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Vitamin D receptor polymorphisms and survival in patients with cutaneous melanoma: a population-based study

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Abstract

Factors known to affect melanoma survival include age at presentation, sex and tumor characteristics. Polymorphisms also appear to modulate survival following diagnosis. Result from other studies suggest that vitamin D receptor (VDR) polymorphisms (SNPs) impact survival in patients with glioma, renal cell carcinoma, lung, breast, prostate and other cancers; however, a comprehensive study of VDR polymorphisms and melanoma-specific survival is lacking. We aimed to investigate whether *VDR* genetic variation influences survival in patients with cutaneous melanoma. The analysis involved 3566 incident single and multiple primary melanoma cases enrolled in the international population-based Genes, Environment, and Melanoma Study. Melanoma-specific survival outcomes were calculated for each of 38 *VDR* SNPs using a competing risk analysis after adjustment for covariates. There were 254 (7.1%) deaths due to melanoma during the median 7.6 years follow-up period. *VDR* SNPs rs7299460, rs3782905, rs2239182, rs12370156, rs2238140, rs7305032, rs1544410 (BsmI) and rs731236 (TaqI) each had a statistically significant (trend *P* values < 0.05) association with melanoma-specific survival in multivariate analysis. One functional SNP (rs2239182) remained significant after adjustment for multiple testing using the Monte Carlo method. None of the SNPs associated with survival were significantly associated with Breslow thickness, ulceration or mitosis. These results suggest that the *VDR* gene may influence survival from melanoma, although the mechanism by which VDR exerts its effect does not seem driven by tumor aggressiveness. Further investigations are needed to confirm our results and to understand the relationship between VDR and survival in the combined context of tumor and host characteristics.

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Abbreviations

GEM	Genes, Environment, and Melanoma
HR	hazard ratio
LD	linkage disequilibrium
SNP	single-nucleotide polymorphism
VDR	vitamin D receptor

Introduction

The 5-year survival rate of melanoma ranges from 98% for localized disease to <20% in patients with distant metastases at the time of diagnosis (1,2). Factors known to affect progression and survival include age at presentation; sex (3–5); anatomic site of the tumor (6); primary tumor characteristics, such as tumor thickness, presence of ulceration and presence of mitoses; and presence or absence of nodal or distant metastases at the time of diagnosis (7). In addition, host genetic factors also appear to have an effect on outcome (8–14).

The major circulating form of vitamin D, 25-hydroxyvitamin D, is inversely related to incidence and mortality of several cancers, including colorectal, lung, breast and prostate cancer (15–28). In support of its potential tumor-suppressor action, this hormone has been shown to suppress cell adhesion and migration (29,30), induce apoptosis (31) and suppress growth of melanoma cells *in vitro* and in xenografts (30,32–34). Observational studies have also suggested that vitamin D and its putative surrogates, such as season, geographic latitude and evidence of continuous sun exposure, are associated with more favorable outcomes in individuals with melanoma, despite the fact that exposure to ultraviolet radiation, which activates the precursor of vitamin D present in skin, increases the risk for developing melanoma (6,35–37).

The biologically active form of Vitamin D, 1,25-dihydroxyvitamin D, exerts its effects through binding to the nuclear Vitamin D receptor (VDR), which in turn regulates the transcription of many other genes. VDR expression is decreased in several advanced solid tumors, and higher VDR expression has been associated with better survival in patients with lung (38,39) and breast cancer (40,41). In melanoma, VDR expression was lower in tumors relative to that in nevi or normal skin, with a marked reduction of expression in vertical versus radial growth phases (42). VDR expression has also been inversely associated with tumor progression (43), indicating that VDR signaling pathway may be relevant in preventing melanocytic progression. VDR expression and function can be modified by epigenetic and genetic changes (44,45).

The *VDR* gene contains numerous variants, some of which are hypothesized to influence the expression, stability or downstream transactivation by the translated protein. Only a few common polymorphisms in the *VDR* gene have been included in most studies to date, and reports suggest that some variants might modify disease-specific outcomes in patients with breast cancer (46), lung cancer (47,48), renal cell carcinoma (49), ovarian cancer (50), prostate cancer (51,52), head and neck cancers (53,54), colorectal cancer (26) and glioma (55); however, others reported no effect (56–58). In melanoma patients, Newton-Bishop *et al.* (36) investigated the *VDR* variants Cdx-2, GATA, FokI, BsmI, ApaI and TaqI in a cohort of 872 cases and found no main effect on overall survival, although the authors concluded that BsmI and polymorphisms in high linkage disequilibrium (LD) with this single-nucleotide polymorphism (SNP) modified the risk for relapse in individuals with lower levels (≤50.4 nm/l) of serum Vitamin D. We have previously carried out the analysis of 38 common SNPs in relation to melanoma risk (59). Here, we investigate their influence on prognosis. To our knowledge, this is the first study addressing the effect of a comprehensive set of common *VDR* polymorphisms on melanoma-specific survival in a large population-based study.

Materials and methods

Study subjects

Subjects were recruited between 1998 and 2003 into an international multicenter population-based study of melanoma, the Genes, Environment and Melanoma (GEM) Study. The GEM study population, identified in eight population-based registries in Australia, Canada, Italy and the USA, and one hospital center in the USA, consists of incident single primary melanoma cases (n = 2361) and incident multiple primary melanoma cases (n = 1205). Details of the study design and its rationale have been published (60,61). The human research oversight committees at each of the GEM study sites approved the study protocol. The sites include those at the British Columbia Cancer Agency, Vancouver, British Columbia, Canada; Cancer Care Ontario, Toronto, Ontario, Canada; Center for Cancer Prevention, Turin, Italy; Memorial Sloan Kettering Cancer Center, New York, NY; Menzies Cancer Center, Hobart, Tasmania, Australia; University of California, Irvine, CA; University of Michigan, Ann Arbor, MI; University of North Carolina, Chapel Hill, NC and University of Sydney, Sydney, New South Wales, Australia. All the participants signed written informed consent.

Outcomes

A thorough search for deaths and their causes was completed at each ascertainment center, and the information on vital status (alive at the end of follow-up, dead of disease or dead of other causes) was obtained for all the participating individuals with melanoma. Patient follow-up for vital status ended on 31 December 2007 in all centers, except for British Columbia and Turin, where follow-up period ended on 31 December 2008. Date and cause of death were obtained from National Death Indexes (62,63), cancer registries and municipal records. Cases were considered alive if not in the National Death Index.

Genotyping

All aspects of the genotyping pipeline in GEM including selection of *VDR* SNPs, DNA extraction, genotyping details and quality control procedures have been described (59). Briefly, we included SNPs with known or suspected impact on the transcription, stability and/or activity of the VDR; SNPs reported as significant in other association studies and the minimal set of tagging SNPs described among Caucasians by others (64,65) with minor allele frequency >10% in Caucasians. Germline (buccal) DNA was genotyped with the Sequenom MassARRAY iPLEX genotyping Platform (Sequenom, San Diego, CA, currently Agena Bioscience) to test 36 SNPs and with pyrosequencing and melting temperature analysis to test 2 SNPs, for a total of 38 SNPs (59). Standard quality control procedures were implemented and included, among others, the use of internal laboratory controls and 5–10% randomly selected repeats. A plate containing discordant results for at least one pair of duplicate samples or internal controls was newly assayed, thus complete concordance between replicas was achieved. Assays were considered optimal according to degree of clustering, specificity and reproducibility. Hardy–Weinberg equilibrium was calculated to identify major genotyping issues (59). However, as the cohort consists solely of cases with the disease absence of Hardy–Weinberg equilibrium could be due to the SNP conferring risk for the disease. We also evaluated the potential functional relevance of the investigated *VDR* SNPs in relation to known and predicted regulatory elements in the intergenic regions of the human genome. Known and predicted regulatory DNA elements including regions of deoxyribonuclease hypersensitivity, binding sites of transcription factors and promoter regions have been biochemically characterized to regulate transcription by accessing publicly available data using the RegulomeDB as a source of information from the public Gene Expression Omnibus database, the ENCODE project, and published literature (66).

Analysis of survival outcomes

Survival outcomes were calculated for each SNP using competing risk models. The endpoint was either date of death or end of follow-up for the censored patients. Patients were censored at the time of death from other (non-melanoma) causes as recorded in the vital records. There was no loss of follow-up based on the study design. Since the parent study involved population-based ascertainment of incident single primary melanoma cases and incident multiple primary melanoma cases, survival time was accumulated from the diagnosis date of the index lesion; this is the date of the first primary for patients with single primary lesions and the date of the index (more recent) lesion for patients with multiple primaries.

Analysis of associations between *VDR* genotypes and survival

Proportionality was evaluated for each SNP using a competing risk model in which the constancy of the time dependency of the survival rate ratio of wild-type versus non-wild-type genotypes was tested individually. For each SNP, we arbitrarily refer to the least frequently occurring allele as the variant allele, and the most common allele as the referent allele. Log-additive trend tests were used to assess the statistical significance of the per allele hazard ratios (HRs), which represents the risk associated with carriage of each additional variant allele.

We found no differences in the effects of *VDR* SNPs on survival by primary status (single versus multiple primary melanoma), and therefore the associations of *VDR* SNPs with survival were examined in all patients in order to improve precision, while adjusting for primary status as described elsewhere (67,68).

Our primary analytic strategy involved analyses in which we tested the associations of the SNPs with melanoma-specific survival adjusted for factors that have the potential to confound the association of VDR genotype with survival. To account for the competing risk of death from other causes, we performed proportional subdistribution hazards regression models according to ref. (69) to assess the effects of covariates on the subdistribution hazard for death as a result of melanoma. We adjusted for age, sex, anatomic site of the primary and study design variables (study center, case–control status and a time-dependent covariate for single primary melanomas who developed subsequent melanoma) in our analysis. For those who developed multiple primary lesions, the anatomic site and pathological characteristics of the deepest tumor were used in the analysis. It is biologically plausible that Vitamin D may act via components of tumor stage; however, tumor stage was missing in one-third of our cases. Instead, Breslow thickness, which was highly correlated with tumor stage (r^2 = 0.91), was included as a surrogate for tumor stage. We tested for potential associations between *VDR* SNPs and Breslow thickness using Spearman rank correlation tests and explored the associations between *VDR* SNPs and melanoma death in a second multivariable model that included Breslow thickness. We also tested the associations between *VDR* SNPs and other known markers of melanoma progression including mitosis and ulceration, using the Wilcoxon Rank Sum test for participants without missing data for these variables.

We employed the Monte Carlo approach to adjust for multiple testing (70,71). The joint distribution of the test statistics for the SNPs was first evaluated by an efficient Monte Carlo procedure, and the joint distribution was then used to determine the multiple-testing adjusted significance threshold. This approach properly accounts for the LD between SNPs and is slightly less conservative than the traditional Bonferroni correction method.

Analysis of associations between haplotypes and survival

We investigated the effect of haplotypes within blocks 1–6 (Supplementary Figure S1, available at *Carcinogenesis* Online) as determined by Haploview (72). We also investigated the previously published haplotype rs11568820–rs2228570–rs144410 (Cdx2–FokI–BsmI) (47,48) by using PHASE version 2.1 software to impute haplotype frequencies (73,74). Each GEM patient was assigned the haplotype with the highest probability and patients in these haplotype groupings were compared collectively with respect to survival.

Analyses were performed using SAS statistical software version 9.2 (SAS Institute, Cary, NC). The competing risk models was implemented using the mstate package in R statistical software (Vienna, Austria) (75), and the Monte Carlo procedure was implemented using the R scripts provided by the He *et al*. (70). All tests were two-tailed and *P* values of <0.05 were deemed statistically significant, unless multiple comparisons were considered.

Results

There were 3566 cases consisting of 1562 females and 2004 males. During the follow-up period (range 0.4–10.6 years, median 7.6 years), there were 562 (15.7%) deaths, of which 254 (7.1%) were due to melanoma. Of these 254 deaths, 103 (40.6%) occurred following a diagnosis of second or higher order melanoma. Adjusted risks of death from melanoma for host and tumor characteristics of GEM study participants are presented in Table 1. Risk for dying from melanoma was increased in older individuals, men, individuals with thicker tumors and those with melanomas on the head and neck. No differences in survival were observed comparing cases with single primary to those with multiple primary melanoma (HR 0.99, 95% confidence interval: 0.75–1.31, *P* = 0.95). When including Breslow thickness of the deepest primary melanoma in the model, age

Table 1. Effect of demographic and clinicopathologic characteristics of melanoma patients in the GEM study on disease-specific mortality

Variable	*N* (%)	*N* events	HR[a]	95% CI[a]	*P* value[a]
Gender					
Male	2004 (56.2)	183	1		<0.001
Female	1562 (43.8)	71	0.57	0.43–0.76	
Age at diagnosis					
Continuous	—		1.03	1.02–1.04	<0.001
Site					
Head/neck	575 (16.1)	76	1		<0.001
Trunk/pelvis	1580 (44.3)	107	0.55	0.41–0.75	
Arms	665 (18.7)	34	0.47	0.31–0.70	
Legs	746 (20.9)	37	0.51	0.34–0.78	
Histology					
SSM	2294 (64.3)	105	1		<0.001
NM	332 (9.3)	70	4.34	3.19–5.92	
LM	364 (10.2)	18	0.84	0.51–1.40	
NOS	495 (13.9)	40	1.83	1.26–2.67	
Other	81 (2.3)	21	5.20	3.23–8.36	
Breslow thickness					
0.01–1.00	2223 (62.3)	44	1		<0.001
1.01–2.00	723 (20.3)	79	5.55	3.83–8.04	
2.01–4.00	359 (10.1)	75	10.44	7.16–15.22	
>4.00	175 (4.9)	52	15.50	10.32–23.29	
Missing	86 (2.4)	4			
Ulceration					
Absent	2475 (69.4)	140	1		<0.001
Present	262 (7.4)	69	4.81	3.58–6.47	
Missing	829 (23.2)	45			
Mitoses					
Absent	1520 (42.6)	41	1		<0.001
Present	1227 (34.4)	169	5.05	3.59–7.11	
Missing	819 (23.0)	44			

Patients who entered the study with single primary melanoma and developed a subsequent melanoma were treated as time dependent. LM, lentigo maligna; NM, nodular melanoma; NOS, not otherwise specified; SSM, superficial spreading melanoma.

[a]Adjusted for study center, presence of multiple primary melanomas, time-dependent crossover status (patients who entered the study with single primary melanoma and developed a subsequent melanoma) and age at diagnosis of the first primary melanoma and sex.

and anatomic site remained significantly associated with melanoma death adjusted for all study design features.

VDR SNPs and tumor characteristics

There was little evidence of association between the 38 *VDR* SNPs and tumor markers of progression. Only one SNP (rs1989969) was associated with tumor thickness ($P = 0.04$), but this SNP was not associated with survival. No significant associations were found between any of the studied SNPs with either ulcerations or mitosis (Supplementary Table S1, available at *Carcinogenesis* Online).

VDR SNPs and melanoma-specific death

All *VDR* SNP genotype frequencies were in Hardy–Weinberg equilibrium. Table 2 shows the per allele subdistribution HRs for each individual SNP for melanoma-specific death in Fine and Gray models for the subdistribution of competing risks. With few exceptions, the models were observed to satisfy the proportional hazards assumption. Individually, SNPs rs7299460, rs3782905, rs2239182, rs12370156, rs2238140, rs7305032, rs1544410 (BsmI) and rs731236 (TaqI) were significantly associated with melanoma-specific survival after accounting for competing risk of death from other causes and after adjusting for age at first diagnosis, sex, anatomic site and study design features (Table 2). Some of the SNPs are in strong LD. The LD plot showing all 38 SNPs is provided in Supplementary Figure S1, available at *Carcinogenesis* Online. The SNP with the most statistically significant association exceeded the Monte Carlo multiple comparison threshold (rs2239182, P value = 0.0018). Publicly available data reveal that rs2239182 overlaps with the binding site of the FOXA1 protein and that this SNP affects VDR expression (76). When analyses were repeated additionally adjusting for tumor thickness, five SNPs were significantly associated with melanoma death (rs4760674, rs2239182, rs7305032, rs1544410 and rs731236), and one SNP had a borderline significant association (rs2189480); in total, four SNPs overlapped with those in our primary analysis, including rs2239182.

VDR haplotypes and melanoma-specific survival

We investigated the association between *VDR* haplotypes defined by the six haplotype blocks (Supplementary Figure S1, available at *Carcinogenesis* Online) and melanoma-specific death adjusting for competing risk of death from other causes. The haplotype C–C–G formed by rs12370156–rs2238140–rs7305032 was associated with death of melanoma after adjusting for covariates (per haplotype HR: 1.22, 95% confidence interval: 1.02–1.45), and the global test of association was significant ($P = 0.04$) (Supplementary Table S2, available at *Carcinogenesis* Online). We also investigated the association between previously published rs11568820/Cdx-2, rs2228570/FokI and rs144410/BsmI allelotypes and melanoma-specific death and found no significant associations (Supplementary Table S3, available at *Carcinogenesis* Online).

Discussion

In this comprehensive evaluation of common *VDR* polymorphisms in relation to melanoma survival in a large cohort of melanoma cases, we found nominally significant associations between eight SNPs mostly located on the coding region [rs7299460, rs3782905, rs2239182, rs12370156, rs2238140, rs7305032, rs1544410 (BsmI) and rs731236 (TaqI)] and melanoma-specific death, and one SNP (rs2239182) remained significant after adjustment for multiple testing. Rs2239182 is known to have functional importance, and thus, our results are biologically plausible considering that vitamin D exerts a variety of tumor suppression effects through its receptor (29–34), VDR expression has been linked to improved survival in other cancers (38–41) and to melanoma progression (42,43) and frequent polymorphisms can, in principle, have a modest to moderate effect on its function.

None of the SNPs that we found to be associated with melanoma-specific survival were associated with Breslow thickness, ulceration or mitosis, indicating that the possible effect of *VDR* SNPs on survival does not occur directly through these tumor features. Considering the wide distribution of VDR throughout the body and that its ligand (vitamin D) has a plethora of effects including, among many others the regulation of adaptive and innate immune system (77,78), it is possible to propose that VDR modulates the tumor microenvironment and exerts its effect though other (non-melanoma) cell types (79).

There is limited published data on the relationship between VDR polymorphisms and outcomes in melanoma patients, and other investigations included small cohorts, few polymorphisms and used tumor thickness (80–83), metastasis (80) or relapse and overall survival as endpoints (36). Among these, the largest study conducted by Newton-Bishop *et al.* (36), investigated five (Cdx-2, FokI, BsmI, TaqI and rs4516035) of the 38 SNPs reported here and 1 SNP (rs7975232) that we did not genotype in a prospective study of 872 individuals with melanoma. Their study found no main effect of *VDR* genotypes on relapse or overall survival, although the authors found increased risk for relapse in carriers of the BsmI A allele in patients with low serum vitamin D levels (36). Similar to Newton-Bishop *et al.*, we found no effect for FokI (rs2228570). In contrast, our study found the BsmI A allele and TaqI 't'(C) allele to be protective with regard to melanoma death (Table 2). It is possible that the results differ between these two studies due to the study populations with different proportion of cases with thin (<1 mm) tumors (62.3% versus 36.3%), follow-up time (7.6 versus 4.7 years), selected endpoint and statistical power. As we did not measure circulating levels of vitamin D, it is not possible to assess its potential modifying effect on genetic associations in the GEM study. Newton-Bishop *et al.* did not genotype the functional SNP with the strongest association in GEM, rs2239182.

In a prior study of 316 melanoma cases and *VDR* in relation to Breslow tumor thickness as outcome, Hutchinson *et al.* (81) reported that the combined TaqI and FokI variant alleles 'ttff' (CCTT) increased the risk for having tumors >1.5 mm. Santonocito *et al.* (82) investigated the effect of BsmI, FokI and rs4516035 (A-1012G) in 100 cases and reported a significant association between the BsmI variant 'b' (G) and tumors >1.5 mm. In contrast, two studies, one of 1001 melanoma cases (84), that investigated Cdx-2, FokI, BsmI, ApaI, rs4516035 and TaqI, and another study that investigated four *VDR* SNPs (TaqI, rs757343, rs2107301 and rs7975232) in 305 melanoma cases (83) found no associations with Breslow thickness, findings in agreement with the present study.

The intronic BsmI is one of the most studied *VDR* SNPs, although the observed functional impact of this polymorphism is not consistent across investigations (45). This SNP is in high LD with TaqI (rs731236), a silent SNP that localizes to a nucleotide conserved across species, and like BsmI, is also inversely associated with death from melanoma in this study.

To the best of our knowledge, several of the significant SNPs found in our study (coding region rs3782905, rs2239182, rs12370156, rs2238140, rs7305032 and promoter region rs7299460) have not been previously reported in relation to melanoma survival or Breslow thickness as the outcomes. The most significant

Table 2. Subdistribution hazard ratios according to *VDR* variants for death of melanoma in GEM accounting for competing risk of death from other causes

Relative 5′ > 3′ position, RefSeq		Chr12: position	Genotype	Total *N* (*N* events)	Per allele adjusted sHR (95% CI)[a]	Trend *P* value[a]	Per allele adjusted sHR (95% CI)[b]	Trend *P* value[b]
Promoter region								
1	rs2071358	46652716	CC	2423 (178)				
			CA	965 (64)				
			AA	121 (4)	0.85 (0.67–1.07)	0.170	0.81 (0.63–1.03)	0.111
2	rs10875712	46649520	GG	1419 (103)				
			GC	1587 (98)				
			CC	493 (45)	1.07 (0.89–1.30)	0.472	1.12 (0.92–1.36)	0.277
3	rs6823	46648679	CC	1102 (78)				
			CG	1673 (118)				
			GG	737 (54)	1.03 (0.86–1.23)	0.752	1.06 (0.89–1.28)	0.501
4	**rs4760674**	46643281	CC	1350 (94)				
			CA	1634 (107)				
			AA	541 (48)	1.11 (0.92–1.34)	0.270	**1.22 (1.01–1.47)**	**0.042**
5	rs1015390	46630305	CC	2581 (192)				
			CT	862 (51)				
			TT	83 (5)	0.81 (0.61–1.07)	0.134	0.84 (0.63–1.12)	0.233
6	rs4237856	46624317	AA	1977 (131)				
			CA	1279 (97)				
			CC	230 (17)	1.11 (0.91–1.35)	0.308	1.02 (0.84–1.24)	0.824
7	rs4073729	46623336	CC	2567 (189)				
			TC	874 (57)				
			TT	80 (3)	0.82 (0.63–1.07)	0.145	0.84 (0.64–1.11)	0.221
8	rs11168314	46616896	GG	2276 (173)				
			GA	1067 (69)				
			AA	164 (8)	0.80 (0.64–1.01)	0.060	0.84 (0.67–1.06)	0.150
9	rs10459217	46602528	TT	2230 (168)				
			TC	1120 (73)				
			CC	160 (9)	0.85 (0.68–1.07)	0.166	0.88 (0.69–1.11)	0.280
10	rs11568820 Cdx-2	46588812	GG	2193 (164)				
			AG	822 (57)				
			AA	137 (7)	0.85 (0.67–1.09)	0.203	0.88 (0.68–1.12)	0.297
11	rs7139166	46586601	CC	1162 (78)				
			GC	1616 (108)				
			GG	682 (55)	1.12 (0.93–1.33)	0.224	1.16 (0.96–1.41)	0.114
12	rs4516035 –1012|GATA	46586093	TT	1166 (76)				
			CT	1661 (117)				
			CC	687 (54)	1.13 (0.95–1.34)	0.182	1.16 (0.96–1.40)	0.116
13	**rs7299460**	46582535	CC	1734 (136)				
			CT	1421 (96)				
			TT	345 (16)	**0.80 (0.66–0.97)**	**0.021**	0.85 (0.70–1.03)	0.091
14	rs11168287	46571681	GG	903 (67)				
			AG	1710 (120)				
			AA	882 (59)	0.97 (0.81–1.15)	0.718	1.01 (0.83–1.22)	0.949
15	rs11168284	46569316	AA	1439 (111)				
			GA	1628 (112)				
			GG	430 (24)	0.87 (0.72–1.04)	0.134	0.86 (0.71–1.06)	0.154
16	rs10875694	46567927	TT	2454 (176)				
			TA	978 (68)				
			AA	119 (7)	0.94 (0.75–1.19)	0.605	0.95 (0.75–1.21)	0.695
17	rs4760648	46566932	CC	1100 (87)				
			CT	1776 (119)				
			TT	639 (44)	0.93 (0.77–1.11)	0.398	0.94 (0.77–1.14)	0.528
18	rs2238135	46564457	GG	2024 (147)				
			GC	1281 (88)				
			CC	227 (15)	0.95 (0.78–1.17)	0.650	0.96 (0.78–1.18)	0.672
19	rs1989969	46564277	CC	1308 (81)				
			CT	1667 (124)				
			TT	565 (46)	1.14 (0.96–1.36)	0.128	1.14 (0.95–1.37)	0.153
Coding region								
20	rs2228570 FokI	46559162	CC (FF)	1258 (98)				
			CT (Ff)	1616 (98)				
			TT (ff)	514 (37)	0.93 (0.76–1.13)	0.467	0.96 (0.77–1.19)	0.682

Table 2. *Continued*

Relative 5′ > 3′ position, RefSeq		Chr12: position	Genotype	Total *N* (*N* events)	Per allele adjusted sHR (95% CI)[a]	Trend *P* value[a]	Per allele adjusted sHR (95% CI)[b]	Trend *P* value[b]
21	rs11168275	46558542	AA	2029 (152)				
			GA	1302 (81)				
			GG	218 (18)	0.97 (0.78–1.20)	0.756	0.96 (0.77–1.20)	0.701
22	rs7974708	46556432	TT	1478 (125)				
			CT	1637 (96)				
			CC	412 (30)	0.83 (0.68 – 1.02)	0.078	0.87 (0.70–1.07)	0.176
23	**rs3782905**	46552434	CC	1603 (132)				
			CG	1562 (96)				
			GG	365 (22)	**0.80 (0.65–0.98)**	**0.031**	0.84 (0.67–1.04)	0.100
24	rs2189480	46550095	CC	1453 (89)				
			CA	1575 (117)				
			AA	471 (38)	1.17 (0.98–1.40)	0.082	1.18 (0.98–1.42)	0.076
25	rs886441	46549231	TT	2297 (157)				
			CT	1066 (80)				
			CC	149 (11)	1.09 (0.88–1.36)	0.429	1.06 (0.83–1.34)	0.650
26	rs2239181	46542216	TT	2817 (201)				
			GT	672 (48)				
			GG	33 (1)	0.97 (0.73–1.29)	0.821	1.04 (0.77–1.41)	0.788
27	rs2107301	46541837	CC	1842 (125)				
			CT	1416 (98)				
			TT	267 (28)	1.18 (0.97–1.44)	0.094	1.10 (0.89–1.35)	0.365
28	**rs2239182**	46541678	GG	965 (51)				
			AG	1676 (121)				
			AA	816 (72)	**1.33 (1.12–1.58)**	**0.001**	**1.25 (1.05–1.49)**	**0.013**
29	**rs12370156**	46540400	TT	946 (55)				
			CT	1708 (126)				
			CC	866 (69)	**1.19 (1.00–1.41)**	**0.046**	1.15 (0.96–1.38)	0.136
30	**rs2238140**	46538931	TT	944 (54)				
			CT	1719 (127)				
			CC	866 (69)	**1.19 (1.01–1.42)**	**0.042**	1.15 (0.96–1.39)	0.127
31	**rs7305032**	46536127	AA	928 (54)				
			AG	1730 (130)				
			GG	670 (55)	**1.22 (1.02–1.46)**	**0.028**	**1.22 (1.01–1.48)**	**0.042**
32	**rs1544410 BsmI**	46526102	GG (bb)	1229 (100)				
			GA (bB)	1625 (107)				
			AA (BB)	562 (31)	**0.78 (0.65–0.94)**	**0.008**	**0.79 (0.64–0.96)**	**0.017**
33	**rs731236 TaqI**	46525024	TT (TT)	1263 (105)				
			TC (Tt)	1633 (107)				
			CC (tt)	594 (33)	**0.78 (0.65–0.94)**	**0.010**	**0.81 (0.67–0.99)**	**0.037**
3′ UTR								
34	rs11574139	46521822	AA	3275 (238)				
			TA	247 (11)				
			TT	9 (1)	0.70 (0.39–1.26)	0.237	0.67 (0.36–1.24)	0.205
35	rs7965281	46517877	GG	810 (48)				
			AG	1840 (133)				
			AA	694 (54)	1.18 (0.97–1.43)	0.098	1.15 (0.94–1.42)	0.171
36	rs2544027	46502796	CC	977 (74)				
			CT	1664 (118)				
			TT	892 (58)	0.93 (0.79–1.10)	0.415	0.92 (0.78–1.10)	0.373
37	rs2544028	46502697	TT	1234 (90)				
			TA	1626 (121)				
			AA	654 (38)	0.92 (0.78–1.09)	0.323	0.94 (0.79–1.12)	0.468
38	rs2544038	46501500	TT	1112 (82)				
			CT	1637 (115)				
			CC	752 (49)	0.94 (0.79–1.11)	0.460	0.93 (0.78–1.11)	0.403

Patients who entered the study with single primary melanoma and developed a subsequent melanoma were treated as time dependent. SNP position on Chr12 is shown for the hg19. Statistically significant associations ($P < 0.05$) are shown in bold. 3′ UTR, 3′ untranslated region; Chr12, Chromosome 12; CI, confidence interval; hg19, human genome version 19; *N*, number; sHR, subdistribution hazard ratio; SNP, single-nucleotide polymorphism.

[a]Adjusted for age at first diagnosis, sex, anatomic site of the deepest primary melanoma and study design features (study center, presence of multiple primary melanoma, time-dependent crossovers).

[b]Adjusted for age at first diagnosis, sex, anatomic site of the deepest primary melanoma, Breslow thickness and study design features (study center, presence of multiple primary melanoma, time-dependent crossovers).

SNP in this study (rs2239182) is located in the coding region of the *VDR* gene. This locus is often found near active regulatory elements, and eQTL analysis indicates that rs2239182 affects VDR expression in a *cis* fashion (66,76), which further supports our observed association, although future studies will have to confirm the direction of the effect in melanoma tumors. SNP rs2239182 overlaps with the binding site of the FOXA1 protein. Of interest, in a recent whole transcriptome RNA-seq analysis of melanoma-derived cell lines, this transcription factor was listed in the top cancer-associated category and can interact with either transcription factors or cytokines known to be involved in the development or progression of other malignancies (85).

We reported previously eight of these *VDR* SNPs significantly associated with melanoma risk (59). When we compared these candidate melanoma 'susceptibility' SNPs with those found in our present analysis of melanoma-specific survival only two overlap: rs7305032, associated with worse survival but with lower melanoma risk and BsmI, associated with improved survival but higher risk of melanoma. An adverse effect on risk allied to improved survival and vice versa has also been observed in other studies (55,86). It is possible that *VDR* plays different roles in melanoma initiation and in progression (e.g. via tumor suppression versus cell migration, inflammation or other processes), depending on the cell context and the availability of transcription factors, in which case polymorphisms that predict disease outcome might be expected to differ from those that predict melanoma risk.

The strengths of our study include its large size that allows us to detect relatively small HRs, the population-based nature of the study, geographically diverse populations that allow our results to be generalized and inclusion of 38 SNPs spanning the promoter to the 3′ untranslated region of the gene, which constitutes a more comprehensive coverage of the gene than previously reported. A limitation of our study is that we did not study polymorphisms in other genes involved in the vitamin D pathway, which might also play a role in melanoma survival.

In conclusion, we found that several SNPs mostly located in the coding region of the *VDR* gene, including a functional SNP that exceeded the multiple comparisons threshold, were associated with melanoma-specific survival. Although the observed genetic effect size is modest, our results provide evidence in support of the hypothesis that variants in the vitamin D pathway may play an important role in melanoma survival. Our finding that the SNPs are not associated with Breslow thickness, ulceration or mitosis, indicates that the mechanism by which VDR may exert its effect does not seem driven locally by these tumor characteristics. Although an effect on a particular yet undefined etiologic melanoma subtype should not be ruled out, it is also possible that VDR modulates survival by a systemic mechanism. Further studies are needed to confirm our findings and investigate the role of the vitamin D pathway, including potential downstream effectors, in relation to melanoma outcome in the context of tumor and host characteristics.

Supplementary material

Supplementary Tables 1–3 and Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

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