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Vitamin D and melanoma

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Recreational sun exposure and sunburn are causal for melanoma but the risk is strongly genetically determined. Health promotion advice about sun protection should be aimed at susceptible individuals (pale skin, freckles, large numbers of melanocytic nevi and a family history). We discuss here the evidence that sun-sensitive people have lower vitamin D levels and that, in practice, it is very difficult for such individuals to achieve sufficient levels without supplementation in the UK at least. We conclude that melanoma susceptible sun-avoidant individuals should be advised to avoid insufficiency by supplementation.

Vitamin D is anti-proliferative in vitro for some melanoma cell lines. In a large melanoma cohort we have observed that lower serum 25-hydroxyvitamin D_2/D_3 levels at diagnosis were associated with thicker tumors and poorer prognosis (study as yet not validated). In the UK, melanoma patients commonly have sub-optimal 25-hydroxyvitamin D_2/D_3 levels at and post diagnosis; we discuss approaches to management of such patients based on some new data from our group.

Vitamin D and Melanoma Susceptibility

There is unequivocal evidence that the major environmental exposure which increases susceptibility to melanoma of the skin, is sun exposure. However, sun exposure is critical for vitamin D synthesis. Suboptimal vitamin D levels are unequivocally associated with reduced bone health and accumulating literature suggests that suboptimal levels of vitamin D increase risk of many other diseases so that a balance between sun protection and exposure is needed. We explore the evidence that sun exposure might also protect against melanoma by resulting in photoadaption or higher vitamin D levels. In the article we use the name vitamin D generally and 25-hydroxyvitamin D_2/D_3 when levels were measured in the blood (although techniques and approaches do vary between studies).

The evidence that melanoma is caused by sun exposure comes from epidemiological data showing, first that melanoma incidence is highest where pale skinned people (susceptible to sunburn) live closest to the equator.[1] Second, that within white skinned peoples, the risk is highest for those who report sunburn and sun exposure/sun bathing on holidays.[2] Evidence that sunbathing is a significant risk factor for melanoma was shown in a pooled data study of 15 case-control melanoma studies reported by Ruby Chang et al. in 2009 in which recreational sun exposure was a risk factor for melanoma on the trunk, pooled odds ratio (pOR = 1.7; 95% confidence interval (CI): 1.4–2.2) and limbs (pOR = 1.4; 95% CI: 1.1–1.7), but not on the head and neck (pOR = 1.1; 95% CI: 0.8–1.4), across latitudes from Australia to Northern Europe.[3]

The relationship between sun exposure and melanoma risk is however complex. There is less evidence of a "dose-response" effect for cumulative sun exposure over time, with the exception of continually exposed skin in very sunny countries, in that occupational sun exposure appears to be associated only with melanoma of the head and neck at low latitudes such as in Australia and Hawaii. In Chang's pooled data analysis, the pooled odds ratio for occupational sun exposure for melanoma on the head and neck at low latitudes was 1.7 (95% CI: 1.0–3.0).[3] When a measure of total lifetime exposure was computed in that same, pooled data analysis, total sun exposure was associated only with increased risk of melanoma on the limbs at low latitudes (pOR = 1.5; 95% CI: 1.0–2.2), but not at other body sites or other latitudes.

Putting all these data together then, it is evident that most melanoma is explained by intermittent sun exposure: the sort experienced on sunny holidays and which is often associated with sunburn. The enormous increase in this type of population exposure since the 1920s is consistent with the observed exponential increase in melanoma in most countries where the majority of the population is white skinned. **Figure 1** shows data produced by Veronique Poirier (from the South West Public Health Observatory in the UK) for the UK National Cancer Intelligence Network (NCIN) (www.ncin.org.uk) in which a continued increase in incidence is demonstrated in recent years especially in older men. Whether this increase in older men in the UK is related to sunny holidays or a change in the epidemiology remains to be seen.

Phenotypes associated with increased melanoma risk include skin types associated with a tendency to burn in the sun such as red or blond hair and freckles[4] and the presence of increased numbers of melanocytic nevi.[5] These phenotypes are largely genetically determined and it is therefore not surprising that the genetic determinants of red hair (the melanocortin receptor 1 gene (*MC1R*) and the agouti locus, are identified as melanoma susceptibility genes by genome wide association studies (GWA).[6,7] Twin studies showed that nevus number is also heritable,[8,9] and GWA studies have identified some of the inherited genetic variation associated both with melanoma risk and with nevi.[6,7]

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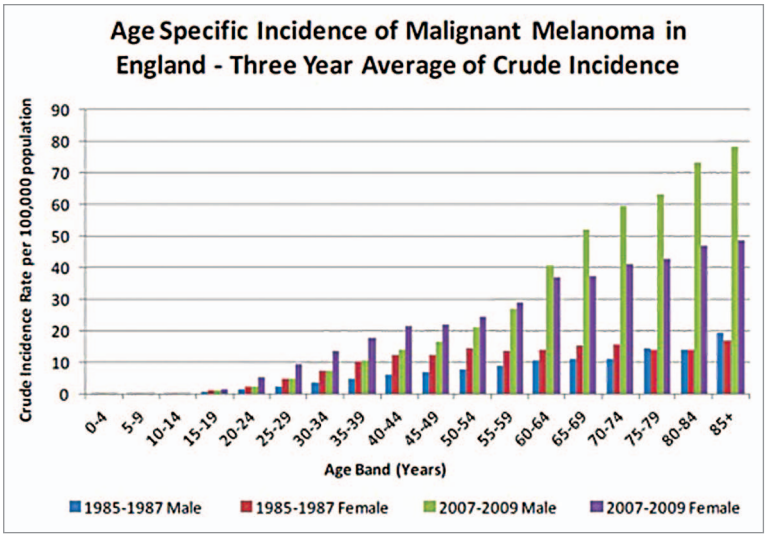

Figure 1. Data produced by Veronique Poirier for the NCIN on melanoma incidence in England in the periods 1985–1987 and 2007–2009 showing an increased incidence at all ages and for both sexes but that the increase was especially marked in those 60 y or older and especially in males.

The genes associated with red hair and freckles increase susceptibility in ways that are viewed as increasingly complex. The type of pigment present in the skin of people with "red hair variants" differs from that of those with wild type *MC1R*, in that the dominant pigment in those with *MC1R* variants is pheomelanin, which is yellow/red and this pigment is less protective against the sun than eumelanin, which is black pigment. Some of the increased risk for people carrying *MC1R* variants is therefore simply because their skin cells are less well protected from UV exposure. The genetic variation is however increasingly recognized to have more complex effects. A very recent paper from David Fisher's group at Harvard described a murine experiment which suggested a deleterious effect of pheomelanin in terms of melanoma risk even in the absence of sunlight.[10]

Overall, the genetic-epidemiological data suggest that intermittent sun exposure and sunburn are the exposures, which have been responsible for the dramatic increase in melanoma incidence in the past 80 y. Phenotypic risk factors are well recognized and the data therefore strongly suggest that sunbathing and sunburn should be avoided in people with those phenotypes to avoid further increases in incidence.

There are some complexities of the epidemiological data, which suggest that sun exposure can be protective, however, in some circumstances. The original data are reasonably old: in a review of 57 case-control studies, Sara Gandini reported a protective effect for melanoma of high occupational sun exposure.[2] It is difficult of course to interpret these associations. The protective effect may be because the occupationally sun exposed develop epidermal thickening and tanning in the course of their work (photoadaption) which might reduce their risks of sunburn when they go on holiday, or perhaps the occupationally sun exposed are less likely to take sunny holidays or less likely to adopt "risky" behaviors on holiday. The complexity of such observational epidemiological studies mean that it is difficult to distinguish different sources of sun exposure and hence to be able to attribute risk to one exposure over another.

In a very detailed analysis of sun exposure and melanoma risk reported by our group in 2011 from a large Leeds (North of UK) melanoma case-control study, we identified further complexities in that overall the clearest relationship between reported sun exposure and risk was for average weekend sun exposure in warmer months, which was actually protective (odds ratio (OR) 0.67, 95% CI: 0.50–0.89 for highest vs. lowest tertile of exposure). Serum 25-hydroxyvitamin D_2/D_3 levels were strongly associated with increased weekend and holiday sun exposure.[11] In this data set, sunburn was a robust risk factor for melanoma as were the well-described inherited susceptible phenotypes of sun sensitivity and increased numbers of nevi[11,12] so the data showed overall consistency with other studies including the pooled analyses. The Leeds study data are therefore reminiscent of the previous case-control data in which a protective effect of occupational sun exposure was seen in that regular sun exposure at least was protective. We postulated three potential explanations of these observations: (1) people who spend many hours outside at weekends in the summer might be less likely to behave in such a way as to risk sunburn on holiday, (2) photoadaption (tanning and thickening of the skin after regular sun exposure) would reduce the risk of sunburn on chronically sun exposed body sites at least and therefore might reduce melanoma risk in these sites or (3) vitamin D could have a protective effect for melanoma as weekend sun exposure was associated with higher vitamin D levels.

There are few strong additional epidemiological data to give insight into the hypothesis that higher vitamin D levels might protect from melanoma, although a number of cohort studies have addressed a possible protective effect of vitamin D. A recent review describes the data in detail.[13] Asgari et al. reported melanoma risk among 68,611 men and women who were participants of the Vitamins and Lifestyle cohort study, a study based in Washington state which is close in latitude to Leeds and hence expected to have similar sun exposure,[14] although the population is likely to encounter more supplemented foods. Participants reported dietary vitamin D intake over the previous year and 10-year use of multivitamin and individual vitamin D supplements on a baseline questionnaire (but importantly the study did not measure serum 25-hydroxyvitamin D_2/D_3 levels). After follow-up through 2006, 455 incident melanomas were identified. Cox proportional hazards regression models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for vitamin D intake after adjustment for melanoma risk factors. Compared with the lowest quartile, no risk reduction of melanoma was detected in the highest quartiles of dietary vitamin D intake (RR = 1.31, 95% CI = 0.94–1.82, i.e., a non-significantly increased risk of melanoma among those on the highest doses of vitamin D), 10-year average supplemental vitamin D intake (RR = 1.13, 95% CI = 0.89–1.43) or combined dietary and supplemental intake (RR = 1.05, 95% CI = 0.79–1.40). In this

large prospective cohort then, no association was seen between vitamin D intake and melanoma risk. Major et al.[15] reported a nested case-control study within the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study. From the ATBC cohort, 368 subjects were chosen; 92 participants that developed melanoma and 276 matched control subjects. At study baseline, lifestyle questionnaires and blood samples had been collected. Serum 25-hydroxyvitamin D_2/D_3 was modeled as three sets of categorical variables: clinically-defined categories, season-specific quartiles and season-adjusted residual quartiles. Conditional logistic regression was used to obtain odds ratios (ORs) and 95% confidence intervals to estimate the association between circulating vitamin D and melanoma risk. Overall no association of serum 25-hydroxyvitamin D_3 and melanoma risk was observed. A decreased risk of developing melanoma was observed in the middle categories compared with the lowest category, but this was not statistically significant. While this study by its design is of notable relevance, the small numbers of melanoma cases means than it could only detect a strong association between vitamin D levels and risk of melanoma.

The possible role of vitamin D, assessed as reported dietary intake, in melanoma risk was reviewed by Gandini et al. in a meta-analysis.[16] They excluded one study on the basis of heterogeneity and in the remaining studies showed some evidence of a protective effect for highest verses lowest intake, summary RR 0.63 (95% CI 0.42–0.94). A small previous study had failed to show lower levels of vitamin D in melanoma patients than in healthy individuals.[17]

Interventional studies being more persuasive than observational ones, a study of melanoma risk in postmenopausal women age 50 to 79 y (n = 36,282) enrolled onto the Women's Health Initiative (WHI) calcium/vitamin D clinical trial, randomly assigned to receive 1,000 mg of elemental calcium plus 400 IU of vitamin D_3 (CaD) daily or placebo for a mean follow-up period of 7.0 y is of interest.[18] This was a relatively small dose of vitamin D, additional supplementation was allowed in controls and no measurements of serum 25-hydroxyvitamin D_2/D_3 took place, limiting the interpretation of the study. Melanoma rates did not differ overall between treatment groups [hazard ratio (HR), 1.02; 95% CI, 0.95 to 1.07] and placebo groups (HR, 0.86; 95% CI, 0.64 to 1.16). In subgroup analyses however, women with a history of non-melanoma skin cancer assigned to calcium and vitamin D had a reduced risk of melanoma vs. those receiving placebo [HR, 0.43; 95% CI, 0.21 to 0.90; P (interaction) = 0.038]. Furthermore the Kaplan Meier curve for the development of melanoma in the whole sample set does not exclude an emerging protective effect taking into account the observation that chemoprevention effects typically take many years to become apparent.

Vitamin D Receptor (VDR) Genetic Polymorphisms and Risk

Epidemiological studies designed to look at risk associated with factors such as 25-hydroxyvitamin D_2/D_3 levels in the serum have many difficulties including the fact that vitamin D levels likely change over time so that a single measure does not necessarily convey the total picture. It is also not clear what is the most relevant measure—average vitamin D over the year or lowest level of vitamin D to indicate the most at risk period. The risk could also be modified by the body's ability to utilize the circulating vitamin D. Several groups have therefore performed candidate gene association studies comparing polymorphisms in the gene coding for the vitamin D receptor and melanoma risk. Overall the data are suggestive, but not conclusive of an association between variants in *VDR* and risk. **Figure 2** shows a Forest plot derived from data reported previously by the Leeds group in which no relationship was seen between these variants and risk in the Leeds data but supportive evidence was seen in a meta-analysis of that and other data sets.[19]

Vitamin D Levels and Sun Avoidance

Many studies have shown that population levels of serum 25-hydroxyvitamin D_2/D_3 are lower than that reported to be necessary for bone health. Most groups report the lower level of optimal range to be around 60 nmol/L of 25-hydroxyvitamin D_2/D_3, based upon an approximate plateauing of the curve of parathyroid hormone levels in relation to 25-hydroxyvitamin D_2/D_3 levels[20] which was consistent with a consensus statement generated by the US Institute of Medicine of the National Academies in 2010 which stated that a level of 20 ng/ml (50 nmol/L) was needed for bone health. The US Endocrine Society's Clinical Guidelines published in 2011 however defined levels below 50 nmol/L as deficient and levels of 52.5–72.5 nmol/L as insufficient.[21,22]

Levels have been reported to be especially low in populations with low dietary intake of foods containing vitamin D such as fatty fish, those living in institutions having little exposure to the sun and others with dark skin living in temperate climates especially if their skin is covered for social or religious reasons.

In fair skinned peoples, curiously, several studies have now shown that sun-sensitivity is associated with lower vitamin D levels,[23-25] presumably because of the difficulties of staying out in the sun for long enough to synthesize sufficient vitamin D without burning (see Discussion and **Fig. 3** below). In a study of UK adult twins, when winter 25-hydroxyvitamin D_2/D_3 levels were examined, the differences were marked; low 25-hydroxyvitamin D_2/D_3 levels (defined as < 70 nmo/l in this study) were found in 60% of the Fitzpatrick skin type 1 compared with 38% of the skin type 4 ($p < 0.0001$).[25] In **Figure 3** we show data derived from a study we reported previously in which the determinants of serum 25-hydroxyvitamin D_2/D_3 levels were studied in participants in the Leeds Melanoma Case-Control study.[24] In this figure we show the relationship between serum levels and reported time outdoors in warmer weather for sun-sensitive and non-sun-sensitive participants who were not taking supplements. It is of relevance that in the UK few foods have to date been supplemented with vitamin D although there is a trend to increased supplementation of cereals.

Vitamin D and Survival from Melanoma

There has been interest in vitamin D and melanoma treatment for some time. Vitamin D has clear anti-proliferative effects on

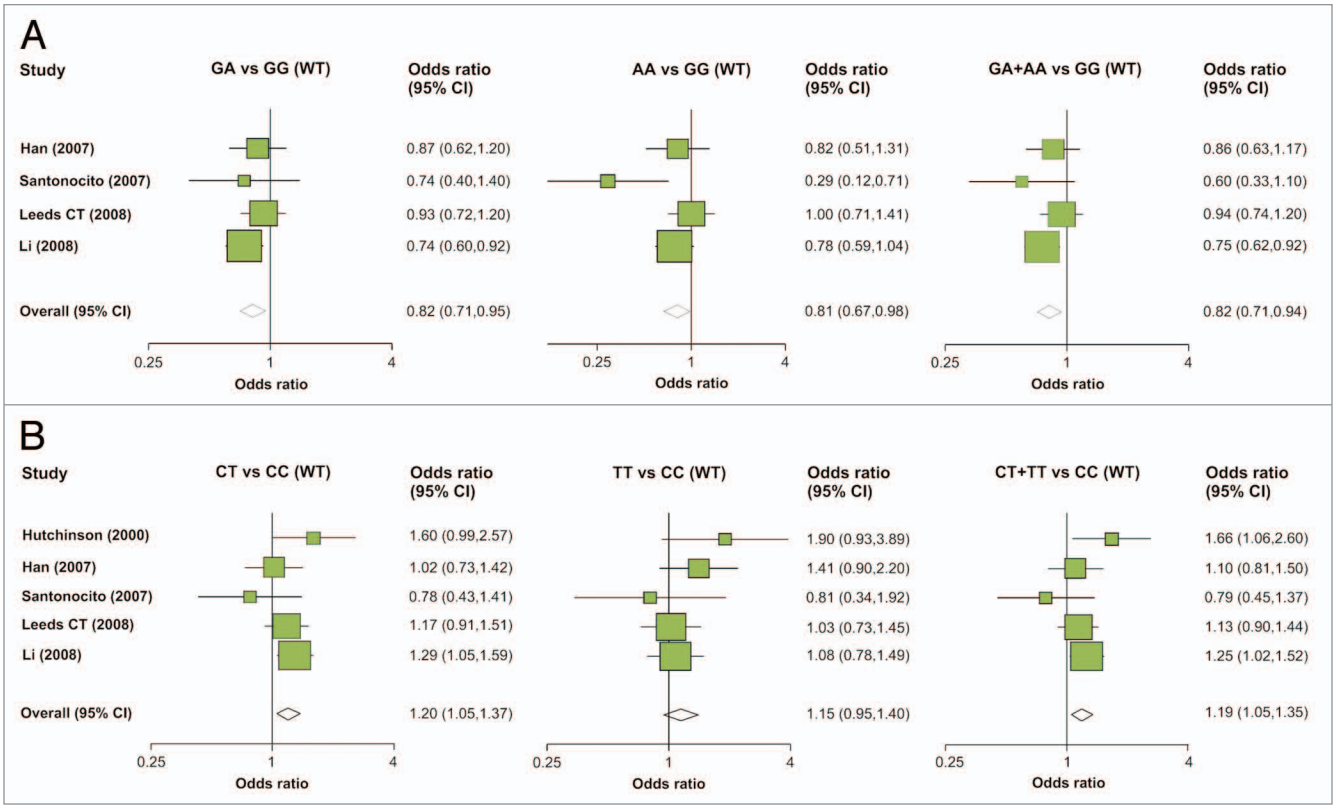

Figure 2. Forest plots showing the association between (**A**) inherited BsmI and (**B**) FokI polymorphisms in data from 5 association studies[19,36-39] which were suggestive of a small association between VDR polymorphisms and melanoma risk.

a proportion of melanoma cell lines in vitro and the variability of those effects, has been reported to be related to integrity of the vitamin D signaling pathway.[27] There is also some evidence of reduced expression of the vitamin D receptor with progression from nevi through primary to metastatic melanoma[28] suggesting that if vitamin D is anti-proliferative for melanoma cells in vivo, then those cells might be less likely to respond to any putative anti-proliferative effects of vitamin D as progression occurs.

The in vitro data suggest that vitamin D is none-the-less of interest in melanoma patients. We therefore, in Leeds, collected data on vitamin D supplementation in a case-control study of 271 late relapsing melanoma patients which was a pilot study designed to identify environmental exposures which might impact on relapse from melanoma.[29] We compared exposures in melanoma patients who had relapsed and were interviewed a median of 7 years after diagnosis compared with patients who had survived a similar period of time without relapse. 62/149 (42%) non-relapsers and 28/91 (31%) relapsers reported regular intake of supplemental vitamin D one year prior to interview (OR 0.6, 95% CI (0.4, 1.1)). Non-relapsers had higher (non-significant) mean 25-hydroxyvitamin D_2/D_3 levels than relapsers, (49 nmol/L compared with 46, t-test, p = 0.3). There was no statistically significant relationship therefore between reported supplementation or serum levels and relapse in this small-scale study, but it was judged sufficient evidence to merit investigation in a larger data set.

We therefore collected data on 25-hydroxyvitamin D_2/D_3 levels in the serum at recruitment to the Leeds Melanoma Cohort. A cohort has been recruited in the period 2000 till 2012, now comprised of 2180 cases. We analyzed the data on serum 25-hydroxyvitamin D_2/D_3 levels in the time around diagnosis and reported this in 2009.[29] In the cohort higher 25-hydroxyvitamin D_2/D_3 levels were associated with lower Breslow thickness at diagnosis (p = 0.002) and were independently protective of relapse and death: the hazard ratio (HR) for relapse-free survival (RFS) was 0.79 (95% CI, 0.64 to 0.96; p = 0.01) for a 20 nmol/L increase in serum level.[29] A subsequent small study in stage IV melanoma patients reported that lower vitamin D levels were associated with poorer survival,[30] but in the absence of further published data the Leeds study remains unvalidated.

We therefore have persuasive (but not proven) evidence that 25-hydroxyvitamin D_2/D_3 levels at diagnosis may predict outcome for melanoma patients. A randomized clinical trial (RCT) would be the only means of determining if vitamin D supplementation would then be of benefit for melanoma patients at diagnosis. The widespread publicity and discussion around the merits of vitamin D supplementation for health generally mean however that melanoma patients may supplement anyway making a placebo controlled RCT difficult. Such a trial would also in our view require individualized dosage as we have shown marked variation in levels according to body mass index, genotype in the gene coding for the vitamin D binding protein, sun sensitivity and sun exposure.[24] If, the albeit rather theoretical arguments

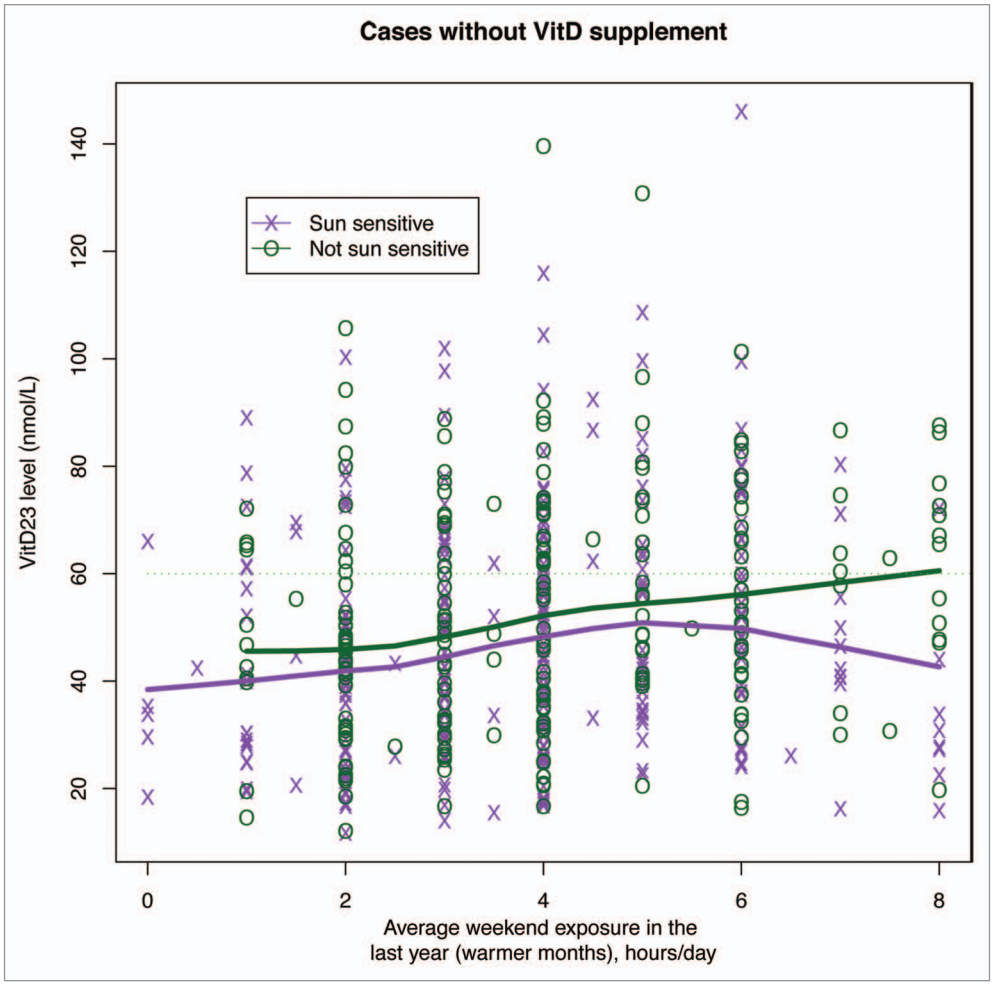

Figure 3. Average levels of 25-hydroxyvitamin D_2/D_3 in participants in the Leeds Melanoma Case-control study who were not taking supplements and in relation to the number of hours spent outside in the warmer months.[24] The solid green line represents those defined as non-sun-sensitive and the purple, the sun-sensitive. It can be seen that the majority of individuals failed to reach the "optimal" 60 nmol/L even after reporting spending on average 6 h per day outside on both Saturdays and Sundays and that this was especially true of the sun-sensitive. Reported sun exposure in the UK was in this population insufficient to result on average in optimal levels of 25-hydroxyvitamin D_2/D_3.

below of aiming for target levels of vitamin D between 60 and 85 nmol/L are accepted, then these or a predefined target serum level should be achieved in the supplemented arm of the RCT. This would necessitate monitoring of serum 25-hydroxyvitamin D_2/D_3 levels and adjustment of individualized supplementation dosage through the period of the study. In our view it is important therefore to further explore the possible relationship between vitamin D and survival in the interim by developing the biological evidence of relevance to such a trial. Indeed the reported effects of vitamin D are so pleomorphic that if a RCT was based on dosage alone, dosage selection would prove very difficult especially if the designers of such a RCT had little evidence for the potential critical mode of action of the vitamin D.

The first issue is whether the observed relationship between 25-hydroxyvitamin D_2/D_3 levels and survival for melanoma patients is causal. The reasons why the link between vitamin D and survival would not be causal, would be if both factors were separately attributable to a third factor. For example, 25-hydroxyvitamin D_2/D_3 levels tend to be higher in leaner, wealthier, more active individuals and therefore the demonstrated relationship between those levels and survival might reflect the beneficial effects of healthier lifestyles generally on survival rather than a direct effect of vitamin D itself. In the epidemiology literature, this is termed "confounding." Again, in observational studies, it is very difficult to distinguish between a causal relationship and confounding in the absence of knowledge of all the confounders. One statistical approach to postulate causation is termed "Mendelian Randomization." Mendelian randomization tests the relationship between the inheritance of a genetic variant known to predict the variable of interest (in this case serum vitamin D levels) and the outcome of interest, here melanoma survival, arguing that the demonstration of a relationship between the genetic variant and survival would establish causality. The weakness of studies taking this approach is usually that the genetic variant explains such a small proportion of the factor of interest e.g., vitamin D levels, that prohibitively large studies are needed. International collaborative work is currently ongoing to address this within BioGenoMEL (www.biogenomel.

eu), but the study may prove inconclusive given the small proportion of the variance in 25-hydroxyvitamin D_2/D_3 levels explained by the variant gene. Only results from a RCT would be conclusive. In fact RCT called MeleVID of adjuvant vitamin D for stage 2 melanoma is registered as recruiting in a study organized by the European Institute of Oncology in Milan. We discuss below some concerns however that we have about the design of RCTs with vitamin D in general.

One issue would be which of the many described biological effects of vitamin D might have a protective effect for melanoma patients and what might be the optimal blood level to achieve those putative beneficial effects. The in vitro anti-proliferative effect of vitamin D added to melanoma cell cultures is persuasive, and the Leeds Melanoma Cohort data suggest that primaries in individuals recruited to the study were thinner in individuals with higher 25-hydroxyvitamin D_2/D_3 levels, which is consistent with an anti-proliferative effect. Extrapolating from in vitro data to appropriate blood levels to achieve putative anti-proliferative effects on melanoma cells in man would be however very difficult. Vitamin D moreover is also reported to have pleomorphic effects including those on new blood vessel formation and immunity and some of these effects might actually be deleterious for cancer patients (discussed below).

Possible Deleterious Effects of Vitamin D in Cancer Patients

The reported effects of vitamin D on the immune system are extremely complex and the literature extensive. Of concern for immunogenic tumors like melanoma however, is the observation that vitamin D may suppress adaptive immunity[31] reviewed in 32. Melanoma is an immunogenic tumor and the positive prognostic value of the development of vitiligo in patients treated with chemotherapy suggests that immune responses to melanoma are important. If vitamin D supplementation was to suppress adaptive immunity then that would be a potentially harmful effect for melanoma patients.

There has been a suggestion from studies of risk in breast cancer[33] and cardiovascular disease[34] of a U or J-shaped relationship between vitamin D levels: that is that there was a suggestion in these studies of a deleterious effect of vitamin D at high dosage. In a recent observational study from Denmark,[35] the results of approximately 250,000 serum vitamin D levels ordered by primary health care physicians were plotted against all cause mortality and the curve was convincingly reverse J-shaped. Low vitamin D levels were strongly associated with higher all cause mortality but there was also a suggestion that levels above 80 or 90 nmol/L were also associated with higher mortality. Interestingly the lowest mortality was at around 60 nmol/L, the level commonly viewed as the lower level associated with bone health. The data are however very difficult to interpret as there is no information provided on why the samples were taken: perhaps those with high levels were unwell and over supplementing? The apparently deleterious effect of higher vitamin D levels might be artifactual.

Given however that we have concerns about the immunosuppressive effects of vitamin D demonstrated in vitro, our view is that high doses of vitamin D are also to be avoided. In fact the recent publication of the Durup paper (with all its difficulties in terms of interpretation) means that we consider that the range to be aimed for may be more like 60 to 85 nmol/L (allowing for a seasonal variation) rather than 60 to 100 nmol/L which we suggested in 2011.[32]

Advice for Melanoma Patients in the Meantime

In 2009 after reporting that patients with lower 25-hydroxyvitamin D_2/D_3 levels around the time of melanoma diagnosis had thicker poorer prognosis tumors, in the Leeds Melanoma Service we advised our patients to sun protect without becoming vitamin D depleted. We provided an information leaflet to that effect and information was also available online on our web page, www.genomel.org. The information suggested increasing intake through food such as oily fish and to consider oral supplementation with 400 IU/day vitamin D_3 daily based on the update on vitamin D by UK Scientific Advisory Committee on Nutrition 2007 which recommended daily intake of vitamin D (10 μg/day = 400 IU/day) for adults at risk of deficiency such as those over 65 years of age, all pregnant and breast feeding women and people not exposed to much sun. The latter category would include people confined indoors and those who cover the skin for cultural reasons; melanoma patients avoiding sun exposure following diagnosis would also fall into this category. We did not prescribe vitamin D or measure levels initially but over time the department decided to measure levels because of some anxieties that patients might be over supplementing and we report here the data from an audit performed of these results.

We assessed 746 patients with a diagnosis of melanoma attending the melanoma follow up clinic to determine how successfully our advice resulted in vitamin D levels in the intended range (60–85 nmol/L), in the period prior to routine testing and after routine testing. After analysis of test results the departmental policy was adjusted to recommend supplementation with 1000 IU per day of vitamin D_3 for levels below 40 nmol/L with repeat levels at 6 months and 400 IU per day of vitamin D_3 for levels between 40 nmol/L and 60 nmol/L with repeat levels at six months. No supplementation was recommended for levels over 60 nmol/L although the advice was given for the patient to review if they changed their sun avoidance subsequently.

The median age at diagnosis was 57.7 years (range 15.6–95.0). Stage was defined on 646, 326 had stage I disease, 181, 127 and 12 had stage II, III and IV disease respectively. 614 cases had at least one 25-hydroxyvitamin D_2/D_3 serum level recorded with a median time from diagnosis to measurement of 370 days (range 0–10,471). 168 had a second level [median time between their first and second level was 192 days (range 41–1376)]. The median level unadjusted for season at first measurement was 48.7 nmol/L (n = 614, range 10–121). 8% (49/614) had levels < 20 nmol/L and 67.4% (414/614) had levels < 60 nmol/L, 2.1% (13/614) had levels > 100 nmol/L. In those reported to be supplementing, median levels were higher (n = 233, median 56.6 nmol/L, range 15–120) than those not supplementing [n = 312, median 38.9 nmol/L (range 10–121)]. 80% (247/312) of patients not on

supplements had suboptimal levels (< 60 nmol/L). Supplement usage data were not available on 69 cases (median 60.1 nmol/L, range 19–105).

If patients were not supplementing and their levels were taken more than six months after diagnosis they were lower compared with levels in individuals taken within six months of diagnosis but this difference was not statistically significant when levels were adjusted for age at diagnosis and season of blood draw (< 6 months median 34.1 nmol/L, > 6 months 30.9 nmol/L, Mann-Whitney ranked test, p = 0.2, assuming blood was drawn in winter from a 50-year-old).

Eighty-eight patients who were not supplementing at first testing, were recommended supplementation and had a second level measured; 93.2% (82/88), 5.7% (5/88), 1.1% (1/88) were below, within and above target range at first testing. Their 25-hydroxyvitamin D_2/D_3 levels on retesting taken a median of 86 days later (range 41–497) approximating to their three monthly melanoma review were statistically significantly higher (n = 88 1st level median 35.6 nmol/L, range 12.7–83.6, 2nd level median 66.95 nmol/L, range 22–120, Wilcoxon signed rank test p = < 0.001) (**Fig. 4**). Based on our 60–85 nmol/L target range a significantly higher proportion achieved optimal levels (38.6% (34/88), sign test p = < 0.001); 43.2% (38/88), 13.6% (12/88), 4.6% (4/88) were below, above but < 100 nmol/l and > 100 nmol/l respectively.

Of those supplementing at first measure, whose supplementation was adjusted in response to blood levels and had a second measure, only 14.5% (8/55) were within the optimal range at first testing; 78.3% (43/55), 3.6% (2/55), 3.6% (2/55) were below, above range but < 100 nmol/L and > 100 nmol/L respectively. This group achieved a statistically significantly higher level of 25-hydroxyvitamin D_2/D_3 on retesting after adjustment to dosage (n = 55 1st level median 48.3, range 15–120, 2nd level median 62.2, range 18.1–116, Wilcoxon signed rank test p = 0.009) (**Fig. 4**) and a significantly higher proportion reached optimal levels (41.8% (23/55), sign test p = 0.002); 41.8% (23/55), 9.1% (5/55), 7.3% (4/55) were below, above but < 100 nmol/L and > 100 nmol/L respectively.

Thirteen patients had 25-hydroxyvitamin D_2/D_3 levels over 100 nmol/L at first measure, 7 were supplementing, 5 were not (2/5 recent sun exposure in a hot country, 1/5 diet rich in eggs/fish) and 1 supplement status was unknown. 5/13 had repeat levels at time of writing: 4/5 had been recommended to stop supplementing and in one supplementation was reduced. All except one individual whose supplementation was not stopped achieved a level below 100 nmol/L within 6 months (median 187 days, range 91–286), 2 reached target and 1 dropped marginally below (56.6 nmol/L).

In summary, the majority of patients with a diagnosis of melanoma had insufficient 25-hydroxyvitamin D_2/D_3 levels after diagnosis regardless of their supplementation practices. These patients had all been given written advice on the avoidance of vitamin D depletion so that the data suggest that if vitamin D levels are not measured then a significant proportion of UK patients tend not to be motivated to take supplements and levels are predicted therefore to fall even further over time as greater sun avoidance is frequently reported after a melanoma diagnosis.

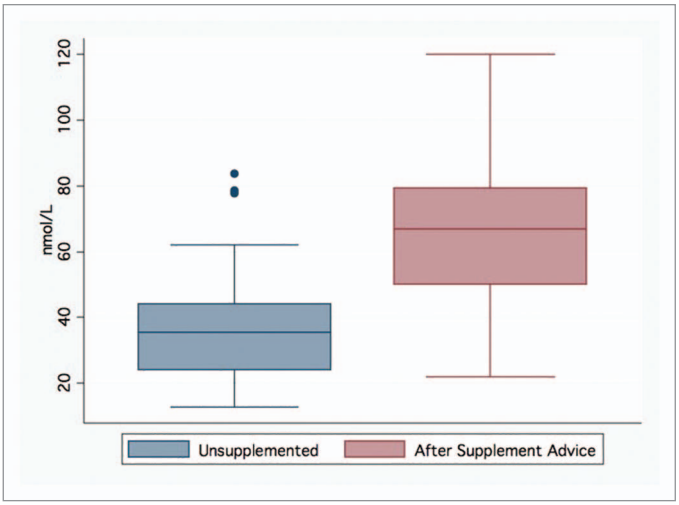

Figure 4. Box and whisker plot showing (in blue) levels in melanoma patients not supplementing a median of approximately 1 year after diagnosis and (in pink) after testing and tailored advice on supplementation with 400 to 1000 IU of vitamin D3.

The audit showed that vitamin D supplementation advice given after testing now results in significantly higher proportion of our patients achieving a vitamin D level within the target range of 60–85 nmol/L. The audit did show that a small proportion of patients had levels higher than our target range of 60 to 85 nmol/L. In some this occurred in the absence of supplementation and appeared to be related to sun exposure or was unexplained. In around 17% levels exceeded the target range after supplementation requiring dose adjustment.

Many melanoma patients have suboptimal 25-hydroxyvitamin D_2/D_3 levels at diagnosis although this is also true of the general population. After diagnosis, patients tend to adopt greater sun avoidance measures and therefore the likelihood is that levels would fall further if no supplements are taken. In the audit reported here we found that melanoma patients often had low levels after diagnosis despite advice to avoid vitamin D depletion. By measuring levels and giving tailored advice on supplementation we achieved median levels within the range we have defined as optimal (60 to 85 nmol/L), with few reaching levels which we have defined as higher than optimal. Where levels were greater than the target range, supplementation dosage correction resulted in regression toward the optimal level but in a proportion higher levels had occurred in the absence of supplementation.

Our advice now, pending a RCT, is that melanoma patients should consider supplementing their diet to avoid further reduction in their vitamin D levels over time as they adopt sun avoidant behaviors. We would suggest however that supplementation should be performed only after a serum 25-hydroxyvitamin D_2/D_3 level has been measured and that the levels we might aim for might be 60 to 85 nmol/L allowing for seasonal variation. Measurement of blood levels after supplementation for 6 months was shown furthermore to identify those who had developed levels higher than the target range so it appears necessary to us to

measure levels at least once. Patients should know that this advice is based upon a pragmatic interpretation of published data rather than clinical trial data.

Summary

The relationship between sun exposure and melanoma risk is complex. There is unequivocal evidence that recreational sun exposure increases melanoma risk especially if associated with sunburn. For the genetically predisposed, avoidance of sun exposures likely to result in sunburn is crucial if the rising incidence of melanoma is to be reversed. In many populations, the genetically predisposed include very large proportions of the population. If we take the presences of freckles as a marker of inherited *MC1R* variants, then dermatologists will recognize that this is so. Genetic studies have proved that this is so as: in an Australian study for example 73% of controls were reported to carry at least one "r" or "R" *MC1R* variant.[26] Sensible advice from health protection agencies would therefore be that all people should avoid sunburn and those with freckles, red or blond hair, who tend to burn in the sun, who are "moley," who have "moley" parents or who have a family history of melanoma should take particular care to protect themselves from sunburn and should avoid sunbathing because that is the behavior most strongly associated with sunburn.

We have shown above that 25-hydroxyvitamin D_2/D_3 levels tend to be lower in sun-sensitive patients and because of the known deleterious effects of sub-optimal vitamin D on bone health alone, it seems sensible to us that public health advice should also be that the sun avoidant should assess their diet (intake of fatty foods or supplemented foods) and consider if supplementation is required. Our data suggest that in the UK at least where the diet is poor in fatty fish and food supplementation is rare, supplementation would be necessary in the majority to achieve blood levels of 60 nmol/L. The Endocrine Society's Clinical Guidelines do not recommend population screening for vitamin D insufficiency in individuals not at risk, presumably cost implications having appropriately been considered here (www.endo-society.org). However, in clinical practice we have found it difficult to predict levels without measuring (see below), and one might argue that the data reported above suggest that the sun-sensitive are at risk of sub-optimal levels at least in the UK.

The evidence that vitamin D levels might influence melanoma risk remains circumstantial therefore as no direct evidence is available from the epidemiological literature; however, it should also be pointed out that the studies of sufficient size to address this issue have not been conducted.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001; 94:153-6; PMID:11668491; http://dx.doi.org/10.1002/ijc.1440
2. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005; 41:45-60; PMID:15617990; http://dx.doi.org/10.1016/j.ejca.2004.10.016
3. Chang YM, Barrett JH, Bishop DT, Armstrong BK, Bataille V, Bergman W, et al. Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. Int J Epidemiol 2009; 38:814-30; PMID:19359257; http://dx.doi.org/10.1093/ije/dyp166
4. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer 2005; 41:2040-59; PMID:16125929; http://dx.doi.org/10.1016/j.ejca.2005.03.034
5. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005; 41:28-44; PMID:15617989; http://dx.doi.org/10.1016/j.ejca.2004.10.015
6. Bishop DT, Demenais F, Iles MM, Harland M, Taylor JC, Corda E, et al. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet 2009; 41:920-5; PMID:19578364; http://dx.doi.org/10.1038/ng.411
7. Barrett JH, Iles MM, Harland M, Taylor JC, Aitken JF, Andresen PA, et al.; GenoMEL Consortium. Genome-wide association study identifies three new melanoma susceptibility loci. Nat Genet 2011; 43:1108-13; PMID:21983787; http://dx.doi.org/10.1038/ng.959
8. Wachsmuth RC, Gaut RM, Barrett JH, Saunders CL, Randerson-Moor JA, Eldridge A, et al. Heritability and gene-environment interactions for melanocytic nevus density examined in a U.K. adolescent twin study. J Invest Dermatol 2001; 117:348-52; PMID:11511314; http://dx.doi.org/10.1046/j.0022-202x.2001.01415.x
9. Easton DF, Cox GM, Macdonald AM, Ponder BA. Genetic susceptibility to naevi--a twin study. Br J Cancer 1991; 64:1164-7; PMID:1764382; http://dx.doi.org/10.1038/bjc.1991.483
10. Mitra D, Luo X, Morgan A, Wang J, Hoang MP, Lo J, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature 2012; 491:449-53; PMID:23123854; http://dx.doi.org/10.1038/nature11624
11. Newton-Bishop JA, Chang YM, Elliott F, Chan M, Leake S, Karpavicius B, et al. Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. Eur J Cancer 2011; 47:732-41; PMID:21084183; http://dx.doi.org/10.1016/j.ejca.2010.10.008
12. Newton-Bishop JA, Chang YM, Iles MM, Taylor JC, Bakker B, Chan M, et al. Melanocytic nevi, nevus genes, and melanoma risk in a large case-control study in the United Kingdom. Cancer Epidemiol Biomarkers Prev 2010; 19:2043-54; PMID:20647408; http://dx.doi.org/10.1158/1055-9965.EPI-10-0233
13. Berwick M, Erdei EO. Vitamin D and melanoma incidence and mortality. Pigment Cell Melanoma Res 2013; 26:9-15; PMID:22947439; http://dx.doi.org/10.1111/pcmr.12015
14. Asgari MM, Maruti SS, Kushi LH, White E. A cohort study of vitamin D intake and melanoma risk. J Invest Dermatol 2009; 129:1675-80; PMID:19194478; http://dx.doi.org/10.1038/jid.2008.451
15. Major JM, Kiruthu C, Weinstein SJ, Horst RL, Snyder K, Virtamo J, et al. Pre-diagnostic circulating vitamin D and risk of melanoma in men. PLoS One 2012; 7:e35112; PMID:22558121; http://dx.doi.org/10.1371/journal.pone.0035112
16. Gandini S, Raimondi S, Gnagnarella P, Doré JF, Maisonneuve P, Testori A. Vitamin D and skin cancer: a meta-analysis. Eur J Cancer 2009; 45:634-41; PMID:19008093; http://dx.doi.org/10.1016/j.ejca.2008.10.003
17. Reichrath J, Querings K. No evidence for reduced 25-hydroxyvitamin D serum level in melanoma patients. Cancer Causes Control 2004; 15:97-8; PMID:15049326; http://dx.doi.org/10.1023/B:CACO.0000016676.23024.e8
18. Tang JY, Fu T, Leblanc E, Manson JE, Feldman D, Linos E, et al. Calcium plus vitamin D supplementation and the risk of nonmelanoma and melanoma skin cancer: post hoc analyses of the women's health initiative randomized controlled trial. J Clin Oncol 2011; 29:3078-84; PMID:21709199; http://dx.doi.org/10.1200/JCO.2011.34.5967

19. Randerson-Moor JA, Taylor JC, Elliott F, Chang YM, Beswick S, Kukalizch K, et al. Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. Eur J Cancer 2009; 45:3271-81; PMID:19615888; http://dx.doi.org/10.1016/j.ejca.2009.06.011
20. Björkman M, Sorva A, Tilvis R. Responses of parathyroid hormone to vitamin D supplementation: a systematic review of clinical trials. Arch Gerontol Geriatr 2009; 48:160-6; PMID:18243368; http://dx.doi.org/10.1016/j.archger.2007.12.005
21. Dietary Reference Intakes for Calcium and Vitamin D. Food and Nutrition Board: The National Academies Press, 2011.
22. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96:1911-30; PMID:21646368; http://dx.doi.org/10.1210/jc.2011-0385
23. Malvy DJ, Guinot C, Preziosi P, Galan P, Chapuy MC, Maamer M, et al. Relationship between vitamin D status and skin phototype in general adult population. Photochem Photobiol 2000; 71:466-9; PMID:10824599; http://dx.doi.org/10.1562/0031-8655(2000)071<0466:RBVDSA>2.0.CO;2
24. Davies JR, Chang YM, Snowden H, Chan M, Leake S, Karpavicius B, et al. The determinants of serum vitamin D levels in participants in a melanoma case-control study living in a temperate climate. Cancer Causes Control 2011; 22:1471-82; PMID:21853245; http://dx.doi.org/10.1007/s10552-011-9827-3
25. Glass D, Lens M, Swaminathan R, Spector TD, Bataille V. Pigmentation and vitamin D metabolism in Caucasians: low vitamin D serum levels in fair skin types in the UK. PLoS One 2009; 4:e6477; PMID:19649299; http://dx.doi.org/10.1371/journal.pone.0006477
26. Cust AE, Armstrong BK, Goumas C, Jenkins MA, Schmid H, Hopper JL, et al. Sunbed use during adolescence and early adulthood is associated with increased risk of early-onset melanoma. Int J Cancer 2011; 128:2425-35; PMID:20669232; http://dx.doi.org/10.1002/ijc.25576
27. Reichrath J, Rech M, Moeini M, Meese E, Tilgen W, Seifert M. In vitro comparison of the vitamin D endocrine system in 1,25(OH)2D3-responsive and -resistant melanoma cells. Cancer Biol Ther 2007; 6:48-55; PMID:17172823; http://dx.doi.org/10.4161/cbt.6.1.3493
28. Brożyna AA, Jozwicki W, Janjetovic Z, Slominski AT. Expression of vitamin D receptor decreases during progression of pigmented skin lesions. Hum Pathol 2011; 42:618-31; PMID:21292298; http://dx.doi.org/10.1016/j.humpath.2010.09.014
29. Newton-Bishop JA, Beswick S, Randerson-Moor J, Chang YM, Affleck P, Elliott F, et al. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. J Clin Oncol 2009; 27:5439-44; PMID:19770375; http://dx.doi.org/10.1200/JCO.2009.22.1135
30. Nürnberg B, Gräber S, Gärtner B, Geisel J, Pföhler C, Schadendorf D, et al. Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients. Anticancer Res 2009; 29:3669-74; PMID:19667163
31. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94:26-34; PMID:18854395; http://dx.doi.org/10.1210/jc.2008-1454
32. Field S, Newton-Bishop JA. Melanoma and vitamin D. Mol Oncol 2011; 5:197-214; PMID:21371954; http://dx.doi.org/10.1016/j.molonc.2011.01.007
33. Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. J Clin Oncol 2009; 27:3757-63; PMID:19451439; http://dx.doi.org/10.1200/JCO.2008.20.0725
34. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008; 117:503-11; PMID:18180395; http://dx.doi.org/10.1161/CIRCULATIONAHA.107.706127
35. Durup D, Jørgensen HL, Christensen J, Schwarz P, Heegaard AM, Lind B. A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice: the CopD study. J Clin Endocrinol Metab 2012; 97:2644-52; PMID:22573406; http://dx.doi.org/10.1210/jc.2012-1176
36. Han J, Colditz GA, Hunter DJ. Polymorphisms in the MTHFR and VDR genes and skin cancer risk. Carcinogenesis 2007; 28:390-7; PMID:16950800; http://dx.doi.org/10.1093/carcin/bgl156
37. Santonocito C, Capizzi R, Concolino P, Lavieri MM, Paradisi A, Gentileschi S, et al. Association between cutaneous melanoma, Breslow thickness and vitamin D receptor BsmI polymorphism. Br J Dermatol 2007; 156:277-82; PMID:17223867; http://dx.doi.org/10.1111/j.1365-2133.2006.07620.x
38. Li C, Liu Z, Zhang Z, Strom SS, Gershenwald JE, Prieto VG, et al. Genetic variants of the vitamin D receptor gene alter risk of cutaneous melanoma. J Invest Dermatol 2007; 127:276-80; PMID:16990805; http://dx.doi.org/10.1038/sj.jid.5700544
39. Hutchinson PE, Osborne JE, Lear JT, Smith AG, Bowers PW, Morris PN, et al. Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. Clin Cancer Res 2000; 6:498-504; PMID: 10690530.