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Sun exposure, vitamin D receptor polymorphisms *FokI* and *BsmI* and risk of multiple primary melanoma

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ABSTRACT

Background: Sunlight exposure increases risk of melanoma. Sunlight also potentiates cutaneous synthesis of vitamin D, which can inhibit melanoma cell growth and promote apoptosis. Vitamin D effects are mediated through the vitamin D receptor (*VDR*). We hypothesized that genetic variation in *VDR* affects the relationship of sun exposure to risk of a further melanoma in people who have already had one. *Methods*: We investigated the interaction between *VDR* polymorphisms and sun exposure in a population-based multinational study comparing 1138 patients with a multiple (second or subsequent) primary melanoma (cases) to 2151 patients with a first primary melanoma (controls); essentially a case–control study of melanoma in a population of melanoma survivors. Sun exposure was assessed using a questionnaire and interview, and was shown to be associated with multiple primary melanoma. *VDR* was genotyped at the *FokI* and *BsmI* loci and the main effects of variants at these loci and their interactions with sun exposure were analyzed. *Results*: Only the *BsmI* variant was associated with multiple primary melanoma (OR = 1.27, 95% CI 0.99–1.62 for the homozygous variant genotype). Joint effects analyses showed highest ORs in the high exposure, homozygous variant *BsmI* genotype category for each sun exposure variable. Stratified analyses showed somewhat higher ORs for the homozygous *BsmI* variant genotype in people with high sun exposure than with low sun exposure. *P* values for interaction, however, were high. *Conclusion*: These results suggest that risk of multiple primary melanoma is increased in people who have the *BsmI* variant of *VDR*.

1. Introduction

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

Sunlight potentiates the synthesis of the steroid hormone vitamin D_3 from precursors in the skin. It does so by way of UVB-

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mediated conversion of 7-dehydrocholesterol and isomerization in basal epidermal keratinocytes to vitamin D_3, with subsequent hydroxylation to the biologically active 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) [6]. There is evidence to suggest that $1,25(OH)_2D_3$ plays a role in the development and progression of melanoma [7,8]. $1,25(OH)_2D_3$ promotes melanocyte differentiation and apoptosis [9,10] and inhibits cell growth, adhesion, migration, metastases and angiogenesis in vitro and in vivo [11–15].

Polymorphic variants of the vitamin D receptor (*VDR*) gene have been associated with increased risk of melanoma in a number of recent hospital-based studies [16–20] and a nested-case–control study within the Nurses Health Study [21]. However, while measures of sun sensitivity including tanning ability and sunburn history were included in some of these studies, none incorporated sun exposure. Given that the *VDR* may only be activated by vitamin D and that sunlight exposure is intimately linked with vitamin D_3 production, consideration of the interaction between them is essential to understanding the impact of these factors on melanoma risk. In North America and based on the typical western diet, sun exposure is the fundamental source of vitamin D [22].

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

2. Methods

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

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

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

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

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

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

2.1. VDR genotyping

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

The *VDR-FokI* specific fragments (267 bp) were amplified in a PCR mix containing 10–100 ng DNA, 200 μM dNTPs, 0.4 μM forward primer (5′-CTGAGCCAGCTATGTAGGGC-3′), 2.0 μM reverse primer (5′-GGTCAAAGTCTCCAGGGTCA-3′), 0.2 μM fluorescein labeled probe (5′-CTTGCTGTTCTTACAGGGACGGAG-3′), 1.5 mM $MgCl_2$, 1 M betaine and 0.05 U/μl of Taq Polymerase. The cycling conditions included a denaturation and Taq activation step at 95 °C for 10 min followed by 5 cycles at 95 °C–25 s, 64 °C–20 s, 72 °C–30 s, 5 cycles at 95 °C–25 s, 60 °C–20 s, 72 °C–30 s, 40 cycles at 95 °C–25 s, 56 °C–20 s, 72 °C–30 s, and a post cycling extension at 72 °C for 5 min.

The *VDR-BsmI* specific fragments (209 bp) were amplified in a PCR mix containing 10–100 ng genomic DNA, 1× buffer II (10 mM Tris–HCl, pH 8.3, 50 mM KCl) (PE, Roche Molecular Systems Inc., Branchburg, NJ), 200 μM dNTP, 0.42 μM forward primer (5′-CCTCACTGCCCTTAGCTCTG-3′) and reverse primer (5′Biotin-CCATCTCTCAGGCTCCAAAG-3′), 2.5 mM $MgCl_2$, 5% DMSO and 0.05 U/μl Taq Polymerase. Cycling conditions included a denaturation step at 95 °C for 5 min followed by 2 cycles at 95 °C–20 s, 59 °C–20 s, 72 °C–25 s, 40 cycles at 95 °C–20 s, 57 °C–20 s, 72 °C–