Polymorphisms in the MTHFR and VDR genes and skin cancer risk

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Abstract
Folate and vitamin D have been shown to be influenced by ultraviolet (UV) radiation. UVA radiation can break down plasma folate, whereas vitamin D can be synthesized in UVB-exposed skin. Folate metabolism is involved in DNA synthesis and repair, and vitamin D processes anti-proliferative effects. The functions of both nutrients are implicated in skin carcinogenesis. We evaluated genetic polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene (C677T and A1298C) and the vitamin D receptor (VDR) gene (Fok1, Bsm1 and Cdx2) with skin cancer risk in a nested case–control study within the Nurses’ Health Study [219 melanoma, 286 squamous cell carcinoma (SCC), 300 basal cell carcinoma (BCC) and 873 controls]. No significant associations were observed for the two MTHFR polymorphisms on skin cancer risk. We observed an interaction between the C677T polymorphism and total folate intake on SCC risk (P, interaction = 0.04); the highest risk was observed among women with TT genotype and low folate intake (OR = 2.14; 95% CI = 1.01–4.50). The VDR Bsm1 BB genotype was significantly associated with an increased SCC risk (OR = 1.51; 95% CI = 1.00–2.28). An interaction between the Bsm1 polymorphism and total vitamin D intake on SCC was observed, with the highest risk seen in women with the BB genotype and high vitamin D intake (OR = 2.38; 95% CI = 1.22–4.62) (P, interaction = 0.08). This study suggests a possible role of the polymorphisms in MTHFR and VDR interacting with dietary intakes of folate and vitamin D in skin cancer development, especially for SCC. Due to a large number of comparisons and tests, the possible associations should be interpreted with caution and confirmed by other studies.

Introduction
Skin cancer is the most common neoplasm in Caucasians in the United States. There are three main types of skin cancers: the most common is basal cell carcinoma (BCC), followed by squamous cell carcinoma (SCC) and melanoma. Ultraviolet (UV) radiation has been widely recognized as a strong risk factor for both melanoma and non-melanocytic skin cancer (1,2). UV radiation has a wide range of carcinogenic effects on skin tissue, such as causing DNA and tissue damage, oxidative stress and inflammation. In addition, blood levels of two nutrient factors, folate and vitamin D, have been proposed to be influenced by UV radiation. UVA radiation can breakdown plasma folate (3). It was shown that exposure of human plasma in vitro to UV causes loss of folate and light-skinned subjects exposed to UV have low-serum folate concentrations, suggesting that photolysis may also occur in vivo (3). Vitamin D can be synthesized in UVB-exposed skin, and the lack of adequate sun exposure can cause vitamin D deficiency (4).

Folate can prevent neural tube defects and vitamin D is essential to bone development and the immune system. Because both are associated with human reproduction, it has been proposed that human skin has evolutionarily gained appropriate color under selection pressure, at least in part, to optimize levels of these two UV-related nutrients (5,6). In addition, folate metabolism is involved in DNA synthesis and repair (7), and vitamin D processes anti-proliferative effects. The functions of both nutrients are implicated in the carcinogenesis of each type of skin cancer. The methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The vitamin D receptor (VDR) is the nuclear receptor that mediates the effects of vitamin D through regulating the transcriptions of other genes. Variation in either nutrient, in the forms of dietary intake, plasma levels and genetic polymorphisms in these two genes, is associated with a number of chronic diseases, including cancer at sites of breast, prostate and colon (8,9). However, the associations with skin cancer risk are relatively unknown. We selected candidate polymorphisms in these two genes (C677T and A1298C in the MTHFR gene, and Fok1, Bsm1 and Cdx2 in the VDR gene). These five polymorphisms have been reported to be associated with altered function or cancer risks in earlier epidemiologic studies. We assessed them in relation to skin cancer risk (melanoma, SCC and BCC) within the Nurses’ Health Study. We further investigated gene–diet interactions between these genetic variants and the nutrient intakes on skin cancer risk.

Materials and methods
Study population
The Nurses’ Health Study (NHS) was established in 1976, when 121 700 female registered nurses between the ages of 30 and 55 years completed a self-administered questionnaire on their medical histories and baseline health related exposures. Updated information has been obtained by questionnaires every 2 years. Between 1989 and 1990, blood samples were collected from 32 826 of the cohort members. Eligible cases in this study consisted of women with incident skin cancer from the subcohort who gave a blood specimen, including SCC and BCC cases with a diagnosis anytime after blood collection up to June 1, 1998 and melanoma cases (including in situ
cases) up to June 1, 2000 with no previously diagnosed skin cancer. All available pathologically confirmed melanoma and SCC cases and 300 self-reported BCC cases randomly selected from ~2600 available self-reported BCC cases were included. The validity of self-report of BCC is high in this medically sophisticated population (90%) (10). All the SCC and BCC cases had no history of melanoma diagnosis. A common control series (case: control = 1:1) was randomly selected from participants who gave a blood sample and were free of diagnosed skin cancer up to and including the questionnaire cycle in which the case was diagnosed. One control was matched to each case by year of birth (±6 years) and race (Caucasian/Hispanic). More than 95% of cases and controls were Caucasian. At the time we selected cases and controls, 47 cases and 69 controls were deceased. In order to obtain additional information by supplementary questionnaires, we randomly selected a second matched living control when the first control was deceased, and collected supplementary questionnaires from these second living controls. The nested case–control study consisted of 219 melanoma cases (including 77 in situ cases), 286 SCC cases, 300 BCC cases and 873 matched controls. Because of the absence of African-American cases, one African-American control was excluded to avoid potential population stratification. We mailed a supplementary questionnaire on lifetime sun exposure and other skin cancer risk factors to 758 living cases and 804 living controls. A total of 695 cases responded, 15 cases refused to participate and 48 cases did not respond after three mailings (participation rate = 92%). Among controls, 713 responded, 9 refused and 82 did not respond (participation rate = 89%). The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women’s Hospital, Boston, MA.

Host factors and sun exposure data
Information regarding skin cancer risk factors was obtained from the prospective biennial questionnaires and the retrospective supplementary questionnaire. Information on natural hair color and childhood and adolescent tendency to sunburn or tan was asked in the 1982 prospective questionnaire; ethnic group in the 1992 questionnaire. The retrospective supplementary questionnaire consisted of questions in three major areas: (i) history of constitutional and susceptibility factors; (ii) history of residence (states and towns), sun exposure habits and severe sunburns at different ages; and (iii) family history of skin cancer (father, mother and siblings). In addition, the 11 states of residence of cohort members at baseline were grouped into three regions: Northeast (CT, MA, MD, NJ, NY and PA), Northcentral (MI and OH), and West and South (CA, TX and FL). In order to estimate sunlight exposure for each subject, an UV database for 50 US states was developed. The database used reports from the Climatic Atlas of the US, which reported mean daily solar radiation (in Langley) at the earth’s surface for weather stations around the country (11). A cumulative lifetime sun exposure was developed by combining the UV database and the information obtained from the supplementary questionnaire. Sunburns (painful or severe sunburns that blistered) and sun exposure history were used to define a cumulative lifetime intermittent (recreational) sun exposure variable for this behavior (12,13).

Dietary data
In 1980 a dietary component was added to the follow-up questionnaire. The validity and reliability of the food-frequency questionnaires (FFQ) in the NHS have been described elsewhere (14). Since 1980, the FFQ has been expanded to include ~130 individual food items plus vitamin and mineral supplement use accounting for >90% of intake of most major nutrients. This expanded questionnaire was administered to the cohort in 1984, 1986, 1990, 1994, 1998 and 2002. Nutrient intakes were computed by multiplying the frequency of response by the nutrient content of the specific portion sizes. We also asked questions on the use of specific vitamins and brand and type of multivitamins as well as dose and duration of use; vitamin supplement use was updated biennially. Values for nutrients in foods were derived from the USDA sources (15) and supplemented with information from manufacturers. Total energy-adjusted dietary nutrient intakes were used in analysis. To reduce within-person variation and represent long-term dietary intake of participants, we modeled skin cancer risk in relation to the cumulative average of dietary intake up to the diagnosis from all available dietary questionnaires up to the start of each 2-year follow-up interval (16).

Laboratory assays
Genotyping was performed by the S’ nuclease assay (TaqMan®), using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. TaqMan® primers and probes were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case–control status and blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded samples was 100%. Primers, probes and conditions for genotyping assays are available upon request.

Statistical methods
We used a χ²-test to assess whether the genotypes were in Hardy–Weinberg equilibrium. Unconditional logistic regression was employed to calculate odds ratio (OR) and 95% confidence interval (CI) to assess the risk of skin cancer for genotypes among all women. A test for trend was calculated across the three categories of genotype for each polymorphism. In this test, we treated the genotype as an ordinal variable (wild-type = 0, heterozygotes = 1 and homozygotes = 2). We used a likelihood ratio test (LRT) to evaluate heterogeneity in the effects of the genotypes on different types of skin cancer in polytomous logistic regression models (17).

To summarize multiple variables for constitutional host factors, we constructed a multivariate confounder score to create a constitutional susceptibility score for skin cancer (18). Briefly, we applied the logistic regression coefficients from a multivariate model including age, race, natural skin color, natural hair color, child or adolescent tendency to burn and the number of palpably raised moles on arms, to each individual’s values for the latter four of these variables and summed the values to compute a susceptibility risk score in the logit scale. The association of tendency to tan with skin cancer risk was abolished after the above constitutional risk factors were mutually adjusted for, and therefore it was not integrated into the constitutional susceptibility score. Higher score predicted higher risk of skin cancer according to the combined effect of these risk factors. Each component contributed to the score to a different extent. For example, women with ≥6 moles on arms had an age-adjusted OR of 3.53 (95% CI, 2.01–6.19) for melanoma risk. Red hair color was strongly associated with melanoma risk in our study (age-adjusted OR, 4.74; 95% CI, 2.47–9.09). The other two components of the score, i.e. natural skin color and childhood or adolescent tendency to burn had risk estimates of ~2 for melanoma. The risk for the highest tertile of the susceptibility score was ~3.5-fold for melanoma, and 3-fold for SCC and BCC, compared with the lowest tertile. In this study, we used median value of this score among controls to define women with low and high constitutional susceptibility.

In the multivariate analyses, we included skin cancer risk factors, including the constitutional susceptibility score, family history of skin cancer, sunburn history, UV exposure history and geographic region. In addition, in the analyses of the MTHFR polymorphisms, we included folate metabolism-related covariates, such as total folate intake, alcohol intake, vitamin B6 and B12 intake and multivitamin use. In the analyses of the VDR polymorphisms, we included vitamin D metabolism-related covariates, such as total vitamin D intake, calcium intake and multivitamin use.

To test statistical significance of interactions between the environmental and dietary exposures and the genotypes, we performed a LRT comparing the models that included terms for all combinations of the genotype and levels of exposure to the models with indicator variables for the main effects only. We performed statistical analyses using SAS 8.0 (SAS Institute, Cary, NC). All statistical tests were two-sided.

Results
Descriptive characteristics of cases and controls
Detailed description of characteristics of cases and controls was reported previously (12). At the beginning of the follow-up of this nested case–control study, the women were between 43 and 68 years with the mean age of 58.7 years. The mean age at diagnosis of melanoma cases was 63.4 years and that of SCC cases and BCC cases was 64.7 and 64.0 years, respectively. Women in the West and South regions were more likely to be diagnosed with SCC or BCC compared to those in Northeast. Cases of each type of skin cancer were more likely to have used sunlamps or attended tanning salons. A family history of skin cancer was a risk factor for the three types of skin cancer. Cases of each type of skin cancer were more likely to have had higher cumulative sun exposure with a bathing suit, more lifetime severe sunburns with a bathing suit, more lifetime severe sunburns that blistered and higher constitutional susceptibility risk score (12). Skin cancer cases, especially melanoma cases, were more likely to possess red hair color and fair skin color. The median total folate intake was
We did not observe an interaction of the observed with dietary folate intake without supplements. Those who had high folate intake. No interaction was significantly associated with an increased risk of SCC among women with low total folate intake (OR, 2.14; 95% CI, 1.01–4.39), and this excess risk was substantially attenuated among women with high constitutional susceptibility, namely with lighter hair, fair skin color, greater tendency to burn and sunlamp use.

Associations of the polymorphisms with skin cancer risk
The genotype distributions of the five polymorphisms examined were in Hardy–Weinberg equilibrium among controls. No significant main effects were observed between the two polymorphisms in the MTHFR gene and skin cancer risk in multivariate models (Table I). Among the polymorphisms in the VDR gene, the Fok1 ff genotype was non-significantly associated with increased risk of each type of skin cancer (Table II). The Bsm1 BB genotype was significantly associated with an increased risk of SCC (OR, 1.51; 95% CI, 1.00–2.28), but not with melanoma or BCC risk. No association was observed between the Cdx2 polymorphism with any type of skin cancer risk. There was no significant heterogeneity in the main effect of each polymorphism on the three types of skin cancer.

Interactions between the polymorphisms and dietary intake with SCC risk
We evaluated gene–diet interactions between the polymorphisms and dietary intake on skin cancer risk. The cumulative average intakes of folate and vitamin D were dichotomized as ‘high versus low’ based on the medians in controls. For total folate intake, the means for high and low categories in controls were 551.5 µg/day and 294.5. For total vitamin D intake, the means for high and low categories in controls were 486.2 IU/day and 211.1.

An interaction was observed between the MTHFR C677T polymorphism and total folate intake on SCC risk (P for interaction, 0.04) (Table III). The TT genotype was significantly associated with an increased risk of SCC among women with low total folate intake (OR, 2.14; 95% CI, 1.01–4.50), and this excess risk was substantially attenuated among those who had high folate intake. No interaction was observed with dietary folate intake without supplements.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (%)</th>
<th>Melanoma</th>
<th>SCC</th>
<th>BCC</th>
<th>Multivariate OR</th>
<th>Multivariate OR</th>
</tr>
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<tbody>
<tr>
<td>C677T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>334 (43.6)</td>
<td>30 (47.5)</td>
<td>104 (59.5)</td>
<td>92 (71.2)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>CT</td>
<td>395 (57.1)</td>
<td>69 (59.1)</td>
<td>126 (72.2)</td>
<td>120 (82.4)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TT</td>
<td>92 (10.7)</td>
<td>27 (31.4)</td>
<td>33 (18.8)</td>
<td>37 (24.8)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The percentages may not sum to 100 due to rounding.

We observed an interaction between the VDR Bsm1 polymorphism and total vitamin D intake on SCC risk (Table IV). The significantly positive association of the B allele with SCC risk was limited to women with high vitamin D intake (OR for BB genotype, 2.38; 95% CI, 1.22–4.62; P for trend = 0.02), but not among women with low vitamin D intake (P for interaction, 0.08). This interaction pattern was similar to that of the Fok1 polymorphism on SCC risk; compared to women with the FF genotype and low vitamin D intake, women with the ff genotype and high vitamin D intake had a significantly increased risk of SCC (OR, 2.46; 95% CI, 1.23–4.90), whereas no association among women with the ff genotype and low vitamin D intake (OR, 1.25; 95% CI, 0.63–2.51). No interaction was observed with dietary vitamin D intake without supplements.

In addition, we also observed a positive association between the Bsm1 polymorphism and SCC risk among women with high constitutional susceptibility, namely with lighter hair, fair skin color, greater tendency to burn and sunlamp use.

Table I. MTHFR genotype and skin cancer risk

<table>
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<th>Genotype</th>
<th>Cases (%)</th>
<th>Melanoma</th>
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</tr>
</thead>
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<td></td>
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<th>Controls (%)</th>
<th>Melanoma</th>
<th>SCC</th>
<th>BCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases (%)</td>
<td>Multivariate OR b</td>
<td>Multivariate OR c</td>
</tr>
<tr>
<td>Fok1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FF</td>
<td>325 (38.1)</td>
<td>77 (35.8)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Ff</td>
<td>418 (49.0)</td>
<td>101 (47.0)</td>
<td>1.01 (0.72–1.41)</td>
<td>0.96 (0.67–1.37)</td>
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<td>ff</td>
<td>111 (13.0)</td>
<td>37 (17.2)</td>
<td>1.37 (0.87–2.14)</td>
<td>1.40 (0.86–2.27)</td>
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<td>Trend</td>
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<tr>
<td>Heterogeneity d</td>
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<tr>
<td>Bsm1</td>
<td></td>
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<tr>
<td>bb</td>
<td>312 (37.1)</td>
<td>85 (40.9)</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Bb</td>
<td>398 (47.4)</td>
<td>94 (45.2)</td>
<td>0.89 (0.64–1.24)</td>
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<td>29 (13.9)</td>
<td>0.81 (0.51–1.30)</td>
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<td>0.49</td>
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<td>Heterogeneity d</td>
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<td>Cdx2</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>548 (64.2)</td>
<td>132 (64.4)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>GA</td>
<td>269 (31.5)</td>
<td>68 (33.2)</td>
<td>1.03 (0.74–1.44)</td>
<td>1.11 (0.78–1.59)</td>
</tr>
<tr>
<td>AA</td>
<td>36 (4.2)</td>
<td>5 (2.4)</td>
<td>0.57 (0.22–1.48)</td>
<td>0.58 (0.21–1.58)</td>
</tr>
<tr>
<td>Trend</td>
<td></td>
<td></td>
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<td>0.85</td>
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<tr>
<td>Heterogeneity d</td>
<td>0.44</td>
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</tbody>
</table>

The percentages may not sum to 100 due to rounding.

aThe number of participants does not sum to total women because of missing data on genotype.

bUnconditional logistic regression adjusted for the matching variables: age and race (Caucasian/missing).

cUnconditional logistic regression adjusted for the matching variables, total vitamin D intake (tertiles), calcium intake (tertiles), multivitamin use (yes/no), constitutional susceptibility score (tertiles), family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.

dLikelihood ratio test to evaluate heterogeneity in the effects of the genotypes on different types of skin cancer in polytomous logistic regression models adjusted for variables in the multivariate model 3.
more moles (BB versus bb OR, 1.85; 95% CI, 1.12–3.06, P trend across the three genotype categories, BB, Bb, bb = 0.03), whereas there was no association between this polymorphism and SCC risk among those with low constitutional susceptibility (BB versus bb OR, 1.05; 95% CI, 0.49–2.28, P trend across the three genotype categories, BB, Bb, bb = 0.92). The test for interaction was not significant (P for interaction, 0.43).

We did not observe any interactions between the five polymorphisms examined and geographic region, lifetime sunburns or sun exposure while wearing a bathing suit, on skin cancer risk.

### Discussion

In this nested case–control study, no significant main effects were observed between the two polymorphisms in the MTHFR gene and skin cancer risk in multivariate models. For the polymorphisms in the VDR gene, the Bsm1 BB genotype was significantly associated with increased risk of SCC risk. The Fok1 ff genotype was non-significantly associated with increased risk of each type of skin cancer. We observed interactions between the MTHFR C677T polymorphism and total folate intake on SCC risk. The nested case–control design, high follow-up rate, and prospective dietary exposure assessment strengthen the validity of this study.

Folate is essential for DNA synthesis and repair in dividing cells because folate deficiency causes excessive uracil misincorporation into human DNA and DNA strand breaks (19). Additionally, in rat colonocytes (20), Chinese hamster ovary cells (21) and in peripheral blood lymphocytes in a cancer-free population (22), folate deficiency was shown to impair nucleotide excision repair capacity, which is the primary repair mechanism to remove UV-induced DNA photoproducts. Therefore, folate is particularly important in rapid proliferating cells, such as skin keratinocytes, for maintaining DNA integrity and efficient repair of DNA damage. The MTHFR is a critical enzyme in folate metabolism. The substrate 5,10-methylenetetrahydrofolate is the intracellular folate required for DNA synthesis and repair, whereas the product 5-methyltetrahydrofolate is the plasma form of folate providing the methyl group for de novo methionine synthesis and DNA methylation. No significant main effects were observed for the two MTHFR polymorphisms in this study. In a case–control study of 197 BCC cases and 548 controls from Sweden and Finland, no significant associations were found for BCC risk (23). The 677 TT genotype had a risk of 1.69 (95% CI, 0.84–3.38), and the risk for the 1298 CC genotype was 1.24 (95% CI, 0.70–2.18). The MTHFR C677T polymorphism causes an Alanine to Valine substitution, which is associated with decreased enzymatic activity (24). Thus, with high level of folate status, cells with the T allele may accumulate 5,10-methylenetetrahydrofolate, maintaining efficient DNA synthesis and repair, and in turn minimizing the DNA damage. On the other hand, low folate intakes as well as the TT genotype have been associated with genomic DNA hypomethylation (25,26). Our interaction data are consistent with the functional relevance of the C677T polymorphism. Compared with the CC genotype, the TT genotype was associated with an increased risk of SCC among women with low folate intake, whereas no such an association was observed among those with high folate intake. The highest risk was observed among women with the TT genotype and low folate intake. This interaction pattern is consistent with previous findings for breast cancer (27) and colon cancer (28). We did not have plasma measurement of folate, which reflects both genetic and dietary variation. The TT genotype has been shown to be significantly associated with lower plasma folate levels (28–30). Plasma levels of folate are positively correlated with folate intakes. The correlation coefficients were much stronger for intakes of folate from foods and supplements (0.49) than from foods.

### Table III. Interaction between the MTHFR C677T polymorphism and total folate intake on SCC risk

<table>
<thead>
<tr>
<th></th>
<th>Cases/controls</th>
<th>OR</th>
<th>Cases/controls</th>
<th>OR</th>
<th>Cases/controls</th>
<th>OR</th>
<th>P, trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>42/143</td>
<td>1.00</td>
<td>43/157</td>
<td>0.94 (0.56–1.58)</td>
<td>19/26</td>
<td>2.14 (1.01–4.50)</td>
<td>0.26</td>
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<tr>
<td>High</td>
<td>56/154</td>
<td>1.36 (0.77–2.42)</td>
<td>41/145</td>
<td>0.95 (0.52–1.73)</td>
<td>11/39</td>
<td>0.78 (0.33–1.88)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Unconditional logistic regression adjusted for the matching variables, alcohol intake (0, >0 to <5, ≥5 to <15, ≥15 to <30, ≥30 g/day), vitamin B6 intake (tertiles), vitamin B12 intake (tertiles), multivitamin use (yes/no), constitutional susceptibility score (tertiles), family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.

### Table IV. Interaction between the VDR Bsm1 polymorphism and total vitamin D intake on SCC risk

<table>
<thead>
<tr>
<th></th>
<th>Cases/controls</th>
<th>OR</th>
<th>Cases/controls</th>
<th>OR</th>
<th>Cases/controls</th>
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<tbody>
<tr>
<td>bb</td>
<td>36/139</td>
<td>1.00</td>
<td>58/162</td>
<td>1.29 (0.78–2.14)</td>
<td>14/61</td>
<td>0.89 (0.43–1.83)</td>
<td>0.85</td>
</tr>
<tr>
<td>High</td>
<td>40/131</td>
<td>1.17 (0.63–2.15)</td>
<td>59/180</td>
<td>1.29 (0.73–2.27)</td>
<td>33/52</td>
<td>2.38 (1.22–4.62)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Unconditional logistic regression adjusted for the matching variables, calcium intake (tertiles), multivitamin use (yes/no), constitutional susceptibility score (tertiles), family history of skin cancer (yes/no), the number of lifetime severe sunburns which blistered (none, 1–5, 6–11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.
only (0.33), reflecting the major contribution of multivitamin supplements to circulating levels of folate (31).

In addition to dietary intake, vitamin D can be synthesized in the skin after exposure to UV. Keratinocytes have the capacity to convert vitamin D to the active form of 1,25(OH)2D3 through the vitamin D-25 hydroxylase (25OHase) and the 25-hydroxyvitamin D-1-hydroxylase (1OHase), which are the same enzymes present in the liver and kidney (32,33). The expression of the two genes was also detected in melanoma cell lines (34). The 1,25(OH)2D3 has shown multiple cellular effects in keratinocytes and melanocytes, including cell-cycle arrest, anti-proliferation and pro-differentiation (35–39). The 1,25(OH)2D3 and its analogs were demonstrated to be clinically effective for the treatment of psoriasis by inhibiting keratinocyte growth and inducing differentiation (40). Low serum levels of 1,25(OH)2D3 have been reported in melanoma patients (41). However, conflicting data exist on the effect of 1,25(OH)2D3 on apoptosis in keratinocytes. Lower concentrations of 1,25(OH)2D3 were shown to protect keratinocytes from apoptosis (42,43); whereas 1,25(OH)2D3 at high doses induced apoptosis (42,44,45). Different apoptotic responses to 1,25(OH)2D3 were also observed in melanocytes and melanoma cells (46,47).

The cellular effect of 1,25(OH)2D3 is mediated through the VDR, which is a member of the steroid/nuclear receptor superfamily. The VDR is heterodimerized with the retinoid X receptor (RXR) to bind to specific vitamin D-response elements in the promoter region of VDR genes. Several polymorphic sites have been identified in the VDR gene. The Fok1 polymorphism in exon 2 results in an altered translation start site. Compared with the F allele encoding the VDR protein without first three amino acids at N-terminus, the F allele encodes the full-length protein, and has decreased ability to interact with transcription factor IIB, induce transactivation in vitro, and inhibit growth of human peripheral blood mononuclear cells (8). The fj genotype was associated with an increased risk of melanoma (OR, 1.90; 95% CI, 0.88–4.15) in a hospital-based case–control study (316 cases and 108 controls) (48). Our data were compatible with this report on melanoma risk, and we also observed that the fj genotype was positively associated with the risks of SCC and BCC. For SCC risk, the association for the fj genotype was stronger among women with high vitamin D intake. The Cdx2 polymorphism is located in the 1e promoter region in a consensus response element for the caudal-related homeobox transcription factor Cdx2, with the allele displaying higher affinity with Cdx2 and higher expression level (49). No association was observed between this polymorphism and skin cancer risk in this study.

Four polymorphic sites in the VDR gene are in linkage disequilibrium, including Bsm1 and ApaI restriction sites in the intron between exons 8 and 9, TaqI restriction site in exon 9 as well as a poly(A) microsatellite length polymorphism in the 3’ UTR region. No apparent association has been found between the Bsm1 polymorphism and altered functional activities (8). The function relevance of the Bsm1/ApaI/TaqI haplotype was previously examined. The baT haplotype inserted in transfection constructs yielded lower reporter gene activity compared with BAt (50), which is in agreement with the reported association of the baT haplotype with low VDR mRNA expression (51).

We observed that the Bsm1 BB genotype was significantly associated with an increased risk for SCC, but not for melanoma or BCC. We observed an interaction between the Bsm1 polymorphism and total vitamin D intake on SCC risk, suggesting that women with the BB genotype and high vitamin D intake have the highest risk of SCC. The BB genotype was associated with higher levels of 1,25(OH)2D3 than the Bb or bb genotypes (50,52). Most of the vitamin D requirement for most people comes from exposure to sunlight. In a recent evaluation of predictors of vitamin D status (53), physical activity and skin pigmentation (estimated by race) were the strongest predictors of 25(OH)2D3 levels, followed by dietary vitamin D intake, and vitamin D from supplements increased the 25(OH)2D3 levels only slightly. In lacking of good understanding of the function of the Bsm1 polymorphism and the role of dietary vitamin D in skin cancer, we should interpret this interaction with caution. There were a large number of comparisons presented in this study and the magnitude of the effect was small; we cannot rule out the possibility that this may be a chance finding.

Compared to melanocytes, keratinocytes, especially squamous cells, have high levels of cell cycling and proliferation. Apoptosis is a major protective mechanism for squamous cells from UV-induced DNA damage. Fast cell turnover may make nutrients, such as folate and vitamin D, particularly important for squamous cells. Unlike internal organs, the genotoxic effect of UV exposure on skin tissues adds more complexity to the relationships of UV exposure, these two nutrients and skin cancer risk. The nested case–control design, high follow-up rate and prospective dietary questionnaire data with repeated measurements strengthen the validity of this study. The limitations of the study include the lack of plasma measurements of folate and vitamin D. There is potential limitation in generalizability of the results in our cohort of nurses, e.g. outdoor occupations are underrepresented. This present study, to our knowledge, is the first report suggesting a possible role of the polymorphisms in MTHFR and VDR with interactions of dietary nutrient intakes in the development of SCC. Due to a large number of comparisons and tests, the possible associations should be interpreted with caution and confirmed by other studies.

Acknowledgements

The authors thank Dr Hardeep Ranu, Craig Labadic, and Patti Soule for their laboratory assistance. Carolyn Guo for her programming support. The authors also thank the participants in the Nurses’ Health Study for their dedication and commitment. This work is supported by NIH grants CA113100 and CA87969. J.H. is partially supported by the Harvard SPORE in Skin Cancer.

Conflict of Interest Statement: None declared.

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Received June 25, 2006; revised August 10, 2006; accepted August 18, 2006