| Mild tan                                | 635 (29.5)         | 349 (30.7)         |
| No tan                                  | 237(11.0)          | 180 (15.8)         |
| Missing                                 | 49 (2.3)           | 25 (2.2)           |
| <b>Number of nevi on back (counted)</b> |                    |                    |
| 0                                       | 337(15.7)          | 157 (13.8)         |
| 1-10                                    | 918 (42.7)         | 464 (40.8)         |
| 11-25                                   | 554 (25.8)         | 302 (26.5)         |
| 26-50                                   | 142 (6.6)          | 91(8.0)            |
| 50+                                     | 161 (7.5)          | 94 (8.3)           |
| Missing                                 | 39 (1.8)           | 30 (2.6)           |

**Table 2**

Genotype frequencies, allele frequencies and odds ratios for VDR polymorphisms\*

| SNP                | SPM<br>N (%) | MPM<br>N (%) | Odds ratio* (95% CI) | P-value<br>(trend) |
|--------------------|--------------|--------------|----------------------|--------------------|
| FokI <sup>†</sup>  |              |              |                      |                    |
| FF                 | 747 (37)     | 395 (37)     | ref                  |                    |
| Ff                 | 956 (48)     | 499 (47)     | 0.97 (0.81-1.15)     |                    |
| ff                 | 303 (15)     | 164 (16)     | 1.00 (0.78-1.29)     | 0.91               |
| Allele proportions |              |              |                      |                    |
| F                  | 0.61         | 0.61         |                      |                    |
| f                  | 0.39         | 0.39         |                      |                    |
| HWE P-value        | 0.92         | 0.76         |                      |                    |
| BsmI <sup>†</sup>  |              |              |                      |                    |
| bb                 | 751 (37)     | 358 (34)     | ref                  |                    |
| Bb                 | 965 (48)     | 493 (47)     | 1.07 (0.89-1.29)     |                    |
| BB                 | 314 (15)     | 200 (19)     | 1.27 (0.99-1.62)     | 0.11               |
| Allele proportions |              |              |                      |                    |
| b                  | 0.61         | 0.58         |                      |                    |
| B                  | 0.38         | 0.42         |                      |                    |
| HWE P-value        | 0.89         | 0.19         |                      |                    |

\* Adjusted for age (continuous), sex, age\*sex, center, ability to tan, family history of melanoma



Table 3

Main effects of sun exposure and joint effects of sun exposure and VDR polymorphisms\*

|   | SPM  | MPM | OR   | 95% CI     | p-value | BsmI | SPM | MPM | OR   | 95% CI     | FokI | SPM | MPM | OR   | 95% CI      |      |
|---|------|-----|------|------------|---------|------|-----|-----|------|------------|------|-----|-----|------|-------------|------|
| <b>UVE10<sup>†</sup></b>                |      |     |      |            |         |      |     |     |      |            |      |     |     |      |             |      |
| Low                                     | 1109 | 437 | 1.00 | Ref        |         | bb   | 397 | 150 | 1.00 |            | FF   | 401 | 142 | 1.00 |             |      |
|   |      |     |      |            |         | bb   | 527 | 201 | 0.99 | 0.76, 1.31 | Ff   | 498 | 212 | 1.15 | 0.87, 1.52  |      |
|   |      |     |      |            |         | BB   | 154 | 74  | 1.34 | 0.93, 1.94 | ff   | 162 | 66  | 1.20 | 0.82, 1.76  |      |
| High                                    | 937  | 650 | 1.37 | 1.01, 1.84 | 0.02    | bb   | 315 | 194 | 1.22 | 0.83, 1.79 | FF   | 319 | 233 | 1.53 | 1.05, 2.23  |      |
|   |      |     |      |            |         | bb   | 404 | 277 | 1.39 | 0.96, 2.02 | Ff   | 416 | 267 | 1.33 | 0.92, 1.92  |      |
|   |      |     |      |            |         | BB   | 151 | 117 | 1.47 | 0.97, 2.22 | ff   | 130 | 96  | 1.50 | 0.97, 2.33  |      |
| <i>P</i> for interaction                |      |     |      |            |         |      |     |     |      |            |      |     |     |      |             | 0.27 |
| <b>Waterside Recreation<sup>†</sup></b> |      |     |      |            |         |      |     |     |      |            |      |     |     |      |             |      |
| <b>n<sup>†</sup></b>                    |      |     |      |            |         |      |     |     |      |            |      |     |     |      |             |      |
| None                                    | 511  | 204 | 1.0  | Ref        |         | bb   | 175 | 63  | 1.00 |            | FF   | 177 | 72  | 1.00 |             |      |
|   |      |     |      |            |         | bb   | 232 | 92  | 1.00 | 0.67, 1.51 | Ff   | 235 | 93  | 0.83 | 0.55, 1.23  |      |
|   |      |     |      |            |         | BB   | 92  | 41  | 1.14 | 0.68, 1.89 | ff   | 83  | 26  | 0.74 | 0.42, 1.29  |      |
| Any                                     | 1533 | 881 | 1.54 | 1.25, 1.89 | <0.0001 | bb   | 556 | 288 | 1.42 | 0.99, 2.02 | FF   | 554 | 317 | 1.35 | 10.96, 1.89 |      |
|   |      |     |      |            |         | bb   | 715 | 395 | 1.56 | 1.10, 2.21 | Ff   | 698 | 396 | 1.36 | 0.97, 1.89  |      |
|   |      |     |      |            |         | BB   | 217 | 155 | 1.92 | 1.30, 2.85 | ff   | 214 | 135 | 1.53 | 1.04, 2.26  |      |
| <i>P</i> for interaction                |      |     |      |            |         |      |     |     |      |            |      |     |     |      |             | 0.61 |
| <b>Sunny Holidays<sup>†</sup></b>       |      |     |      |            |         |      |     |     |      |            |      |     |     |      |             |      |
| Low                                     | 881  | 386 | 1.0  | Ref        |         | bb   | 299 | 127 | 1.00 |            | FF   | 314 | 127 | 1.00 |             |      |
|   |      |     |      |            |         | bb   | 715 | 395 | 1.11 | 0.82, 1.49 | Ff   | 388 | 169 | 1.08 | 0.79, 1.46  |      |
|   |      |     |      |            |         | BB   | 217 | 155 | 1.21 | 0.8, 1.80  | ff   | 125 | 62  | 1.15 | 0.77, 1.73  |      |
| High                                    | 860  | 440 | 1.33 | 1.10, 1.61 | <0.005  | bb   | 327 | 137 | 1.21 | 0.88, 1.67 | FF   | 301 | 163 | 1.48 | 1.08, 2.03  |      |
|   |      |     |      |            |         | bb   | 376 | 199 | 1.52 | 1.13, 2.06 | Ff   | 387 | 198 | 1.33 | 0.99, 1.79  |      |
|   |      |     |      |            |         | BB   | 117 | 77  | 1.82 | 1.22, 2.73 | ff   | 121 | 54  | 1.36 | 0.89, 2.08  |      |

|                          | SPM | MPM | OR | 95% CI | p-value | BsmI | SPM | MPM | OR | 95% CI | FokI | SPM | MPM | OR | 95% CI |
|--------------------------|-----|-----|----|--------|---------|------|-----|-----|----|--------|------|-----|-----|----|--------|
| <i>P</i> for interaction |     |     |    |        |         |      |     |     |    |        |      |     |     |    |        |
|                          |     |     |    |        |         |      |     |     |    | 0.41   |      |     |     |    |        |

0.32

\* Adjusted for age (continuous), sex, age\*sex, center, ability to tan and family history of melanoma