Vitamin D Receptor Polymorphisms Are Associated with Altered Prognosis in Patients with Malignant Melanoma

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ABSTRACT
Calcitriol [1,25(OH)2 D3], the hormonal derivative of vitamin D3, is an antiproliferative and prodifferentiation factor for several cell types, including cultured melanocytes and malignant melanoma (MM) cells. Several polymorphisms of the vitamin D receptor (VDR) gene have been described including a FokI RFLP in exon 2, BsmI, and ApoI polymorphisms in intron 8 and an adjacent TaqI RFLP in exon 9. Alterations in vitamin D/1,25(OH)2 D3 levels and polymorphisms of the VDR have been shown to be associated with several systemic malignancies. We hypothesize that polymorphism in this gene may be associated with altered susceptibility and outcome in patients with MM. A hospital-based case-control study, using 316 MM cases and 108 controls, was used to assess associations with MM susceptibility. Breslow thickness, the most important single prognostic factor for patients with cutaneous MM (3), was used as the outcome measure. Polymorphisms at the FokI and TaqI restriction sites were determined using PCR-based methods. Polymorphism at the FokI, but not TaqI, RFLP was associated with an altered risk of MM (P = 0.014). More importantly, variant alleles were associated with increased Breslow thickness. Thus, homozygosity for variant alleles at both RFLP (tt genotype combination) was significantly associated with thicker tumors. (≥3.5 mm; P = 0.001; odds ratio = 31.5). Thus, polymorphisms of the VDR gene, which would be expected to result in impaired function, are associated with susceptibility and prognosis in MM. These data suggest that 1,25(OH)2 D3, the ligand of the VDR, may have a protective influence in MM, as has been proposed for other malignancies.

INTRODUCTION

MM4 is the most serious cutaneous malignancy, and the prognosis of some tumors is very poor (1, 2). It is predominantly a disease of white-skinned people, and exposure to UV light is thought to be critical, although the relationship between risk and exposure is unclear (2). Other important risk factors for the occurrence of MM include presence of excessive numbers of banal nevi, multiple atypical nevi, fair skin, red hair, and blue or green eyes.

Breslow thickness at presentation remains the most important single prognostic factor for patients with cutaneous MM (3). In general, patients with thin tumors have a much longer survival than those with thick lesions; the 5-year survival rate for lesions <1.5-mm thick is 93%, compared with 67% for 1.5 mm-3.49 mm and 37% for ≥3.5 mm (4). Risk factors for thicker tumors, and hence poorer prognosis, include age at initial presentation and tumor site.

Relatively little is known of the genetic factors that mediate susceptibility to, and outcome of, sporadic MM. Several putatively important genes, including the susceptibility genes melanocyte stimulating hormone receptor (5, 6), glutathione S-transferase GSTM1 (7), and cytochrome P450 CYP2D6 (8, 9) as well as the cancer candidate genes, p16INK4a and p15INK4b (10), have been studied, although thus far only the CYP2D6 PM genotype has been associated with increased risk in independent studies.

We propose that the VDR gene may influence susceptibility and outcome in MM. This view is supported by data showing that 1,25(OH)2 D3 (the hormonal derivative of vitamin D3 and the ligand of the VDR) has antiproliferative and prodifferentiation effects in VDR-expressing cell types (11–14). Furthermore, associations have been identified between 1,25(OH)2 D3 and susceptibility to, and outcome of, systemic malignancies such as breast, prostate, and colon. These include association with both serum vitamin D/1,25(OH)2 D3 levels as well as with polymorphisms in the VDR gene (15–19). Similar supportive data exist for MM. Thus, melanocytes and MM cells express the VDR, and 1,25(OH)2 D3 has an antiproliferative effect in vitro (20, 21). For example, stimulation of tyrosinase activity, a specific prodifferentiation stimulus, has been reported in melanocytes exposed to 1,25(OH)2 D3 (21). In vivo, there is currently little evidence of involvement of vitamin D3, although low serum levels of 1,25(OH)2 D3 have been reported in

4The abbreviations used are: MM, malignant melanoma; VDR, vitamin D receptor; OR, odds ratio; 95% CI, 95% confidence interval; calcitriol, 1,25(OH)2 D3; CDK, cyclin-dependent kinase.

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patients with MM (22). The role of sun exposure in MM is unclear. Current literature remains controversial, with most clinicians advocating a causative association between UV exposure and risk, whereas other studies support the view of a possible protective effect of vitamin D (generated at least in part by UV). For example, use of sunscreens is associated with increased MM risk, an all-year tan appears protective, and outdoor occupation appears to demonstrate no association with susceptibility to MM.

Five polymorphic sites have been identified in the VDR. These comprise RFLP in exon 2 (FokI restriction site), the last intron (BsmI and Apal restriction sites), and an adjacent area of exon 9 (TaqI restriction site) as well as a poly(A) microsatellite length polymorphism in the 3′ untranslated region. The FokI polymorphism results in an altered translation start site and has been shown to be functionally relevant (23). The other four sites demonstrate linkage disequilibrium, and there is evidence to suggest functional consequences of these polymorphisms (24).

Because these data support the view that polymorphism in the VDR gene may be an important determinant of susceptibility and outcome in patients with MM, the aim of the present study was to investigate the relationship between the VDR polymorphisms and susceptibility to and prognosis (as estimated by Breslow thickness) of MM. Because there is no evidence of linkage disequilibrium between the FokI RFLP and the cluster of polymorphisms at the 3′ end of the gene and there is evidence to suggest functional consequences of each of these polymorphic regions, we have concentrated on the FokI polymorphism and a representative example of the 3′ cluster (TaqI RFLP).

PATIENTS AND METHODS

Patients. All MM cases (n = 316) were of Northern European Caucasian extraction, originally presented between January 1994 and December 1997 and attended the Dermatology Departments at the Leicester Royal Infirmary, North Staffordshire Hospital or Royal Cornwall Hospitals, between 1996 and 1997. All tumors were histologically diagnosed as in situ or invasive MM. Lentigo maligna and lentigo maligna melanoma were not included, in view of the biological singularity of lentigo maligna. Patients with acral tumors or those with MM and other malignant pathologies (cutaneous or internal) were also excluded. We attempted to recruit all eligible patients, although some were randomly lost in busy clinics. None of the subjects approached refused to participate. This cohort comprises ~80% of all eligible patients and represents a typical sample of MM patients presenting to dermatologists in the participating centers. The controls (n = 108) comprised randomly recruited, hospital-based Northern European Caucasians attending these Dermatology departments with basal cell papillomas and without clinical or histological evidence of malignancy. Subjects with a history of inflammatory pathology were also excluded. The study was performed with local Ethical Committee approval, and informed consent was obtained from all of the individuals recruited.

Cases and controls were interviewed by a dermatologist (J. E. O., J. T. L., A. G. S., or P. W. B.). The following demographic and clinical data were recorded: patient age at presentation, gender, skin type in terms of propensity to sun burning and tanning using the Fitzpatrick classification (25), eye and hair color at age 21 years, tumor site, and Breslow thickness. Breslow thickness (defined as the vertical thickness of the tumor from the granular layer of the epidermis to the deepest part of the melanoma) was determined by specialist pathologists. On the basis of Breslow thickness, patients were divided into five categories: in situ, <0.75 mm, 0.75–1.49 mm, 1.5–3.49 mm, and ≥3.5 mm. Table 1 shows the distribution of these clinical parameters in the total case group. As also indicated in Table 1, complete clinical data could not be obtained from all patients because of insufficient time in busy clinics (74–95% for TaqI and 72–92% for FokI genotyped cases).

Determination of VDR Genotype. All genotyping assays were performed by workers who were unaware of the clinical status of individual subjects. PCR assays to identify VDR genotypes included one DNA sample (selected at random) of known genotype for each batch of eight samples of unknown genotype, at least one homozygous variant DNA (tt or ff) as a control for restriction enzyme digestion, one negative control (no DNA), and molecular weight markers. Approximately 15% of all patient DNA samples were re-assayed on at least one occasion, and the genotype assignment was confirmed. All assignments were validated by an independent, blinded observer examining the agarose gels. DNA was extracted from peripheral blood (5 ml; collected into EDTA) using standard phenol-chloroform methods. PCR- RFLP based assays were used to identify alleles containing the exon 2 (FokI) and exon 9 (TaqI) variants. Primers were selected based on the methods of Gross et al. (Ref. 26; FokI) and Spector et al. (Ref. 27; TaqI) with modifications. The exon 2 wild-type (f) and variant (t) alleles were identified using primers 3′-AGCTGGCCCTGGCACGTCTCTGCTCT-3′ and 5′-ATGGAAAACACCTTGCTTCTTCTTCCCT- C-3′ to amplify a 265-bp product. Amplification of template DNA was performed in an incubation mixture (total volume, 50 µl) comprising 20 pmol each of primer, 200 µM deoxynucleotide triphosphates, 1.5 mM MgCl₂, and 1 unit of Taq polymerase in buffer containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, and 0.1% (v/v) Triton X-100. The PCR conditions were: initial denaturation (94°C for 3 min), followed by 30 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 30 s), followed by a final extension at 72°C for 5 min. PCR products were then digested with FokI (37°C for 20 h), and the products were examined after electrophoresis in 2% agarose gels. The F allele was refractory to digestion, whereas f was identified by fragments of 196 and 69 bp. The TaqI wild-type (T) and variant (t) alleles were identified using the forward primer from Spector et al. (27), 5′-CAGAGCATGGAGCAAGGAGCAAAG-3′, and a novel reverse primer, 5′-CGCAGCGGATGTCAGTGTCGA-3′, to amplify a 345-bp PCR product. The PCR conditions were as for the FokI RFLP. PCR products were then digested with TaqI (65°C for 20 h), and the products were examined after electrophoresis in 2% agarose gels. The T allele was refractory to digestion, whereas t was identified by fragments of 260 and 85 bp. We attempted to obtain genotype data from all samples, but in some earlier cases, DNA was exhausted or refractory to amplification.

Statistical Analysis. Statistical analysis was undertaken using the Stata software package (version 5.0; Stata Corp., College Station, TX). χ² tests were used to test for homogeneity between and within cases and controls (28). Because some frequencies were small, the StatXact-Turbo statistical package (version 3; Cytel Software Corp., Cambridge, MA) was used to obtain exact significance levels (Pₛ). Logistic regression analysis was used to examine differences between cases and controls while simultaneously
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RESULTS

Three hundred and sixteen patients with MM (mean age ± SD, 53.3 ± 16.7 years; 67% female) and 108 controls (mean age ± SD, 55.7 ± 19.7 years; 50% female) were recruited. Correcting for imbalances in age and gender. Logistic regression was also used to examine differences in genotype frequencies between cases stratified by Breslow thickness, while correcting for age at presentation and gender. Significant associations of combined genotypes (e.g., ttff) were only accepted if they remained significant in the presence of the main effects (i.e., a model including tt, tf, and ff). If the significance of the combined genotype disappeared, this would suggest that the factors were acting independently, and the significance of the combined effect was driven by the strength of either (or both) of the component factors. Associations of Breslow thickness were confirmed using logistic regression after transformation of thickness values to normality and correction for age at presentation and gender. Because some tumors were in situ (0 mm thick), transformation was performed using the formula: ln (Breslow thickness + 1).

Case-Control Analysis. Table 2A shows the allele frequencies of TaqI and FokI alleles in controls and MM cases. All allele frequencies conformed to Hardy-Weinberg equilibrium. The F allele was significantly less common in MM cases than controls (P = 0.029; OR, 0.69; 95% CI, 0.50–0.847). Table 2B shows the relationship between TaqI and FokI genotypes in cases and controls. No significant correlations between genotypes at the two sites were identified in either controls (P = 0.365) or cases (P = 0.847), suggesting that the two polymorphisms did not demonstrate linkage disequilibrium. Table 1 shows frequencies of FokI and TaqI genotypes in controls compared with MM cases. There was a decreased proportion of individuals with the FokI FF genotype in cases versus controls. Thus, for FF versus other FokI genotypes, the uncorrected OR for MM was 0.60 (P = 0.026). The findings remained significant after correction for age and gender using multivariate logistic regression (OR, 0.59; P = 0.029). The estimated risk reduction attributable to the FF genotype was 23.7% (95% CI, 1.2–51.3%).

Table 1  Frequency of TaqI and FokI polymorphisms in controls and MM cases

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>TT</th>
<th>Tt</th>
<th>tt</th>
<th>n</th>
<th>FF</th>
<th>Ff</th>
<th>ff</th>
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<tr>
<td>Controls</td>
<td>93</td>
<td>39</td>
<td>41</td>
<td>14</td>
<td>108</td>
<td>52</td>
<td>44</td>
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<tr>
<td>MM cases</td>
<td>261</td>
<td>94</td>
<td>41</td>
<td>14</td>
<td>293</td>
<td>105</td>
<td>142</td>
<td>46</td>
</tr>
</tbody>
</table>

**Table 1**  Frequency of TaqI and FokI polymorphisms in controls and MM cases

**a** Analysis was performed using logistic regression.

**b** Proportion of subjects with the FF genotype in cases versus controls; P = 0.026; OR, 0.69; 95% CI, 0.38–0.94 (uncorrected); and P = 0.029; OR, 0.59; 95% CI, 0.37–0.95 (corrected for age and gender).

**c** Proportion of patients with the tt genotype in MM cases versus controls; P = 0.026; OR, 0.60; 95% CI, 0.38–0.94 (uncorrected); and P = 0.029; OR, 0.69; 95% CI, 0.50–0.847. Table 2A shows the allele frequencies of TaqI and FokI alleles in controls and MM cases. All allele frequencies conformed to Hardy-Weinberg equilibrium. The F allele was significantly less common in MM cases than controls (P = 0.029; OR, 0.69; 95% CI, 0.50–0.847). Table 2B shows the relationship between TaqI and FokI genotypes in cases and controls. No significant correlations between genotypes at the two sites were identified in either controls (P = 0.365) or cases (P = 0.847), suggesting that the two polymorphisms did not demonstrate linkage disequilibrium. Table 1 shows frequencies of FokI and TaqI genotypes in controls compared with MM cases. There was a decreased proportion of individuals with the FokI FF genotype in cases versus controls. Thus, for FF versus other FokI genotypes, the uncorrected OR for MM was 0.60 (P = 0.026). The findings remained significant after correction for age and gender using multivariate logistic regression (OR, 0.59; P = 0.029). The estimated risk reduction attributable to the FF genotype was 23.7% (95% CI, 1.2–51.3%).

**Table 1**  Frequency of TaqI and FokI polymorphisms in controls and MM cases

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>TT</th>
<th>Tt</th>
<th>tt</th>
<th>n</th>
<th>FF</th>
<th>Ff</th>
<th>ff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>93</td>
<td>39</td>
<td>41</td>
<td>14</td>
<td>108</td>
<td>52</td>
<td>44</td>
<td>12</td>
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<tr>
<td>MM cases</td>
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<td>41</td>
<td>14</td>
<td>293</td>
<td>105</td>
<td>142</td>
<td>46</td>
</tr>
</tbody>
</table>
Table 2  Frequency of TaqI and FokI alleles and concordance between genotypes in controls and MM cases

<table>
<thead>
<tr>
<th></th>
<th>TaqI</th>
<th>FokI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>T</td>
</tr>
<tr>
<td>Controls</td>
<td>186</td>
<td>119 (64.0%)</td>
</tr>
<tr>
<td>MM cases*</td>
<td>522</td>
<td>315 (60.3%)</td>
</tr>
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**B. Concordance**

<table>
<thead>
<tr>
<th>FokI</th>
<th>TaqI</th>
<th>MM Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
<td>Tt</td>
</tr>
<tr>
<td>FF</td>
<td>17</td>
<td>12 (33.3%)</td>
</tr>
<tr>
<td>Ff</td>
<td>17</td>
<td>12 (43.6%)</td>
</tr>
<tr>
<td>ff</td>
<td>3</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>44 (36.1%)</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>19 (47.5%)</td>
</tr>
</tbody>
</table>

*Proportion of subjects with the F allele in cases versus controls; P = 0.029; OR, 0.69; 95% CI, 0.50–0.96.

**Association of VDR Genotype with Patient Characteristics.** Table 1 shows the frequency of VDR genotypes in the MM cases stratified by patient characteristics. There was no significant association between genotype frequencies and tumor site, skin type, or eye color. However, the ff and tt genotypes were significantly less common in MM patients with red hair than in patients with other hair colors (P = 0.021, \( \chi^2 = 5.31 \) and \( P = 0.040, \chi^2 = 4.21 \), respectively).

**Association for VDR Genotypes with Breslow Thickness.** Patients were categorized by Breslow thickness (Table 1). In tumors \( \approx 1.5 \)-mm thick, for TaqI, there was an increased proportion of the tt genotype (\( P = 0.047 \)), but there was no obvious effect for FokI (\( P = 0.701 \)). Homozygosity for variant alleles at either FokI and TaqI loci (tt or ff genotypes) was associated with an increased proportion of \( \approx 3.5 \)-mm thick, although this did not achieve statistical significance (tt: \( P = 0.105 \); OR, 2.84; and ff: \( P = 0.266 \); OR, 1.99, uncorrected).

The effects of combinations of the TaqI and FokI polymorphisms are shown in Table 3. There was an association of tff combined genotype with thicker tumors, using either \( \approx 1.5 \) mm (\( P = 0.065 \)) or \( \approx 3.5 \) mm (\( P < 0.001 \)) as the cutoff. These results retained similar significance, particularly for tumors \( \approx 3.5 \)-mm thick, after correction for potential confounding factors (age, gender, and tumor site). Thus, the mean Breslow thickness in patients with the tff genotype combination was 2.9 mm compared with 1.1 mm in patients with other genotype combinations. This association was further confirmed using linear regression analysis, which showed that the tff genotype was correlated with Breslow thickness (\( P = 0.002 \), transformed to normality and corrected for age and gender). Significant associations were also identified between Breslow thickness and combinations of genotypes including the genotypes Tff and tFF, although these were less effective at predicting Breslow thickness.

**DISCUSSION**

We have postulated that polymorphism in the VDR gene is important in MM. This hypothesis is supported by data showing that 1,25(OH)\(_2\)D\(_3\) inhibits cell proliferation (12, 13) and stimulates differentiation (11, 14) and apoptosis (29) in several cell types expressing the VDR. There is evidence that 1,25(OH)\(_2\)D\(_3\) has an anticancer effect in several systemic cancers such as breast (30), prostate (31), colon (32), leukemia (33), and kidney (34). Furthermore, in vitro studies have demonstrated that 1,25(OH)\(_2\)D\(_3\) inhibits growth of cultured malignant cells (11–14, 34) and inhibits experimental carcinogenesis (35, 36). In vivo, decreased mean serum levels of 1,25(OH)\(_2\)D\(_3\) or its precursors have been reported in carcinoma of the breast (15), prostate (17), and colon (19). More recently, polymorphisms of the VDR have been reported associated with cancer of the breast [FokI and poly(A) site RFLP; Ref. 16] and prostate [BsmI and poly(A) site RFLP; Refs. 18 and 37].

Data for MM are similar, although more limited. Normal (38) and malignant melanocytes (20) express the VDR, and 1,25(OH)\(_2\)D\(_3\) has been shown to inhibit normal (21) and malignant melanocyte (20) growth in vitro. In a study of 1,25(OH)\(_2\)D\(_3\) serum levels in MM patients, lower levels were found compared with controls, although this did not achieve statistical significance (22).

In our study, homozigosity for the wild-type (F) allele at the FokI restriction sites was associated with a reduced risk of MM, with a risk reduction attributable to the FF genotype estimated at 23.7%. Furthermore, the proportion of F alleles was significantly lower in the case group compared with controls. The number of controls, however, was relatively small, and larger cohorts would be required to reduce the risk of both type 1 and type 2 errors. In this initial study, we have used hospital-based controls. Selection of control subjects is always difficult, and although the use of “normal” volunteers or blood donors would reduce the risk of potential bias because of occult associations with other disease processes, since they are generally not examined by a clinician, the possibility of undetected malignant or inflammatory pathologies cannot be excluded. By use of hospital-based controls, it was possible to focus only on controls who were clinically free of other malignant or inflammatory pathologies. Furthermore, the control genotype frequencies were similar to those described in other studies (18, 26, 27, 39), supporting the view that our control group is representative of the normal population.

The FokI RFLP has been reported previously to be associated with breast cancer (16), where the FF genotype was
Table 3  Interactions between VDR genotypes and association with Breslow thickness

<table>
<thead>
<tr>
<th>Genotype combination</th>
<th>&lt;1.5 mm</th>
<th>≥1.5 mm</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td><strong>A. &lt;1.5 mm vs. 1.5 mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/158 (1.3%)</td>
<td>3/45 (6.7%)</td>
<td>0.065</td>
<td>5.6</td>
<td>0.9–34.4</td>
</tr>
<tr>
<td>tff&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.023</td>
<td>9.2</td>
<td>1.4–61.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.062</td>
<td>7.2</td>
<td>0.9–57.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff or ttFF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10/158 (6.3%)</td>
<td>9/45 (20.0%)</td>
<td>0.008</td>
<td>3.7</td>
<td>1.4–9.8</td>
</tr>
<tr>
<td>tff or ttFF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.009</td>
<td>3.9</td>
<td>1.4–11.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff or ttFF&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.007</td>
<td>4.3</td>
<td>1.5–12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff or Ttff&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17/158 (10.8%)</td>
<td>6/45 (13.3%)</td>
<td>0.631</td>
<td>1.3</td>
<td>0.5–3.5</td>
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<tr>
<td>tff or Ttff&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.292</td>
<td>1.8</td>
<td>0.6–5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff or Ttff&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.336</td>
<td>1.7</td>
<td>0.6–5.4</td>
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<table>
<thead>
<tr>
<th>Genotype combination</th>
<th>&lt;3.5 mm</th>
<th>≥3.5 mm</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td><strong>B. &lt;3.5 mm vs. 2.3 mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>tff&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/191 (1.1%)</td>
<td>3/12 (25.0%)</td>
<td>&lt;0.001</td>
<td>31.5</td>
<td>4.7–212.7</td>
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<tr>
<td>tff&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>93.2</td>
<td>94–926.6</td>
<td></td>
<td></td>
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<tr>
<td>tff&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>108.5</td>
<td>8.2–1438.8</td>
<td></td>
<td></td>
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<tr>
<td>tff or ttFF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16/191 (8.4%)</td>
<td>3/12 (25.0%)</td>
<td>0.071</td>
<td>3.6</td>
<td>0.9–14.8</td>
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<tr>
<td>tff or ttFF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.075</td>
<td>3.8</td>
<td>0.9–16.4</td>
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<tr>
<td>tff or ttFF&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.090</td>
<td>4.8</td>
<td>0.8–29.5</td>
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<tr>
<td>tff or Ttff&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19/191 (9.9%)</td>
<td>4/12 (33.3%)</td>
<td>0.022</td>
<td>4.5</td>
<td>1.2–16.4</td>
</tr>
<tr>
<td>tff or Ttff&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.005</td>
<td>7.8</td>
<td>1.8–32.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tff or Ttff&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.006</td>
<td>12.3</td>
<td>2.1–73.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Uncorrected data.
<sup>b</sup> Corrected for age at presentation and gender.
<sup>c</sup> Corrected for age at presentation, gender, and head/neck tumor site.

Associated with a decreased risk of ~50% in certain racial groups. The poly(A) polymorphism (classified into long, L, or short, S) has been associated with altered risk of breast (16) and prostate (18) cancer. In breast cancer, LL and LS alleles were also associated with a ~50% reduction in risk (16). However, in prostate cancer, the presence of L, whether in the heterozygous (LL) or homozygous (LS) state, was associated with a 4–5-fold increased risk of prostate cancer (18, 37). Because the TaqI restriction site is in strong linkage disequilibrium with the poly(A) polymorphism (T demonstrates linkage disequilibrium with L; Ref. 39), the findings in breast cancer are comparable with our findings in MM, although our data on the TaqI RFLP did not achieve statistical significance.

Our data also identified an association between VDR genotypes and red hair in patients with MM. There was insufficient hair color data on our control subjects to examine whether this was a general phenomenon. Although the mechanism for this association is not known, other studies have identified links between polymorphism at other loci and hair color in MM (6, 8). These data suggest that the molecular route by which patients with red hair develop MM may differ from patients with other hair colors, supporting the view that these patients represent a high risk subgroup. However, these data require confirmation in independent studies, including in control individuals.

More significantly, we have identified significant associations between VDR genotypes and outcome in patients with MM. Thus, our data suggest that VDR polymorphism is a better determinant of outcome in MM than of its initiation. Melanoma depth is well recognized as an important prognostic indicator with respect to risk of metastatic disease and survival (40). In general, for both restriction sites, the proportion of thick MM (with either ≥1.5 or ≥3.5 mm cutoff) increased with increasing number of variant alleles. The effect of VDR genotypes on Breslow thickness was markedly increased when the two polymorphic sites were considered together. Thus, the combined tff genotype was associated with tumors ≥1.5-mm thick but particularly those ≥3.5-mm thick (P < 0.001; Table 3). We also corrected the data for the potential confounding effects of gender, tumor site, and age at presentation because thicker tumors are associated with male gender, head/neck tumor site, and older age. The association of the tff genotype remained significant, suggesting that the effect on Breslow thickness is independent of these factors. Similar results were obtained with other genotype combinations, although the magnitudes of the effects were smaller, suggesting that the heterozygote genotypes were of intermediate importance in determining Breslow thickness. Similarly, in carcinoma of the prostate, poly(A) microsatellite variants are reported to be associated with more advanced disease (37).

In addition, low serum levels of 1,25(OH)2D3 have been implicated in metastatic rather than in situ disease in prostatic cancer, suggesting an impact on tumor progression rather than development (17).

The polymorphism at the FokI restriction site (T-C transition) produces an ATG start codon resulting in translation initiation 10 bp upstream and therefore the production of a lengthened protein of 427 amino acids (26, 41). The F allele (restriction site absent, ACG), which results in a shorter protein, has been shown to be more effective at activating the transcript...
tion of a VDR reporter construct (23), thereby indicating that the polymorphism is functionally significant. The cluster of polymorphisms at the 3′ end of VDR, which includes TaqI, are in mutual tight linkage disequilibrium and a representative, BsmI, is known to be in linkage disequilibrium with the poly(A) microsatellite (39). It has been suggested that the length of the poly(A) repeat affects mRNA stability or is tightly linked to a further functionally significant site (24). The net effect of the ff and the tt polymorphisms can be envisaged as a reduction in the cellular effect of 1,25(OH)₂D₃ and therefore a growth advantage of the melanocytes. This conclusion is supported by the increased effect of combined homozygosity, which would be expected to have a more profound effect on the VDR protein.

Data from this hypothesis that the VDR genotype has a significant role in determining tumor occurrence and behavior in MM and indicate a role for vitamin D in melanoma cell cycle control and differentiation in vitro. There is evidence of a blocking effect of 1,25(OH)₂D₃ on the transition from G₂ to S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter the S phase of the cell cycle via several mechanisms, such as stimulation of the CDK inhibitory proteins, P21 (42), which enter

**REFERENCES**


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