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Haplotype and Genotypes of the *VDR* Gene and Cutaneous Melanoma Risk in Non-Hispanic Whites in Texas: A Case-Control Study

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Abstract

In a hospital-based case-control study of 805 non-Hispanic whites with cutaneous melanoma and 841cancer-free age-, sex- and ethnicity-matched control subjects, three *VDR* polymorphisms (i.e., *Taq*I, *Bsm*I, and *Fok*I) were genotyped using blood samples collected between 1994 and 2006. We tested the hypothesis that the haplotypes and combined genotypes of these polymorphisms were associated with melanoma risk by interacting with known risk factors. Haplotypes *t-B-F* (adjusted odds ratio [OR], 0.52; 95 percent confidence interval [CI], 0.34–0.80) and *t-B-f* (adjusted OR, 0.51; CI, 0.27–0.94) were associated with a reduced risk when compared with *T-b-f*. The combined genotypes *Tt+tt/Bb+BB/Ff+ff* (adjusted OR, 0.69; CI, 0.52, 0.90) and *Tt+tt/Bb+BB/FF* (adjusted OR, 0.58; CI, 0.43, 0.78) were also associated with reduced risk, whereas the combined genotype *TT/Bb+BB/Ff+ff* genotype (adjusted OR, 2.35; CI, 1.13, 4.98) was associated with increased risk when compared with *TT/bb/Ff+ff* genotypes. On multivariate analysis, only the *Taq*I polymorphism was an independent risk factor, while the *Fok*I polymorphism interacted with skin color ($p = 0.029$), moles ($p = 0.017$), and first-degree relatives with any cancer ($p = 0.013$) in modifying risk. Together, these findings suggest that *VDR* polymorphisms may directly effect or modify the risk associated with known melanoma risk factors. Larger, population-based studies are needed to replicate our findings.

Keywords

case-control studies; vitamin D receptor; genetic polymorphisms; genotypes; melanoma

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Interaction

Vitamin D is involved in a variety of biological processes including bone metabolism, immunomodulation, and regulation of cell proliferation and differentiation [1–4]. Vitamin D is also known to have a potential protective effect against cancers, including cutaneous melanoma [5, 6], a lethal skin cancer that has an increasing incidence in the United States over the last 30 years [7]. Vitamin D exerts its tumor-suppressive effects by binding to the vitamin D receptor (VDR). A ubiquitously expressed intracellular polypeptide that belongs to the steroid/retinoid receptor superfamily of nuclear receptors, VDR specifically binds to $1,25_2D_3$ and interacts with target cell nuclei [8]. The VDR protein is overexpressed in malignant melanocytes responsive to vitamin D's antiproliferative effects [2]. Several studies have suggested that *VDR* polymorphisms may alter the functions of genes involved in cell division and adhesion [2, 9], thus implicating such polymorphisms in melanoma development [10].

Located on chromosome 12q12-q14, *VDR* contains at least five promoter regions [11], eight protein-coding exons, and six untranslated exons, all of these regions bejng alternatively spliced [12]. *VDR* at least has 196 single nucleotide polymorphisms (SNPs) (http://egp.gs.washington.edu/data/vdr/vdrxx.csnps.txt), of which 64 lie in the promoter region, 32 in the 3′ and 5′ untranslated regions, and 2 synonymous and 2 nonsynonymous SNPs in the coding region. *Fok*I (exon 2, rs10735810), *Bsm*I (intron 8, rs1544410), and *Taq*I (exon 9, rs731236) are the three most frequently investigated SNPs for their associations with various cancers [13–15]. Some studies also addressed gene-environment interactions since environmental factors (e.g., sunlight) can influence vitamin D production [16].

Although ultraviolet light plays an important role in melanoma development [17, 18], only three studies to date have assessed the associations between this factor, *VDR* polymorphisms, and melanoma risk. Moreover, no studies have examined whether *VDR* polymorphisms modulate the risk associated with other established melanoma risk factors [19–21]. Therefore, we conducted a relatively large case-control study of non-Hispanic whites (i.e., 805 patients with melanoma and 841 cancer-free controls) to determine whether the haplotypes and combined genotypes of *VDR* polymorphisms *Taq*I, *Bsm*I, and *Fok*I are associated with melanoma risk and whether these polymorphisms can modify the risk associated with known melanoma risk factors.

MATERIALS AND METHODS

Study subjects

The study protocol was approved by The University of Texas M. D. Anderson Cancer Center institutional review board, and written informed consent was obtained from all subjects. The subject recruitment has been described previously [20, 22, 23]. In brief, all patients with newly diagnosed, histologically confirmed [24] and untreated cutaneous melanoma, who were referred to M. D. Anderson Cancer Center between May 1994 and February 2006, were recruited as case subjects. Because most of the patients (~99 percent) were non-Hispanic whites, the few minority subjects who were recruited were excluded from analysis. Although there were no restrictions on patient age or tumor stage, only those patients who were free of metastases or other cancers and agreed to donate a blood sample were included in the present study. Approximately 85 percent of eligible patients recruited for this study agreed to participate. Cancer-free control subjects were recruited during the same period from among cancer-free visitors to M. D. Anderson Cancer Center who were accompanying patients to our outpatient clinics, were not seeking medical care, and were not related by blood to the patients. Approximately 90 percent of eligible control subjects recruited for this study agreed to participate. The control subjects were matched by frequency to case subjects by age (±5 years), sex, and ethnicity.

After giving informed consent, all subjects completed a self-administered questionnaire that elicited information on demographic factors (e.g., age, sex, education, and income), ethnicity, medical history, family history, and sunlight exposure history (i.e., tanning ability, lifetime number of severe sunburns, and freckling in the sun as a child) [25]. Then, each subject was interviewed in person to assess his or her host characteristics (e.g., natural hair, eye, and skin color) as well as self-reported skin conditions (e.g., color, moles, and pigmented nevi). After each interview, a sample of blood (30 mL) was drawn from the subject and collected in a heparinized tube.

Genotyping

Genotyping was performed as follows. First, 1 mL of each whole blood sample was centrifuged to isolate a leukocyte cell pellet from the buffy coat fraction. Genomic DNA was extracted from the pellet, purified using a DNA blood mini kit (Qiagen, Valencia, CA), and assayed for purity and concentration by spectrophotometry (i.e., absorbance at 260 nm and 280 nm). Next, DNA fragments of *VDR* containing the *Taq*I [26], *Bsm*I [27] and *Fok*I [10, 19] polymorphisms were amplified by polymerase chain reaction, subjected to restriction fragment length polymorphism analysis, and sequenced (Figure I). Approximately 10 percent of samples were genotyped a second time; the repeat genotyping results agreed completely with the initial results.

Statistical methods

The χ^2 test was used to evaluate case-control differences in the frequency distributions of selected demographic variables, known risk factors, and each allele and genotype of the *VDR* polymorphisms. Because skin color was self-assessed on the screening questionnaire on a scale of 1 (light) to 10 (dark), skin colors were categorized as fair (1 or 2), brown (3 or 4), , or dark (5–10); the aim was to obtain similar numbers of observations in each stratum to facilitate further stratification analysis. Some subjects did not provide information about some variables (e.g., hair color, eye color, skin color, tanning ability, number of sunburns, freckling, pigmented nevi, and family history of skin cancer); the missing variables for those subjects were treated as missing data on multivariate analysis. The linkage disequilibrium for each SNP of interest (i.e., *Taq*I, *Bsm*I, and *Fok*I) was calculated, and the polymorphism haplotypes for each subject were reconstructed on the basis of the known *Taq*I, *Bsm*I, and *Fok*I genotypes. Because of the potential effect of locus-locus interactions of the polymorphisms on melanoma risk, associations between risk and the haplotypes and combined genotypes of the three polymorphisms were also evaluated.

Crude and adjusted odds ratios [ORs] and associated 95 percent confidence intervals (CIs) were determined by univariate and multivariate unconditional logistic regression analyses. Multivariate adjustments were made, where appropriate, for age, sex, and other known risk factors. Odds ratios, CIs, and *p* values for interactions and trend tests were obtained from multivariate logistic regression models.

The null hypotheses of multiplicative gene-gene interactions were tested, and departures from multiplicative interaction models were assessed empirically. A more-than-multiplicative interaction was suggested when $OR_{11} > OR_{10} * OR_{01}$ [28]. To assess potential departures from a multiplicative model, interaction terms between variables were modeled according to standard unconditional logistic regression techniques. Finally, to determine whether the main effect of the *VDR* polymorphisms was independent of other known risk factors, selected variables were included in the multivariate logistic regression analyses of data from only those subjects who completely answered their questionnaires [29].

Two models were fitted. The first model included age, sex, and the three polymorphisms of interest, the aim being to control for any potential effects due to associations among the

polymorphisms. The second model was to exclude the polymorphism that showed no statistically significant association with risk in the first model and then include all other known risk factors, the aim being to assess further the independent effects of the polymorphisms. A p value of ≤ 0.05 was considered statistically significant. All tests were two-sided and were performed using SAS software (version 9.13; SAS Institute, Cary, NC).

RESULTS

Population characteristics and risk factors

The initial analysis included all cases (n=805) and controls (n=841). The two groups had similar age ($p = 0.37$), sex ($p = 0.16$), education ($p = 0.99$), and household income ($p = 0.35$) (Table I). The similar age and sex distributions implied adequate frequency matching. Because some subjects did not completely answer their questionnaires, the numbers of subjects in some risk factor strata were less than the total number of subjects in the study. Nevertheless, our results were consistent with previous findings by others [30–32]. Except for skin color ($p = 0.16$), the frequencies of known melanoma risk factors were significantly higher among cases than among controls and were associated with 1.55- to 7.78-fold increased melanoma risk (Table II). Subjects with these risk factors were placed into dichotomized groupings for further stratification and assessment of interactions in multivariate logistic regression analyses.

VDR allele and genotype distributions and association with melanoma risk

Allele and genotype frequencies of the polymorphisms of interest are presented in Table III. Genotype distributions among controls were consistent with the Hardy-Weinberg equilibrium ($p = 0.49$ for *Taq*I, $p = 0.31$ for *Bsm*I, and $p = 0.64$ for *Fok*I). *Taq*I alleles *t* and *Bsm*I alleles *B* were significantly less frequent among cases than among controls (0.370 vs. 0.429 [$p < 0.01$] and 0.394 vs. 0.431 [$p = 0.03$], respectively), whereas the *Fok*I allele *f* was more frequent, though not significantly so (0.378 vs. 0.356 [$p = 0.20$]). This suggested that *t*, *B*, and *F* might protect carriers against melanoma or *T*, *b*, and *f* might put them at risk. Moreover, the *t* and *B* genotypes (i.e., *Tt*+*tt* and *Bb*+*BB*) were consistently less frequent among cases than among controls ($p < 0.05$ for both) and were associated with a significantly lower melanoma risk (i.e., a protective effect) for *Tt*+*tt* vs. *TT* genotypes (adjusted OR [CI], 0.72 [0.58, 0.90] and 0.68, [0.56, 0.83] and *Bb*+*BB* vs. *bb* genotypes, respectively (Table III). In contrast, the *f* genotypes (i.e., *ff*+*Ff*) were significantly more frequent among cases than among controls and were associated with a significantly greater melanoma risk than was the *FF* genotype (adjusted OR [CI], 1.25 [1.03, 1.53]) (Table III).

Association between *VDR* haplotypes or combined genotypes and melanoma risk

*Taq*I, *Bsm*I, and *Fok*I polymorphisms were in linkage disequilibrium (*t* and *B* alleles: $D' = 0.918$, $R^2 = 0.855$, $p < 0.001$; *t* and *F* alleles: $D' = 0.039$, $R^2 = 0.001$, $p < 0.001$; *B* and *F* alleles: $D' = 0.027$, $R^2 = 0.001$, $p < 0.001$), suggesting a potentially joint effect of the haplotypes of the three *VDR* polymorphisms on melanoma risk. Eight hypothetical haplotypes were estimated based on the observed genotypes (Table IV). However, the overall distributions of these haplotypes did not significantly differ between cases and controls ($p = 0.381$). When the *Tbf* haplotype was used as the referent (the *T*, *b*, and *f* alleles being putatively associated with increased melanoma risk), the haplotypes *tBF* and *tBf* were both associated with a significantly reduced melanoma risk (adjusted OR [CI], 0.52 [0.33, 0.79]) and (0.51 [0.27, 0.94], respectively) (Table IV). This suggested that the *tB* haplotype was protective, regardless of the *f* allele's presence or absence.

When the putative risk genotypes (i.e., *TT*, *bb*, and *ff*+*Ff*) were combined and used as the referent (*TT/bb/ff*+*Ff*), only the *Tt*+*tt/Bb*+*BB/Ff*+*ff* and *Tt*+*tt/Bb*+*BB/FF* genotypes were associated with a significantly reduced melanoma risk (adjusted OR [CI], 0.69 [0.52, 0.90] and

0.58 [0.43, 0.78], respectively), whereas the *TT/Bb+BB/Ff+ff* genotype was associated with a significantly increased risk (adjusted OR [CI], 2.35 [1.13, 4.98]). Together, these findings suggested that the *Tt+tt/Bb+BB* genotypes were protective, consistent with the effect of the *tB* haplotype, and that the *Bb+BB* genotypes were not protective in the presence of the *TT* genotype (Table IV).

Association between melanoma risk and polymorphism genotypes stratified by known risk factors

Because the *Fok*I variants were associated with increased melanoma risk and the *Taq*I and *Bsm*I variants with reduced risk, subjects bearing the protective *Taq*I and *Bsm*I variant genotypes were further stratified by the *Ff+ff* and *FF* genotypes and all known melanoma risk factors (Table V). In the *Ff+ff* subgroup, the *Tt+tt* genotypes were associated with a significantly lower risk of melanoma than was the *TT* genotype, provided the carriers of the *Tt+tt* genotypes were old, male, and blue-eyed; had not freckled in the sun as a child; or had no pigmented nevi. In contrast, the protective *Bb+BB* genotypes were associated with a significantly lower risk than was the *bb* genotype only if the carriers were old (Table V).

In the *FF* subgroup, the *Tt+tt* genotypes were more likely to be associated with reduced risk in carriers who were young, female, black- or brown-skinned, black- or brown-haired, or non-blue-eyed; had poor tanning ability, had ≥ 1 lifetime sunburn with blistering, had a childhood history of freckling in the sun, had no moles or pigmented nevi, or had no family history of cancer. The same was generally true of the *Bb+BB* genotypes, except that being young was not a risk factor. Further tests for interaction were significant for age ($p = 0.03$ for *Taq*I and $p = 0.01$ for *Bsm*I) among subjects carrying the *Ff+ff* genotype and for sex ($p = 0.05$ for *Bsm*I) and hair color ($p = 0.05$ for *Taq*I and $p = 0.03$ for *Bsm*I) among subjects carrying the *FF* genotype. However, these findings may have been due to chance since multiple tests were performed.

Multivariate analysis of association between polymorphisms and melanoma risk

All variables used in the initial analyses were fitted to two multivariate unconditional logistic models after simultaneous adjustment (Table VI). In the first model, which included the age, sex, and polymorphism genotypes for all subjects, the *t* genotypes (*Tt+tt* vs. *TT*) and *f* genotypes (*Ff+ff* vs. *FF*), but not the *B* genotypes (*Bb+BB* vs. *bb*), were associated with a significantly reduced melanoma risk (OR [CI], 0.57 [0.39, 0.84] for *Taq*I and 1.27 [1.04, 1.55] for *Fok*I). This suggested that the *Bsm*I polymorphism was not an independent melanoma risk factor, consistent with the high linkage disequilibrium between the *t* and *B* alleles. Consequently, *Bsm*I was excluded from the second model, and all other selected risk factors were added to the multivariate logistic regression model. The second model included only data from subjects who provided complete questionnaire data (i.e., 712 case subjects and 707 control subjects). Most of the known risk factors were consequently found to be significant independent predictors of melanoma risk, the exceptions being age, sex, skin color, and childhood freckling. Because skin color may be represented by hair or eye color and freckling by the number of sunburns in the same model, and because there was a high correlation between these variables in our study (data not shown), variance in the model was reduced by excluding skin color and freckling from the final model (Table VI). As a result, the *VDR Taq*I *t* variant genotypes assessed in the final model were associated with a significantly reduced melanoma risk (OR [CI], 0.68 [0.54, 0.86]), whereas the *Fok*I *f* variant genotypes were not (1.14 [0.91–1.44]) (Table VI). Further tests for interaction revealed significant associations between the *Fok*I *f* genotypes and skin color ($p = 0.029$), moles ($p = 0.017$), and a family history of cancer ($p = 0.013$) but not between the *Taq*I *t* genotypes and the same variables (data not shown). Since multiple tests were performed, these interactions are only suggestive and require validation in larger future studies.

DISCUSSION

In this hospital-based case-control study of cutaneous melanoma, we found that *Taq*I *t* and *Bsm*I *B* variant genotypes of the *VDR* gene (*Tt*+*tt* and *Bb*+*BB*, respectively) were associated with a reduced risk of melanoma and *Fok*I *f* variant genotypes (*Ff* and *Ff*+*ff*) with an increased risk when compared with the *TT*, *bb*, and *FF* genotypes, respectively. The *tBF* and *tBf* haplotypes were associated with a significantly lower melanoma risk than was the *Tbf* haplotype. The *VDR Fok*I polymorphisms appeared to interact with other known risk factors to modulate the melanoma risk associated with those factors, while the *VDR Taq*I polymorphism appeared to exert its protective (i.e., risk-reducing) effect independently of other risk factors.

The VDR protein is expressed in both melanocytes and melanoma cells, and 1,25-$[OH]_2D_3$ can apparently inhibit the growth of both normal and malignant melanocytes *in vitro* [2, 5, 33]. However, malignant transformation may inhibit the anticancer actions of $1,25_2D_3$ for reasons that include genetic polymorphisms of the *VDR* gene [34]. The *VDR* gene comprises nine exons harboring several polymorphisms, including a poly-A microsatellite in the 3′ flanking region [35], changes in intron 8 that generate *Bsm*I [36] and *Apa*I restriction enzyme sites [37], a synonymous change at codon 352 in exon 9 that generates a *Taq*I restriction enzyme site [38], and a 5′ FokI site in exon 2 [8]. No apparent association has been found between the *Taq*I or *Bsm*1 polymorphisms and altered functional activities. Nevertheless, both of *Taq*I and *Bsm*I polymorphisms located near to the 3′ end of the gene, thus are thought to affect mRNA stability and *VDR* gene transcription regulation [39]. Among the VDR polymorphisms, the *Fok*I singlenucleotide polymorphism of the translation start site is the only one that results in a VDR protein with a different structure[40]. This polymorphism is characterized by the presence of either two ATG start codons separated by six nucleotides in the long f-VDR or only one start codon due to a T-to-C substitution in the most 50 ATG codon, resulting in a 3-aa shorter F-VDR protein (424 aa in stead of 427 aa)[41].

To date, two other groups have reported their studies on *Taq*I, *Bsm*I, or *Fok*I polymorphisms in evaluating their association with melanoma risk [19–21] but generated mixed results. In the earliest study of the *Taq*I polymorphism in 316 melanoma patients and 108 control subjects, neither the *Tt* nor the combined *Tt*+*tt* genotype was associated with altered melanoma risk when compared with the *TT* genotype [19]. However, in the present large study, we found that the *t* genotypes were in fact associated with a lower melanoma risk. In the Nurses' Health Study, investigators examined the association between melanoma risk and *Bsm*I polymorphism in 219 melanoma patients and 873 controls and found no association between the two[21]. However, in our present study, we found an association between *Bb*+*BB* genotypes and reduced melanoma risk only in women who carried the *FF* genotype and not in men (Table V). We found the *Ff* and *Ff*+*ff* genotypes to be associated with increased melanoma risk. Interestingly, even though this finding was consistent with published data from other group [19], it was not consistent with the finding in the Nurses' Health Study of an association (though not significant) between only the *ff* genotype and higher melanoma risk[21].

There are several possible reasons for the apparent discrepancy between our results and those reported by others. One is the relatively larger size of our control population, and another is the potential for selection bias in our control population. Our present study, with its 805 melanoma cases and 841 control subjects, is the largest study so far to have addressed the possible association between *VDR* polymorphism and melanoma risk; in addition, the *VDR t*, *B*, and *f* allele frequencies we report here are similar to those reported previously in a large meta-analysis of studies in whites [14]. Therefore, we believe it unlikely that the association between the *VDR* polymorphisms and melanoma risk demonstrated in the present study was biased by our selection of controls. A third possible reason for the discrepancy between our

findings and those previously reported is variations in the serum vitamin D levels of study subjects between studies. Indeed, the serum vitamin D level may have affected our results. Unfortunately, none of the studies published so far gathered data on serum vitamin D levels in their subjects. A fourth possible reason is recall biases in exposure data, to which a retrospective study might be prone. Thus, larger, population-based studies are needed to verify our findings.

The functional significance of the VDR TaqI polymorphisms is unknown. As a synonymous change in exon 9, the *Taq*I polymorphism does not cause the amio acid substitution (http://egp.gs.washington.edu/data/vdr/vdrxx.csnps.txt; http://snp500cancer.nci.nih.gov/snplist.cfm). In addition, no apparent association has been found between the *Bsm*I polymorphism and altered functional activities[42]. Nevertheless, these polymorphisms might be functional themselves or in linkage disequilibrium with other functional SNPs and associated with melanoma risk. Indeed, previous in vitro functional studies have revealed the *baT* haplotype (haplotype of *Bsm1/ApaI/TaqI*) inserted in transfection constructs resulted in lower reporter gene activity compared with *BAt* [43] and associated with low VDR mRNA expression [44], which is in agreement with our findings that both of the *Bsm*I *B* allele and *Taq*I *t* allele are protective against melanoma in the present study. VDR *Fok*I is the only polymorphism that is not linked to any of the other VDR polymorphisms[41]. A study recently provided evidence that the VDR *Fok*I polymorphism affects immune cell behavior, with a more active immune system for the short F-VDR[45]. Consistent with this functional study, we found *Ff+ff* genotype associated with significantly increased melanoma risk, which might due to the *f* allele-related reduced anti-tumor immune activity. However, since some in vitro data may not accurately reflect the biologic environment, in which a marker may be acting in humans, our consideration regarding the putative function of VDR polymorphisms should be adequately validated in further functional studies.

As some epidemiologic studies have suggested, adequate vitamin D levels (including sunlight induced) may provide very important protection against colon, breast, and prostate cancers [46–48]. However, its protection against skin cancer is a more complex issue. One potential complication is that ultraviolet light exposure not only promotes vitamin D-3 (cholecalciferol) synthesis in the skin but also increases the risk of skin cancer by inducing DNA damage. Therefore, it is very important to consider gene-environment interactions as well as locus-locus interactions when studying associations between *VDR* polymorphisms and melanoma risk. For example, one recent case-only analysis study revealed an association between the *Taq*I *tt* genotype and reduced prostate cancer risk, but only in association with high sun exposure [16]. However, in the present study, we found that the *Taq*I *t* variant genotypes exerted their protective effects independently of other genotypes and known risk factors. Meanwhile, the effect of *Fok*I genotypes on melanoma risk appeared to be independent of the *Taq*I polymorphism but dependent on other known risk factors, suggesting that some environmental modification of the *VDR* gene may have occurred. Indeed, we found that the *Fok*I polymorphism interacted with the known melanoma risk factors of skin color, moles, and family history of cancer. However, because of our study's limited size and current uncertainty about the biological mechanisms underlying such interactions, these findings are only suggestive. Again, larger, population-based, and functional studies are necessary to validate these interactions.

The present study has several limitations. First, it was a hospital-based case-control study in which selection of the unrepresentive population and retrospective collection of exposure data may have led to uncontrolled biases. Second, despite being the largest study of its kind ever published, the present study was still too underpowered to detect gene-gene or gene-environment interactions. Third, the self-reporting of skin conditions by both case and control subjects created an additional source of potential bias. Finally, like most previous studies on the subject, ours could not account for serum vitamin D and thus did not allow for genotype-

phenotype correlation analysis. These limitations can only be overcome in large, well-designed prospective studies that gather data on both genotypes and phenotypes of vitamin D metabolism.

In summary, the *VDR Taq*I, *Bsm*I, and *Fok*I polymorphisms and their combined variant genotypes do affect melanoma risk. The *VDR Taq*I polymorphism alters risk independently of *Bsm*I, *Fok*I, and other known melanoma risk factors, while the *VDR Fok*I polymorphism may modify it through interaction with sun exposure-related melanoma risk factors. Larger, population-based studies are needed to confirm these findings.

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Abbreviations

CI	95 percent confidence interval
OR	odds ratio
SNP	single nucleotide polymorphism
VDR	vitamin D receptor

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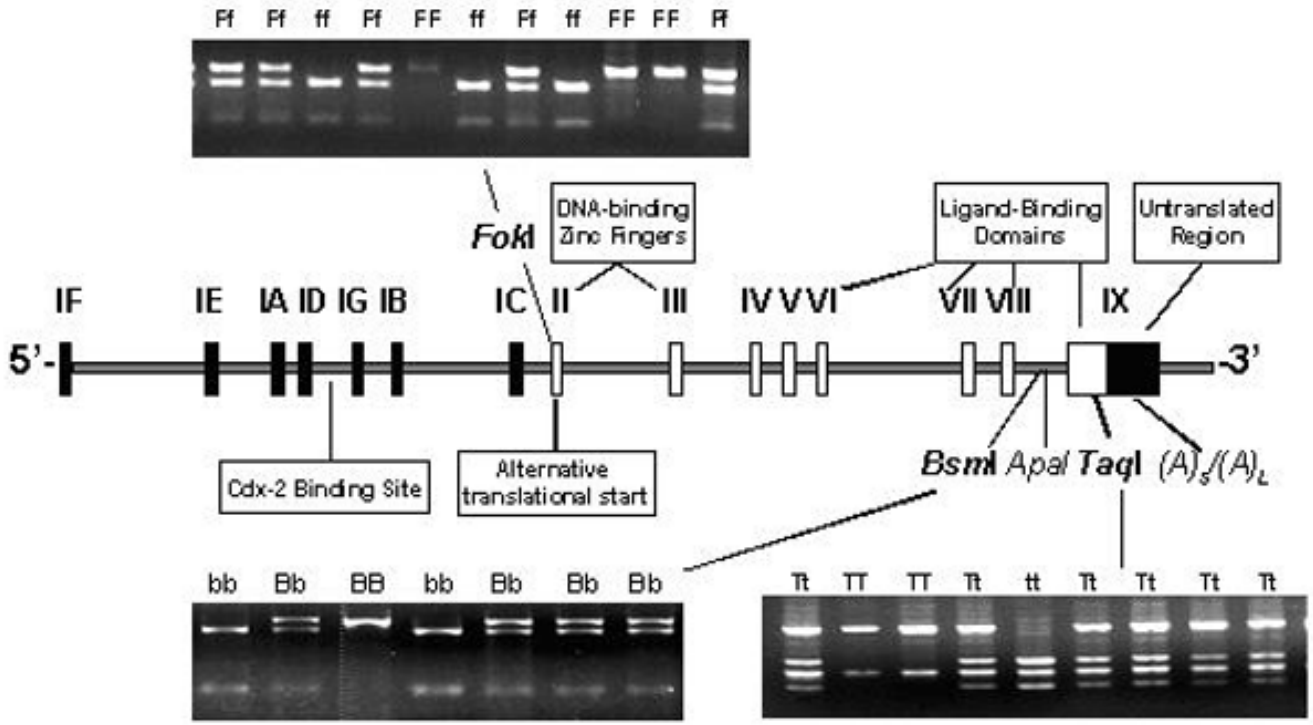

Figure I.

VDR gene structure and locations and genotypes of selected polymorphisms.

Table I

Demographic distributions of non-Hispanic whites in a hospital-based case-control study of cutaneous melanoma, Texas, 1994–2006

	Cases (n = 805)		Controls (n = 841)		
Variables*	**No.**	**%**	**No.**	**%**	***p*†**
Age (years)					0.36
<39	123	15.3	121	14.4	
39–50	195	24.2	189	22.5	
51–60	258	32.0	258	30.7	
>60	229	28.5	273	32.4	
Sex					0.16
Male	514	63.8	565	67.2	
Female	291	36.2	276	32.8	
Education (years)					0.99
≤14	239	32.7	235	32.7	
>14	493	67.4	484	67.3	
Household income (yearly)					0.35
<$35,000	144	20.3	127	18.4	
≥$35,000	564	79.7	564	81.6	

*The numbers of subjects in some of the strata were less than the total number of subjects included in this study because some subjects did not provide complete information in their screening questionnaires.

†Two-sided χ^2 test.

Table II

Age and sex adjusted odds ratios for selected cutaneous melanoma risk factors, Texas, 1994–2006

	Cases* (n = 805)		Controls* (n = 841)			Odds ratio and 95% confidence interval			
Variable	**No.**	**%**	**No.**	**%**	***p*†**	**Crude odds ratio**	**95 percent confidence interval**	**Adjusted odds ratio‡**	**95 percent confidence interval**
Hair color					<0.01				
Black or brown	483	66.0	579	80.9		1.0	Referent	1.0	Referent
Blond(e) or red	249	34.0	137	19.1		2.18	1.71, 2.77	2.15	1.69, 2.74
Eye color					<0.01				
Other	424	58.0	534	74.4		1.0	Referent	1.0	Referent
Blue	307	42.0	184	25.6		2.10	1.68, 2.63	2.09	1.68, 2.62
Skin color					0.16				
Black or brown	328	44.8	347	48.1		1.0	Referent	1.0	Referent
Fair	404	55.2	374	51.9		1.14	0.93, 1.41	1.13	0.92, 1.40
Tanning ability					<0.01				
Good	173	23.6	267	37.1		1.0	Referent	1.0	Referent
Moderate or poor	559	76.4	453	62.9		1.91	1.52, 2.40	1.90	1.51, 2.39
Lifetime sunburn with blistering					<0.01				
None	191	26.4	296	45.5		1.0	Referent	1.0	Referent
≥1 time	533	73.6	421	54.5		1.96	1.57, 2.45	1.95	1.56, 2.45
Freckling in the sun as a child					<0.01				
No	343	47.1	421	58.6		1.0	Referent	1.0	Referent
Yes	385	52.9	297	41.4		1.59	1.29, 1.96	1.55	1.25, 1.91
Moles					<0.01				
No	175	21.7	289	34.4		1.0	Referent	1.0	Referent
Yes	630	78.3	552	65.6		1.89	1.51, 2.35	1.88	1.51, 2.34
Atypical nevi					<0.01				
No	740	91.9	832	98.9		1.0	referent	1.0	referent
Yes	65	8.1	9	1.1		8.12	4.02, 16.4	7.78	3.84, 15.8
First–degree relatives with any cancer					<0.01				
No	343	42.6	431	51.3		1.0	Referent	1.0	Referent
Yes	461	57.4	410	48.7		1.42	1.17, 1.72	1.45	1.19, 1.76

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[*] The numbers of subjects in some of the strata were less than the total number of subjects included in this study because some subjects did not provide complete information in their screening questionnaires.

[†] Two-sided χ^2 test.

[‡] Adjusted by age and sex.

Table III

Genotype and allele frequencies of the *VDR* polymorphisms among non-Hispanic whites in a case-control study of cutaneous melanoma, Texas, 1994–2006

	Case (n = 805)		Control (n = 841)			Odds ratio and 95% confidence interval			
***VDR* genotype**	**No.**	**%**	**No.**	**%**	***p*†**	**Crude odds ratio**	**95 percent confidence interval**	**Adjusted odds ratio‡**	**95 percent confidence interval**
*Taq*I					<0.01§				
TT	330	41.0	269	32.0		1	Referent	1	Referent
Tt	355	44.1	422	50.2		0.69	0.55, 0.86	0.69	0.56, 0.86
tt	120	14.9	150	17.8		0.65	0.49, 0.87	0.66	0.49, 0.87
Tt +*tt*	475	59.0	572	68.0	<0.01§§	0.68	0.55, 0.83	0.68	0.56, 0.83
t allele frequency	0.370		0.429		<0.01§§§				
*Bsm*I					0.02§				
bb	305	37.9	265	31.5		1	Referent	1	Referent
Bb	366	45.5	427	50.8		0.75	0.60, 0.92	0.75	0.60, 0.93
BB	134	16.6	149	17.7		0.78	0.59, 1.04	0.78	0.59, 1.05
Bb+*BB*	500	62.1	576	68.5	<0.01§§	0.75	0.58, 0.92	0.72	0.58, 0.90
B allele frequency	0.394		0.431		0.03§§§				
*Fok*I					0.05§				
FF	287	35.7	344	40.9		1	Referent	1	Referent
Ff	427	53.0	396	47.1		1.29	1.05, 1.59	1.30	1.05, 1.60
ff	91	11.3	101	12.0		1.08	0.78, 1.49	1.08	0.78, 1.49
Ff +*ff*	518	64.4	497	59.1	0.03§§	1.25	1.02, 1.53	1.25	1.03, 1.53
f allele frequency	0.378		0.356		0.20§§§				

*The observed distribution of genotype frequency among the control subjects appeared to be in Hardy Weinberg equilibrium (χ^2 = 0.49, p = 0.49 for *Taq*I; χ^2 = 1.04, p = 0.31 for *Bsm*I; and χ^2 = 0.64, p = 0.43 for *Fok*I).

†Two-sided χ^2 test for distributions of either genotype or allele frequency.

§distribution of three genotypes;

§§distribution of combined genotypes;

§§§allele distribution.

‡Odds ratios were adjusted for age and sex in a logistic regression model.

Table IV

Age- and sex-adjusted odds ratios for association between cutaneous melanoma risk and presence of haplotypes and combined genotypes of *VDR TaqI*, *BsmI*, and *FokI* in non-Hispanic whites, Texas, 1994–2006

Haplotype			Cases (n = 1610 alleles)		Controls (n = 1682 alleles)		Odds ratio and 95% confidence interval			
***Taq*I**	***Bsm*I**	***Fok*I**	**No.**	**%**	**No.**	**%**	**Crude odds ratio**	**95 percent confidence interval**	**Adjusted odds ratio †**	**95 percent confidence interval**
T	*b*	*f*	329	20.4	314	18.7	1.0	Referent	1.0	Referent
T	*b*	*F*	586	36.4	609	36.2	0.69	0.42,1.12	0.69	0.42, 1.11
T	*B*	*f*	16	1.0	8	0.5	3.81	0.51, 28.5	4.02	0.53, 30.4
T	*B*	*F*	36	2.2	29	1.7	1.47	0.50, 4.31	1.48	0.50, 4.36
t	*b*	*F*	19	1.2	22	1.3	0.39	0.09, 1.73	0.39	0.09, 1.72
t	*b*	*f*	13	0.8	12	0.7	1.06	0.14, 7.88	1.09	0.14, 8.07
t	*B*	*F*	378	23.5	424	25.2	0.52	0.33, 0.79	0.52	0.34, 0.80
t	*B*	*f*	233	14.5	264	15.7	0.51	0.27, 0.94	0.51	0.27, 0.94
Combined genotype			Cases (*n* =805)		Controls (*n* = 841		Odds ratio and 95% confidence interval			
*Taq*I	*Bsm*I	*Fok*I	No.	%	No.	%	Crude odds ratio	95 percent confidence interval	Adjusted odds ratio †	95 percent confidence interval
TT	*bb*	*Ff+ff*	181	22.5	142	16.9	1.0	Referent	1.0	Referent
TT	*bb*	*FF*	99	12.3	105	12.5	0.74	0.52, 1.05	0.74	0.52, 1.05
TT	*Bb+BB*	*Ff+ff*	30	3.7	10	1.2	2.35	1.11, 4.98	2.35	1.13, 4.98
TT	*Bb+BB*	*FF*	20	2.5	12	1.4	1.31	0.62, 2.76	1.31	0.62, 2.77
Tt+tt	*bb*	*Ff+ff*	18	2.2	12	1.4	1.18	0.55, 2.52	1.18	0.55, 2.54
Tt+tt	*bb*	*FF*	7	0.9	6	0.7	0.92	0.30, 2.78	0.94	0.31, 2.85
Tt+tt	*Bb+BB*	*Ff+ff*	289	35.9	333	39.6	0.68	0.52, 0.89	0.69	0.52, 0.90
Tt+tt	*Bb+BB*	*FF*	161	20.0	221	26.3	0.57	0.42, 0.77	0.58	0.43, 0.78

† Odds ratios were adjusted for age and sex in a logistic regression model.

‡ *p* values for trend were obtained in a logistic regression model after adjustment for age and sex.

§ *p* values for interaction were obtained from logistic regression models after adjustment for age, sex, and the main effects of the interactive variables.

Table V

Association between cutaneous melanoma risk and *VDR Taq*I, *Bsm*I, and *VDR Fok*I genotypes, stratified by risk factors, in non-Hispanic whites, Texas, 1994–2006

	***FokI Ff+ff* (no. cases/no. controls)**										***FokI FF* (no. cases/no. controls)**									
	***Taq*I**					***Bsm*I**					***Taq*I**					***Bsm*I**				
Variable	***TT***	***Tt+tt***	**Odds ratio[‡]**	**CI**	***p*[§]**	***bb***	***Bb+BB***	**Odds ratio[‡]**	**CI**	***p*[§]**	***TT***	***Tt+tt***	**Odds ratio[‡]**	**CI**	***p*[§]**	***bb***	***Bb+BB***	**Odds ratio[‡]**	**CI**	***p*[§]**
Age (years)					0.03					0.01					0.80					0.71
≤50	39/55	75/78	1.35	0.80, 2.27		35/51	79/82	1.40	0.82–2.38		87/56	117/121	0.62	0.41, 0.95		85/58	119/119	0.68	0.45, 1.04	
>50	80/62	93/149	0.50	0.33, 0.76		71/60	102/151	0.58	0.38–0.89		124/96	190/224	0.66	0.48, 1.92		114/96	200/224	0.76	0.54, 1.05	
Sex					0.18					0.17					0.19					0.05
Male	78/73	104/157	0.62	0.41, 0.93		69/68	113/162	0.69	0.46–1.04		132/108	200/227	0.74	0.54, 1.02		121/111	211/224	0.89	0.64, 1.22	
Female	41/44	64/70	1.00	0.58, 1.72		37/43	68/71	1.11	0.64–1.93		79/44	107/118	0.50	0.32, 0.79		78/43	108/119	0.50	0.32, 0.78	
Skin color										0.07					0.65					0.70
Black or brown	55/43	72/86	0.66	0.39, 1.09		54/38	68/71	0.55	0.32–0.92		82/61	119/157	0.55	0.36, 0.83		74/64	127/154	0.70	0.47, 1.06	
Fair	55/53	81/109	0.74	0.45, 1.19		44/54	73–91	1.08	0.66–1.77		104/62	164/150	0.65	0.44, 0.96		104/61	164/151	0.65	0.44, 0.96	
Hair color					0.45					0.81					0.05					0.03
Black or brown	70/77	105/55	0.76	0.51, 1.15		64/73	111/159	0.81	0.53, 1.22		124/92	184/255	0.54	0.39, 0.75		119/93	189/254	0.58	0.42, 0.81	
Blond(e) or red	40/18	48/39	0.57	0.28, 1.14		34/18	54/39	0.73	0.36, 1.50		62/31	99/49	1.04	0.60, 1.82		59/32	102/48	1.20	0.69, 2.09	
Eye color					0.12					0.26					0.38					0.44
Other	63/77	93/144	0.79	0.52, 1.21		57/73	99/148	0.86	0.56, 1.33		63/46	93/60	0.55	0.39, 0.79		106/90	162/233	0.62	0.44, 0.87	
Blue	46/17	60/51	0.42	0.21, 0.82		40/17	66/51	0.53	0.27, 1.04		77/17	144/51	0.73	0.45, 1.20		72/35	129/81	0.79	0.48, 1.29	
Tanning ability					0.44					0.05					0.74					0.83
Good (high)	30/31	41/75	0.55	0.29, 1.05		31/28	40/78	0.43	0.22, 0.83		40/48	62/113	0.67	0.40, 1.14		40/50	62/111	0.71	0.42, 1.20	
Poor (low)	80/65	112/119	0.77	0.51, 1.17		67/64	125/120	1.00	0.65, 1.53		146/75	221/193	0.60	0.42, 0.84		138/75	229/193	0.66	0.47, 0.92	
Lifetime sunburn with blistering					0.83					0.92					0.42					0.93
None	34/38	49/84	0.63	0.35, 1.14		31/38	52/84	0.73	0.40, 1.32		38/50	70/124	0.75	0.45, 1.25		39/48	69/126	0.68	0.40, 1.14	
≥1	74/156	105/111	0.71	0.45, 1.10		65/52	114/115	0.79	0.50, 1.23		146/173	208/181	0.58	0.41, 0.82		137/77	217/177	0.70	0.49, 0.98	
Freckling in the sun as a child					0.45					0.71					0.10					0.23
No	62/56	71/109	0.57	0.35, 0.91		54/54	79/111	0.69	0.42, 1.11		83/83	127/173	0.74	0.50, 1.08		80/82	130/174	0.77	0.53, 1.13	
Yes	48/39	81/85	0.77	0.45, 1.30		44/37	85/87	0.81	0.47, 1.38		102/40	154/133	0.46	0.30, 0.71		98/43	158/130	0.55	0.36, 0.84	
Moles					0.94					0.67					0.35					0.65
No	25/30	43/71	0.73	0.38, 1.41		21/30	47/71	0.98	0.50, 1.92		40/47	67/141	0.57	0.34, 0.95		39/53	68/135	0.70	0.42, 1.16	

	FokI Ff+ff (no. cases/no. controls)									*FokI FF* (no. cases/no. controls)								
	*Taq*I					*Bsm*I				*Taq*I					*Bsm*I			
Variable	***TT***	***Tt+tt***	**Odds ratio[‡]**	**CI**	***p*[§]**	***bb***	***Bb+BB***	**Odds ratio[‡]**	**CI**	***p*[§]**	***TT***	***Tt+tt***	**Odds ratio[‡]**	**CI**	***p*[§]**	***bb***	***Bb+BB***	**Odds ratio[‡]**	**CI**	***p*[§]**
Yes	94/87	125/156	0.76	0.52, 1.11		85/81	134/162	0.81	0.55, 1.19		171/105	240/204	0.74	0.54, 1.01		160/101	251/208	0.79	0.57, 1.07	
Dysplastic nevi					0.98					0.51					0.28					0.24
No	113/115	155/227	0.70	0.50, 0.97		100/110	168/232	0.80	0.57, 1.12		190/151	282/339	0.66	0.51, 0.86		178/153	294/337	0.75	0.57, 0.98	
Yes	6/2	13/0		NC		6/1	13/1	0.48	0.01, 40.2		21/1	25/6	0.07	0.01, 0.99		21/1	25/6	0.07	0.01, 0.98	
First-degree relatives with any cancer					0.36					0.94					0.49					0.63
No	43/67	67/122	0.86	0.53, 1.40		42/62	68/127	0.79	0.48, 1.29		104/77	129/165	0.58	0.40, 0.85		96/77	137/165	0.68	0.46, 0.99	
Yes	76/50	101/105	0.64	0.41, 1.00		64/49	113/106	0.83	0.52, 1.31		107/75	178/180	0.70	0.49, 1.00		103/77	182/178	0.77	0.53, 1.10	

[*] The numbers of subjects in some of the strata were less than the total number of subjects included in this study because some subjects did not provide the information.

[†] CI, confidence interval.

[‡] Odds ratios were adjusted for age and sex in a logistic regression model.

[§] *p* values for interaction were obtained from logistic regression models after adjustment for age, sex, and the main effects of the interactive variables.

Table VI

Multivariate logistic regression analysis of associations between VDR *Taq*I, *Bsm*I, and *Fok*I genotype frequencies and cutaneous melanoma risk in non-Hispanic whites, Texas, 1994–2006

Variable	β	Wald χ^{2-}	*p*	Odds ratio	95 percent confidence interval
Model 1 (805 cases and 841 controls)					
Age (years)	−0.007	2.59	0.11	0.99	0.99, 1.00
Sex (male vs. female)	0.13	1.51	0.22	1.14	0.93, 1.40
*VDR Bsm*I (*Bb+BB* vs. *bb*)	0.21	1.11	0.29	1.23	0.84, 1.82
*VDR Fok*I (*Ff+ff* vs. *FF*)	0.24	5.39	0.02	1.27	1.04, 1.55
*VDR Taq*I (*Tt+tt* vs. *TT*)	−0.57	8.20	0.004	0.57	0.39, 0.84
Model 2 (712 cases and 707 controls) *					
Age (years)	−0.01	0.11	0.74	1.00	0.99, 1.01
Sex (male vs. female)	0.08	0.42	0.52	1.08	0.88, 1.37
Eye color (other vs. blue)	0.58	21.7	<0.001	1.79	1.40, 2.29
Hair color (blond[e] or red vs. black or brown)	0.46	11.3	<0.001	1.58	1.21, 2.07
Lifetime sunburns with blistering (≥1 vs. 0)	0.45	12.9	<0.001	1.56	1.23, 1.99
Tanning ability after prolonged sun exposure (moderate or poor vs. good)	0.37	8.55	0.003	1.45	1.13, 1.86
Moles (yes vs. no)	0.65	27.9	<0.001	1.92	1.51, 2.44
Pigmented nevi (yes vs. no)	1.70	20.9	<0.001	5.46	2.64, 11.3
Family history of skin cancer (yes vs. no)	0.27	5.25	0.02	1.31	1.04, 1.65
*VDR Fok*I (*Ff+ff* vs. *FF*)	0.15	1.55	0.21	1.16	0.92, 1.46
*VDR Taq*I (*Tt+tt* vs. *TT*)	−0.38	10.3	0.001	0.68	0.54, 0.86

* The numbers of subjects included in this model were less than the total number of subjects included in this study because this model only included subjects who provided complete information in their screening questionnaires.