

# Vitamin D Receptor Polymorphisms and the Risk of Cutaneous Melanoma

## *A Systematic Review and Meta-Analysis*

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It has been hypothesized that polymorphisms in the vitamin D receptor (VDR) gene affect the risk of developing melanoma. However, results often are conflicting, and no meta-analysis has been performed to date on published data. Six studies (cases, 2152; controls, 2410) that investigated the association between 5 VDR polymorphisms (TaqI, FokI, BsmI, EcoRV, and Cdx2) and the risk of melanoma were retrieved and analyzed. The model-free approach was applied to meta-analyze these molecular association studies. Available data suggested a significant association between the BsmI VDR polymorphism and melanoma risk (pooled odds ratio [OR], 1.30; 95% confidence interval [CI], 1.11-1.53;  $P = .002$ ; heterogeneity Cochran Q test,  $P > .1$ ), and the population-attributable risk was 9.2%. In contrast, the FokI polymorphism did not appear to be associated with such risk (OR, 1.09; 95% CI, 0.99-1.21;  $P = .07$ ; heterogeneity Cochran Q test,  $P > .1$ ). For the TaqI and the EcoRV polymorphisms, significant between-study heterogeneity did not support genotype data pooling. Only 1 study investigated the Cdx2 variant, and the findings were negative. Current evidence is in favor of an association between 1 VDR gene polymorphism (BsmI) and the risk of developing melanoma. The current findings prompt further investigation on this subject and indirectly support the hypothesis that sun exposure may have an antimelanoma effect through activation of the vitamin D system. *Cancer* 2008;113:2398-407. © 2008 American Cancer Society.

**KEYWORDS:** melanoma, risk, vitamin D receptor, polymorphism, meta-analysis.

An increasing body of research supports the hypothesis that 1,25-dihydroxycholecalciferol (1,25[OH]<sub>2</sub>D<sub>3</sub> or calcitriol)—the active form of vitamin D—has significant protective effects against the development of cancer.<sup>1,2</sup> This anticancer potential results from the role of vitamin D as a transcription factor that regulates cell growth, differentiation, and apoptosis, which are cellular mechanisms central to the development and progression of cancer. Moreover, epidemiologic studies suggest an inverse association between sun exposure, serum levels of 1,25(OH)<sub>2</sub>D<sub>3</sub>, and vitamin D intake and the risk of developing and/or surviving cancer.<sup>1-4</sup> Because this vitamin exerts its function on engagement of its receptor (ie, vitamin D receptor [VDR]), it is likely that polymorphisms of this nuclear hormone receptor family member affect the ability of interacting with its ligand, which ultimately would lead to different levels of vitamin D biologic activity.<sup>5</sup> Several studies have addressed the issue of whether VDR variants are associated with the risk of developing dif-

ferent types of carcinoma,<sup>6-8</sup> although the only 2 meta-analyses performed on the subject to date rejected this hypothesis for prostate cancer.<sup>9,10</sup>

With regard to melanoma, preclinical evidence supports the role of vitamin D as a modulator of the malignant behavior of this tumor,<sup>11</sup> although conflicting findings also have been reported.<sup>12</sup> Despite evidence that sunburn is a melanoma risk factor,<sup>13</sup> epidemiologic data indicate that sun exposure may improve the survival of patients with melanoma,<sup>3,4</sup> a phenomenon that can be linked reasonably to the finding that sunlight is necessary for the synthesis of a putative anticancer agent like vitamin D<sub>3</sub>. On the other side, studies testing the hypothesis of an association between VDR polymorphisms and melanoma risk often have investigated different allelic variants, sometimes are statistically underpowered (because of small sample sizes), and overall have yielded heterogeneous results. To make the most of the available information on VDR polymorphisms and melanoma and to determine whether this topic was worth further investigation, we undertook a systematic review of published studies and used meta-analysis techniques to pool together and quantitatively summarize the current evidence.

## MATERIALS AND METHODS

### Search Strategy, Eligibility Criteria, and Data Extraction

According to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines for reporting on meta-analyses of observational studies,<sup>14</sup> the following data were extracted from the eligible studies: authors' names, region/country where the study was conducted, year of publication, numbers of cases and controls, mean age (or range) of cases and controls, sex proportion, race, manner in which cases and controls were selected, and number of individuals with the *VDR* genotype in both cases and controls. A systematic review of original articles analyzing the association between VDR polymorphisms and melanoma risk was performed by searching the PubMed, Medline, Embase, Cancerlit, and Cochrane databases. The search strategy included the following keywords (variously combined): 'melanoma,' 'VDR,' 'vitamin D receptor,' 'polymorphism,' 'risk,' 'allele,' and 'gene.' Original and review articles that were published up to March 2008 were sought, and the review articles were used as additional sources for original articles that otherwise were overlooked. Cited references from selected articles also were reviewed as appropriate. The authors of the various series were contacted whenever data that were not reported were useful for inclusion in our system-

atic review or to rule out data that were published in different articles from overlapping series.

Eligibility criteria included: 1) any associated study (regardless of the sample size) in which the outcome was histologically proven, cutaneous melanoma and there were 2 comparison groups (melanoma vs nonmelanoma populations); 2) genotype/allele data necessary for association analyses had to be reported. Care was taken to account for overlapping and duplicate datasets. Where multiple publications from the same study group were identified, the most complete and recent results were considered.

Data were extracted separately by both investigators to ensure homogeneity of data collection and to rule out the effect of subjectivity in data gathering and entry. Disagreements were resolved by iteration, discussion, and consensus.

### Statistical Analysis

Because all of the investigated polymorphisms (FokI, BsmI, TaqI, EcoRV, and Cdx2) were diallelic, single-nucleotide polymorphisms (SNPs), odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were used to assess the strength of association between each VDR variant and the risk of melanoma. Standard meta-analysis methods<sup>15,16</sup> were applied. To evaluate the overall effect of VDR polymorphism genotypes on the risk of melanoma, the so called model-free approach<sup>17</sup> was adopted by taking the following 5 steps:

- 1) In each study, the genotype distribution in the control population is checked for departure from Hardy-Weinberg equilibrium (HWE) using the chi-square goodness-of-fit test; if a departure from HWE is identified in 1 or more studies, then sensitivity analyses are performed with and without those studies to test the robustness of the findings.
- 2) The Cochran Q test for between-study heterogeneity is checked for 3 ORs: OR1 (eg, *AA* vs *aa*), OR2 (eg, *Aa* vs *aa*), and OR3 (eg, *AA* vs *Aa*); if heterogeneity is identified, then potential sources are investigated by sensitivity analyses (eg, leave-one-out analysis), subgroup analyses (considering more homogeneous studies), and metaregression (eg, by fitting a covariate like age, sex, or race into a meta-regression model).
- 3) The main effect of genotype is assessed by logistic regression analysis, which protects against an inflated type 1 error in the context of multiple testing.

- 4) If there is a significant gene effect, then pairwise group comparisons of OR1, OR2, and OR3 are explored, and those results are allowed to indicate the best genetic model according to the following schema:
- If  $OR1 = OR3 \neq 1$  and  $OR2 = 1$ , then a recessive model is suggested;
  - if  $OR1 = OR2 \neq 1$  and  $OR3 = 1$ , then a dominant model is suggested; and
  - if  $OR1 > OR2 > 1$ , or if  $OR1 < OR2 < 1$ , or if  $OR1 > OR3 > 1$ , or if  $OR1 < OR3 < 1$ , then a codominant model is suggested.
- 5) Data then may be pooled according to the model suggested. Heterogeneity is checked again, and its sources are explored as necessary.

Meta-analysis was performed first using the fixed-effect model (Mantel-Haenszel method), which assumes that all studies share the same common effect. The consistency of results (effect sizes) among studies was investigated by means of 2 heterogeneity tests: the chi-square-based Cochran Q test and the  $I^2$  statistic ( $[Q - df]/Q \times 100$ , where Q is the Cochran statistic, and *df* [degrees of freedom] is the number of studies minus 1; it indicates the percentage of the variability in effect estimates caused by heterogeneity rather than sampling error). To be more conservative, we considered that heterogeneity was present when the Q test *P* value was  $<.1$ . In addition, inconsistency across studies was quantified by means of the  $I^2$  statistic, which generally is considered significant (ie, heterogeneity has a significant impact on meta-analysis) for values  $>50\%$ . In case of heterogeneity, meta-analysis was performed by applying the random-effect model according to the DerSimonian and Laird method,<sup>18</sup> which assumes that studies were a random sample of a hypothetical population of studies and assigns a weight to each study taking into account variance within and between studies.

The extent to which the combined risk estimate may be affected by individual studies was assessed by consecutively omitting every study from the meta-analysis (leave-one-out procedure). Subgroup analysis (considering more homogeneous sets of studies), random effects meta-regression (to investigate study features that may affect the magnitude of the effect estimate),<sup>19</sup> and funnel plot analysis (to unravel publication bias)<sup>20</sup> were planned to explore potential sources of heterogeneity, as described in detail elsewhere.<sup>21</sup> Population-attributable risk was calculated using the following formula:  $P(RR - 1)/(1 + P[RR - 1])$ , where P is the proportion of controls exposed to the genotype of interest, and the

relative risk (RR) was estimated by using the OR calculated in the meta-analysis.

Meta-analysis was conducted using RevMan software version 4.2 (The Cochrane Collaboration, Oxford, United Kingdom). The Cochran-Armitage trend test (to assess a linear correlation between genotypes and risk) was performed using StatXact7 software (Cytel Inc., Cambridge, Mass). The other statistical analyses were performed with the SPSS statistical package (version 13.0; SPSS Inc., Chicago, Ill). Probability values  $<5\%$  were considered significant (except in tests for heterogeneity).

## RESULTS

### General Considerations

At the time of this writing, 7 original reports addressed the issue of VDR polymorphisms and the risk of cutaneous melanoma.<sup>22-28</sup> Of these, 1 study<sup>28</sup> was not considered for the current review/meta-analysis, because the data overlapped with a subsequent publication from the same research group.<sup>22</sup>

The main characteristics of the remaining 6 studies (cases, 2152; controls, 2410) are reported in Table 1. The following 5 VDR SNPs were studied: TaqI (2 studies; alleles *T/t*),<sup>22,27</sup> BsmI (3 studies; alleles *B/b*),<sup>22,23,26</sup> FokI (4 studies; alleles *F/f*),<sup>22,23,26,27</sup> EcoRV (3 studies; alleles *A/G*),<sup>23-25</sup> and Cdx2 (1 study; alleles *G/A*).<sup>26</sup> In all studies, the controls were in HWE (Table 2).

The studies were performed in 3 countries: 1 in North European (United Kingdom), 1 in South European (Italy), and 1 in North American (United States), and all cases and controls were Caucasian. In 1 study, only women were enrolled.<sup>26</sup>

### Polymorphisms

#### TaqI polymorphism

The TaqI restriction fragment length polymorphism (RFLP) (SNP reference cluster identification number [rs] 731236<sup>29</sup>) was investigated in 2 series,<sup>22,27</sup> which enrolled 1066 cases and 934 controls. In the larger series,<sup>22</sup> the TaqI polymorphism (*T/t*) was associated with the risk of melanoma, and *T* was identified as the risk allele.

Heterogeneity was significant for OR1 (chi-square Q test, 2.8;  $P = .09$ ) and for OR3 (chi-square Q test, 4.9;  $P = .03$ ), which discouraged us from pooling the genotype data for meta-analysis. Because only 2 studies were available, the search for sources of heterogeneity virtually was impossible. The difference in the *T* allele frequency among controls in the 2 series (57% vs 63%) was not statistically significant ( $P = .07$ ). No other sources of heterogeneity were

**TABLE 1**  
Main Characteristics of the 6 Studies Investigating on the Relation Between Vitamin D Receptor Single Nucleotide Polymorphism and the Risk of Melanoma

Study	Country	Design	No. (Enrollment Site)		Race	Mean Age, y	Sex	SNP
			Cases	Controls				
Li 2008 <sup>22</sup>	USA	C-C	805 (Hospital)	841 (Hospital)	White	No significant C-C difference	Women: 36% of cases and 33% of controls; no significant difference	BsmI, FokI, TaqI
Han 2007 <sup>26</sup>	USA	Nested C-C	215 (Nurses' Health Study)	854 (Nurses' Health Study)	White	58	Women only	BsmI, FokI, Cdx2
Povey 2007 <sup>25</sup>	UK	C-C	596 (Hospital)	441 (Population)	White	48 (Cases) vs 51 (controls); significant difference	Women: 57%; no significant C-C difference	EcoRV
Santonocito 2007 <sup>23</sup>	Italy	C-C	101 (Hospital)	101 (Blood donors)	White	54 (Cases) vs 54 (controls); no significant difference	Women: 53%; no significant C-C difference	BsmI, FokI, EcoRV
Halsall 2004 <sup>24</sup>	UK	C-C	174 (Hospital)	80 (Hospital)	White	54 (Cases), 56 (controls)	Women: 62% of cases and 50% of controls; no significant difference	EcoRV
Hutchinson 2000 <sup>27</sup>	UK	C-C	261 (Hospital)	93 (Hospital)	White	53 (Cases), 55 (controls)	Women: 67% of cases and vs 50% of controls; significant difference	FokI, TaqI

SNP indicates single nucleotide polymorphism; C-C, case-control.

\*An association between genotypes and confounding factors was ruled out by chi-square test and not by multivariate logistic regression.

identified in the study design/features of the 2 reports.

### **BsmI polymorphism**

This RFLP (rs1544410<sup>29</sup>) was investigated in 3 series,<sup>22,23,26</sup> which enrolled 1114 cases and 1782 controls. In 2 series<sup>22,23</sup> the BsmI polymorphism (*B/b*) was associated with melanoma risk, and *b* was identified as the risk allele.

The 3 studies were homogeneous for both OR1 (chi-square Q test, 4.4;  $P = .11$ ), OR2 (chi-square Q test, 4.8;  $P = 1.09$ ), and OR3 (chi-square Q test, 0.59;  $P = .74$ ). To assess the main effect of the BsmI polymorphism, logistic regression analysis was fit to the genotype data: The results suggested an association between this VDR polymorphism and the risk of melanoma (overall model fit: chi-square, 5.05;  $P = .02$ ). Because the model-free approach indicated a recessive genetic model ( $OR1 = OR3 \neq 1$  and  $OR2 = 1$ ), genotype data were pooled accordingly (*bb* vs  $BB + Bb$ ). In this model, the meta-analysis confirmed a significant association between the BsmI polymorphism and the risk of melanoma, with an estimated pooled OR of 1.30 (95% CI, 1.11-1.53;  $P = .002$ ;

heterogeneity: chi-square Q test, 1.1;  $P = .57$ ) (Fig. 1). Based on these findings and considering the *bb* genotype, the calculated population-attributable risk was 9.2%.

### **FokI polymorphism**

This RFLP (rs10735810<sup>29</sup>) was investigated in 4 series,<sup>22,23,26,27</sup> which enrolled 1414 cases and 1904 controls. In 2 series,<sup>22,27</sup> the FokI polymorphism (*F/f*) reportedly was correlated with the risk of melanoma, and *f* was identified as the risk allele. In the report by Li et al,<sup>22</sup> neither the allele frequency nor the genotype frequency (as assessed by the Cochran-Armitage trend test) differed significantly between cases and controls (Table 2). Although genotype pooling according to a dominant model indicated that the *f* allele was associated with an increased risk of disease, the significance of this relation disappeared after adjustment for known melanoma risk factors (Table 2).

The 3 studies that investigated the FokI polymorphism were homogeneous for OR1 (chi-square Q test, 4.8;  $P = .18$ ), OR2 (chi-square Q test, 3.2;  $P = .35$ ), and OR3 (chi-square Q test, 3.6;  $P = .31$ ). Logis-

**TABLE 2**  
**Vitamin D Receptor Genotype Distributions in Patients With Melanoma (Cases) and Controls**

SNP/Study*	Genotype	Cases	Controls	OR (95%CI)	P†	Adjusted P‡
FokI						
Li 2008 <sup>22</sup>	FF	287	344	0.80 (0.65-0.97)		NS [age, sex, sunburn, phototype, skin/eye color]
	Ff	427	396	1.27 (1.04-1.54)	.16	
	ff	91	101	0.93 (0.69-1.26)		
	F, %	62.1	64.4	0.90 (0.78-1.04)	.17	
	HWE			—	.45	
Han 2007 <sup>26</sup>	FF	77	325	0.90 (0.66-1.24)		NS [age, sunburn, vitamin D intake]
	Ff	101	418	0.95 (0.70-1.29)	.21	
	ff	37	111	1.39 (0.92-2.08)		
	F, %	59.3	62.5	0.87 (0.70-1.08)	.22	
	HWE			—	.21	
Santonocito 2007 <sup>23</sup>	FF	47	41	1.27 (0.72-2.22)		NS [age, sex, skin type, eye color]
	Ff	41	46	0.81 (0.46-1.42)	.47	
	ff	13	14	0.91 (0.40-2.06)		
	F, %	66.8	63.3	1.16 (0.77-1.75)	.46	
	HWE			—	.83	
Hutchinson 2000 <sup>27</sup>	FF	105	52	0.60 (0.38-0.94)		Association with age and sex ruled out by chi-square test
	Ff	142	44	1.36 (0.87-2.13)	.02§	
	ff	46	12	1.49 (0.75-2.93)		
	F, %	60.0	68.5	0.69 (0.49-0.96)	.02§	
	HWE			—	.65	
TaqI						
Li 2008 <sup>22</sup>	TT	330	269	1.47 (1.20-1.80)		Significant [age, sex, sunburn, phototype, skin/eye color]
	Tt	355	422	0.78 (0.64-0.95)	.0005§	
	tt	120	150	0.80 (0.62-1.04)		
	T, %	63.0	57.0	1.28 (1.11-1.47)	.0004§	
	HWE			—	.52	
Hutchinson 2000 <sup>27</sup>	TT	94	39	0.77 (0.48-1.26)		Association with age and sex ruled out by chi-square test
	Tt	127	41	1.20 (0.74-1.93)	.38	
	Tt	40	13	1.11 (0.56-2.19)		
	T, %	60.3	63.9	0.85 (0.60-1.21)	.38	
	HWE			—	.65	
BsmI						
Li 2008 <sup>22</sup>	BB	134	149	0.92 (0.71-1.19)		NS [age, sex]
	Bb	366	427	0.80 (0.66-0.98)	.03§	
	bb	305	265	1.32 (1.08-1.62)		
	B, %	39.3	43.1	0.85 (0.74-0.98)	.03§	
	HWE			—	.32	
Han 2007 <sup>26</sup>	BB	29	130	0.88 (0.57-1.36)		NS [age, sunburn, vitamin D intake]
	Bb	94	398	0.91 (0.67-1.24)	.32	
	bb	85	312	1.16 (0.85-1.59)		
	B, %	36.5	39.1	0.89 (0.71-1.11)	.32	
	HWE			—	.88	
Santonocito 2007 <sup>23</sup>	BB	10	24	0.35 (0.15-0.78)		Significant [age, sex, skin type, eye color]
	Bb	54	51	1.12 (0.64-1.95)	.009§	
	Bb	37	26	1.44 (0.78-2.65)		
	B, %	36.6	49.0	0.60 (0.40-0.89)	.01§	
	HWE			—	1.00	
FokI						
Li 2008 <sup>22</sup>	FF	287	344	0.80 (0.65-0.97)		NS [age, sex, sunburn, phototype, skin/eye color]
	Ff	427	396	1.27 (1.04-1.54)	.16	
	ff	91	101	0.93 (0.69-1.26)		
	F, %	62.1	64.4	0.90 (0.78-1.04)	.17	
	HWE			—	.45	
Han 2007 <sup>26</sup>	FF	77	325	0.90 (0.66-1.24)		NS [age, sunburn, vitamin D intake]
	Ff	101	418	0.95 (0.70-1.29)	.21	
	ff	37	111	1.39 (0.92-2.08)		
	F, %	59.3	62.5	0.87 (0.70-1.08)	.22	
	HWE			—	.21	

(continued)

TABLE 2  
(continued)

SNP/Study*	Genotype	Cases	Controls	OR (95%CI)	P†	Adjusted P‡
Santonocito 2007 <sup>23</sup>	FF	47	41	1.27 (0.72-2.22)		NS [age, sex, skin type, eye color]
	Ff	41	46	0.81 (0.46-1.42)	.47	
	ff	13	14	0.91 (0.40-2.06)		
	F, %	66.8	63.3	1.16 (0.77-1.75)	.46	
	HWE			—	.83	
Hutchinson 2000 <sup>27</sup>	FF	105	52	0.60 (0.38-0.94)		Association with age and sex ruled out by chi-square test
	Ff	142	44	1.36 (0.87-2.13)	.02§	
	ff	46	12	1.49 (0.75-2.93)		
	F, %	60.0	68.5	0.69 (0.49-0.96)	.02§	
	HWE			—	.65	
EcoRV Santonocito 2007 <sup>23</sup>	AA	35	43	0.59 (0.33-1.05)		NS [age, sex, skin type, eye color]
	AG	51	45	1.26 (0.72-2.20)	.30	
	GG	15	13	1.18 (0.53-2.62)		
	A, %	59.9	64.8	0.80 (0.54-1.21)	.30	
	HWE			—	.82	
Halsall 2004 <sup>24</sup>	AA	66	22	1.61 (0.90-2.87)		Association with age and sex ruled out by chi-square test
	AG	92	42	1.01 (0.59-1.72)	.015§	
	GG	16	16	0.40 (0.19-0.85)		
	A, %	64.3	53.7	1.55 (1.06-2.27)	.02§	
	HWE			—	.82	
Povey 2007 <sup>25</sup>	AA	196	130	1.05 (0.80-1.38)		NS [age, sex]
	AG	297	195	1.10 (0.85-1.41)	.27	
	GG	103	86	0.78 (0.57-1.08)		
	A, %	57.8	55.3	1.10 (0.92-1.32)	.27	
	HWE			—	.42	
Cdx2 Han 2007 <sup>26</sup>	GG	132	548	1.00 (0.73-1.38)		NS [age, sunburn, vitamin D intake]
	GA	68	269	1.07 (0.77-1.49)	.65	
	AA	5	36	0.56 (0.21-1.46)		
	G, %	80.1	80.0	1.06 (0.80-1.39)	.66	
	HWE			—	.67	

SNP indicates single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NS, not significant; HWE, Hardy Weinberg Equilibrium.

\*FokI, TaqI, BsmI, EcoRV, and Cdx2 are vitamin D receptor SNPs.

†For each study, the *P* values of the following tests are reported sequentially (top to bottom): Cochran-Armitage trend test (for genotype distribution), Fisher exact test (for allele distribution), and HWE.

‡For each study, the statistical significance of the association tests (logistic regression analysis, unless otherwise specified) adjusted by the variables listed in brackets is reported (as described by the authors of the individual studies).

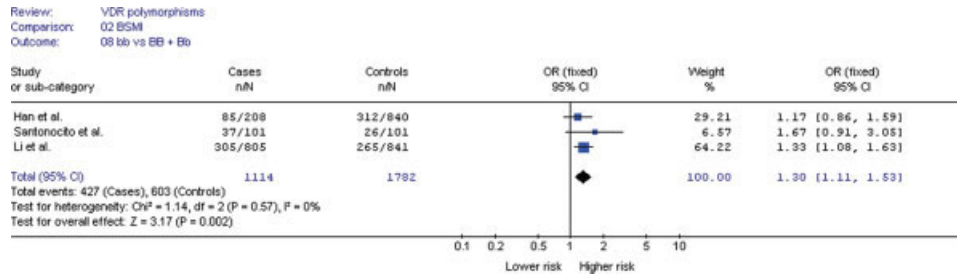
§Significant *P* value.

tic regression analysis was fit to the genotype data and indicated no association between FokI and the risk of melanoma (overall model fit: chi-square, 3.2; *P* = .07; OR, 1.09; 95% CI, 0.99-1.21). Accordingly, there was no indication to collapse the genotype data and meta-analyze them. Nevertheless, because the *P* value of the regression analysis was borderline (.07), we explored the hypothesis that the data may suggest a genetic model: the results (OR1 = OR3 = 1 and OR2 > 1) indicated that no model fit the data, which further discouraged us from pooling the data.

### EcoRV polymorphism

This RFLP (rs4516035<sup>29</sup>) was investigated in 3 series,<sup>23-25</sup> which enrolled 871 cases and 592 controls. In 2 series,<sup>24,25</sup> the EcoRV polymorphism (A/G) reportedly was correlated with the risk of melanoma, and A was identified as the risk allele.

The 3 studies were homogeneous for OR2 (chi-square Q test, 2.12; *P* = .35) and OR3 (chi-square Q test, 2.21; *P* = .33), but they were heterogeneous for OR1 (chi-square Q test, 5.7, *P* = .06), which did not support the pooling of genotype data and their meta-analysis. To assess whether publication bias



**FIGURE 1.** This Forest plot illustrates the odds ratios (OR) for the vitamin D receptor (VDR) BsmI polymorphism and the risk of melanoma. CI indicates confidence interval.

was significant, ORs of allele frequency (*A* vs *G*) were calculated for each study and were used to generate a funnel plot (data not shown): Because the plot results were symmetric (Egger test;  $P = .89$ ), no publication bias could be demonstrated. The leave-one-out sensitivity analysis demonstrated that 1 of 2 North European studies<sup>24</sup> accounted for heterogeneity. No other sensitivity analyses could be performed because of the small number of studies.

#### Cdx2 polymorphism

This SNP (rs11568820<sup>29</sup>) was investigated in only 1 series.<sup>26</sup> Analyzing the data from 205 cases and 853 controls, the authors did not report a significant association between the Cdx2 VDR variant and the risk of melanoma.

## DISCUSSION

This meta-analysis, the first to our knowledge on this topic, indicated that the available evidence is in favor of a significant association between the VDR BsmI polymorphism and the risk of developing cutaneous melanoma (Fig. 1). By contrast, the analysis of pooled data ruled out the melanoma-promoting role of the FokI polymorphism that was hypothesized by some investigators. In addition, available evidence was not sufficient to draw any conclusion regarding the role of the other 3 SNPs (TaqI, EcoRV, and Cdx2) that have been investigated to date. To interpret our findings, we need to briefly summarize the current knowledge regarding the VDR gene organization and the functional effects of the 5 VDR polymorphisms considered.

#### VDR Gene Organization and Polymorphisms

Located on chromosome 12q12-q14, the VDR gene contains 5 promoter regions, 8 protein-coding exons, and 6 untranslated exons; and all of these regions are spliced alternatively. At least 196 VDR SNPs have been described,<sup>29,30</sup> and 64 of them lie in the promoter region, 32 lie in the 3' and 5' untranslated

regions, and 2 synonymous SNPs and 2 nonsynonymous SNPs lie in the coding region.<sup>5,31</sup> The 5 SNPs that were studied for their association with melanoma risk usually are grouped into 2 categories: a cluster of tightly linked polymorphisms at the 3' end (BsmI, intron 8; TaqI, silent site of exon 9) and 3 polymorphisms at the 5' end of the gene (FokI, exon 2; EcoRV and Cdx2, promoter region).

#### FokI polymorphism

FokI is the only SNP that leads to a different gene product: The T-C transition at this restriction site produces an ATG start codon, initiating translation 10 base pairs upstream and, thus, resulting in a 3 amino-acid, extended VDR protein.<sup>32,33</sup> The *f* allele, which results in this longer protein and was associated with increased melanoma risk in 2 of 4 studies (Table 2), appears to be less effective at activating the transcription of a VDR reporter construct,<sup>34</sup> thereby indicating that this SNP is relevant functionally. However, the meta-analysis of available data, which was possible because of the homogeneity among the 4 studies, ruled out a significant association between the FokI polymorphism and melanoma risk.

#### BsmI polymorphism

For this SNP, which is in mutual tight linkage disequilibrium with the other polymorphisms at the 3' end of the VDR gene,<sup>5,31,35</sup> there is no evidence of a functional effect on VDR activity.<sup>36</sup> However, it is believed that the 3'-end polymorphisms (with particular regard to BsmI and TaqI) affect messenger RNA stability and VDR gene transcription regulation<sup>5,37</sup>; and the net effect of *bb*, *ff*, and *tt* genotypes may be envisaged as a reduction in the cellular activity of the vitamin D system. Indeed, in vitro functional studies have demonstrated that the *baT* (BsmI/ApaI/TaqI) haplotype inserted into transfection constructs resulted in lower reporter gene activity compared with *BAI*<sup>38</sup> and was associated with low VDR messenger RNA levels<sup>39</sup>. These findings, along with ours, are

in line with the hypothesis that a reduction in vitamin D system activity may lead to an increased melanoma risk. Otherwise, the BsmI polymorphism may be in linkage disequilibrium with other functionally relevant polymorphisms that will require further investigation.

#### *EcoRV polymorphism*

This SNP of the VDR promoter (A-1012G) may modulate the docking of a transcription factor: in fact, this polymorphism is within the core sequence of a putative glutamyl-transfer RNA amidotransferase subunit A 3 (GATA-3) binding site in the A allele, whereas this binding site is not present in the G allele.<sup>40</sup> Because GATA-3 plays a pivotal role in the polarization of naive T cells into T-helper 2-type lymphocytes<sup>41</sup>—which are believed to hamper an effective anticancer immune response<sup>42</sup>—it has been suggested that the EcoRV polymorphism may tip the balance toward the phenomenon of tumor immune escape.<sup>43</sup> Results from the 3 studies that investigated the relation between this SNP and the risk of melanoma are conflicting; and, unfortunately, the between-study heterogeneity did not support the use of available data for meta-analysis. Therefore, further work is warranted to elucidate the role of this VDR variant in the determinism of melanoma development.

#### *TaqI polymorphism*

The functional effect of this RFLP at the 3' end of the VDR gene is similar to that of BsmI. In analogy to the EcoRV polymorphism, the available data are conflicting, and meta-analysis was not indicated because of heterogeneity. Consequently, in this polymorphism, further work is warranted to assess whether the VDR variant is associated with melanoma risk.

#### *Cdx2 polymorphism*

Cdx2, which is a caudal-related homeodomain transcription factor, is important during the development of the intestine and, in adults, it reportedly regulates VDR expression in the small bowel.<sup>44</sup> Polymorphism at the Cdx2 binding site alters the transcriptional activity of the VDR promoter region: in particular, the A allele is associated with higher VDR gene transcription activity.<sup>45</sup> The available evidence (lack of association) comes from a single study: consequently, further investigation will be required before ruling out a significant impact of this SNP on the risk of melanoma.

#### **Final Considerations**

A significant limitation of the current meta-analysis is that the results could not be adjusted by patient

characteristics (eg, age, sex), environmental factors (eg, sun exposure, dietary vitamin D intake), or any other melanoma risk factors (eg, phototype, skin/eye color). Conversely, only an individual patient data meta-analysis could address this issue, and no study reported such detailed information, which calls for the development of publicly available databases aimed at the collection and analysis of biologic information on a single-patient basis.<sup>46,47</sup>

It must be noted that, as shown in Table 2, the authors of all series in our meta-analysis adjusted their associated findings by age and sex, 3 groups<sup>22,23,26</sup> analyzed the impact of known risk factors (ie, phototype, skin/eye color), and 2 groups<sup>22,26</sup> took into consideration environmental factors that potentially influenced the results (ie, sunburn history and vitamin D intake, vitamin D plasma levels). After these corrections, the results differed from the unadjusted data in only 1 case (the BsmI polymorphism).<sup>22</sup>

With regard to the BsmI polymorphism—the only one that was associated significantly with the risk of melanoma in the current meta-analysis—the 3 available studies performed 3 different genotype comparisons while adjusting the results (*BB* vs *bb*,<sup>26</sup> *bb* vs *BB*,<sup>23</sup> and *Bb* + *BB* vs *bb*<sup>22</sup>), which did not allow us to meta-analyze the adjusted OR by using the generic inverse variance method. Clearly, well designed, population-based, large, multi-institutional studies are warranted to address the issue regarding whether any VDR polymorphism is associated independently with melanoma risk.

In conclusion, current evidence is in favor of the association between 1 VDR gene polymorphism (BsmI) and the risk of melanoma development, although further work will be necessary to validate the risk identified in the current meta-analysis. Although the functional effect of the VDR BsmI SNP has not been elucidated fully, these findings indirectly support the hypothesis that sun exposure may have an antimelanoma effect through activation of the vitamin D system, as proposed for other cancer types.<sup>48,49</sup>

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