Article:
Liyanage, UE, Law, MH, Melanoma Meta-analysis Consortium et al. (3 more authors) (2020) Is there a causal relationship between vitamin D and melanoma risk? : A Mendelian randomization study. British Journal of Dermatology, 182 (1). pp. 97-103. ISSN 0007-0963

https://doi.org/10.1111/bjd.18238

British journal of Dermatology

Title

Is there a causal relationship between vitamin D and melanoma risk? : A Mendelian randomization study

Running head

Vitamin D and melanoma risk: A Mendelian randomization study

Word, table and figure count

Words - 2833, tables -1, figures -2

Authors

Upekha E. Liyanage[1], Matthew H. Law[1], Melanoma Meta-analysis Consortium*, Jennifer H. Barrett[2], Mark M. Iles[2], Stuart MacGregor[1]

Affiliations

[1]Statistical Genetics Lab, QIMR Berghofer Medical Research Institute, 300, Herston road, Brisbane, QLD 4006

[2]Leeds Institute for Data Analytics, School of Medicine, University of Leeds, Leeds, UK

*Full consortium author list can be found in the supplementary document.

Corresponding author

Upekha Liyanage

Address - Statistical Genetics Lab, QIMR Berghofer Medical Research Institute, 300, Herston road, Brisbane, QLD 4006

Telephone - 0738453713

E mail - upekha.liyanage@qimrberghofer.edu.au

A statement of all funding sources

This work was supported by NHMRC project grant APP1123248. Stuart Macgregor was supported by an Australian Research Council Fellowship. The funding sources and acknowledgements for the melanoma meta-analysis can be found in the supplementary document.

Conflict of interest disclosures

All authors report they have no conflicts of interest.

What's already known about this topic?

- Anti-tumour activity of vitamin D has been identified in pre-clinical studies.
- Observational studies link vitamin D deficiency with an increased risk of a range of cancers.
- Hence, there is a growing interest amongst the public for vitamin D supplementation.

- Observational studies of melanoma are with fraught with difficulties because whilst higher ultraviolet radiation levels increase vitamin D levels, such exposure is also associated with increased melanoma risk.
- Results from observational studies are inconclusive regarding the effect of vitamin D on melanoma risk.

What does this study add?

- Using Mendelian randomization, an approach to causal inference which is analogous to a natural randomised controlled trial, we found no causal association between vitamin D levels and melanoma.

Key words/Topic

Mendelian randomization, 25-hydroxyvitamin D, melanoma

Abbreviations

25(OH) D - 25-hydroxyvitamin D; vitamin D

DAG – Direct acyclic graph

CI - Confidence Interval

GWAS - Genome-wide association study

IV - Instrumental variables

IVW - Inverse variance weighted method

MR - Mendelian randomization

OR - Odds Ratio

SNPs - Single nucleotide polymorphisms

RCT - Randomised controlled trial

UVR - Ultraviolet radiation

Summary

Background: Several pre-clinical studies have identified the anti-proliferative effects of 25-hydroxyvitamin D (25(OH) D; vitamin D). Ultraviolet radiation (UVR) is essential for vitamin D synthesis yet increases the risk of melanoma. Observational studies on the association of vitamin D levels with melanoma risk have reported inconclusive results, and are difficult to interpret due to the potential confounding from the dual role of UVR.

Objectives: Our objective was to determine whether there is a causal association between genetically predicted 25(OH) D concentrations and melanoma using a Mendelian randomization (MR) approach.

Methods: We performed MR using summary data from a large genome-wide association study (GWAS) meta-analysis of melanoma risk, consisting of 12,874 cases and 23,203 controls. Five SNPs that are associated with 25(OH) D concentration rs12785878, rs10741657, rs2282679, rs6013897 and rs116970203 were selected as instrumental variables (IVs). Inverse variance weighted method was used to access the evidence for causality. MR results from the melanoma meta-analysis were combined with results from an MR study based on a melanoma risk GWAS using UK Biobank data. Results: A 20 nmol/L decrease in 25(OH) D was not associated with melanoma risk (OR = 1.06, 95% CI = 0.94 – 1.19). Results from the UK Biobank were concordant with this, with meta-analysis of our and UK Biobank derived MR causal estimates showing no association (OR = 1.02, 95% CI = 0.92 – 1.13 for a 20nmol/L decrease).

Conclusions: Our study results suggest that the genetically vitamin D levels may not be causally associated with the risk of melanoma.

Introduction

Vitamin D "the sunshine vitamin" is a prohormone that, following conversion to calcitriol, exerts a wide range of biological functions in the human body [1]. In addition to the well-known association with bone and calcium metabolism disorders, many illnesses have been linked with vitamin D deficiency in recent epidemiological studies [2]. Many studies suggest an inverse relationship between vitamin D levels and cancer risk [3], particularly pancreatic [4], breast [5,6] , prostate [7], and colon [8]. However other studies have reported a null association or an increase in cancer risk with higher vitamin D levels [9–12]. Observational studies are prone to bias due to confounding and reverse causation, which may explain the differing results. Furthermore, the results of these observational studies are difficult to interpret as they have used different biomarkers to determine vitamin D levels e.g. serum levels of 25-hydroxy vitamin D, exposure to ultraviolet radiation (UVR), vitamin D dietary intake, and supplementation.

The causal association between vitamin D and melanoma has been a controversial topic due to the opposing role of UVR in both melanoma aetiology and vitamin D synthesis [13]. UVR is the main environmental risk factor for melanoma [14]. UVR is also essential for synthesis of vitamin D through conversion of 7-dehydrocholesterol to vitamin D3 (cholecalciferol) in the skin [15]. UVR synthesis supplies the majority of vitamin D required by humans (Figure 1), with a limited amount sourced from the diet e.g. fatty fish, milk, and eggs. Vitamin D has been suggested to have anticancer effects e.g. inhibition of proliferation and induction of apoptosis of cells [16]. Several studies have suggested an increased susceptibility for melanoma, and decreased survival due to vitamin D deficiency [17–20], while others suggests a

null association [21]. In contrast, a further study reported that increasing vitamin D levels may increase the risk of melanoma [22].

A well designed randomised controlled trial (RCT) would be the ideal design to determine if there is a causal association between vitamin D and melanoma. However such an RCT would be extremely expensive to conduct. A feasible alternative is a Mendelian randomization study (MR). Random allocation of alleles during meiosis is used in MR, analogous to the allocation to intervention and control groups in RCTs (Supplementary Figure 1). The MR approach is less affected by confounding and reverse causation, which may lead to biased results in observational studies. In MR, the causality of the exposure of interest on the outcome (melanoma) is inferred using the genetic variants as instrumental variables (IVs) which are associated with the exposure (in this case, vitamin D levels). Some key assumptions are required for MR to provide valid inferences. This method assumes that the IVs are reliably associated with the exposure (strong instrument assumption), are independent from the confounding factors of exposure and outcome (independence assumption), and associated with the outcome only through the exposure (exclusion restriction assumption) [23]. A direct acyclic graph (DAG) of our MR study is illustrated in Figure 2. A limitation of MR is the need for large samples to provide adequate statistical power. The ready availability of summary data from genome-wide association studies (GWAS) makes it feasible to conduct MR analysis on sufficiently large sample sizes. Here we conducted MR analysis on summary data using the inverse variance weighted method (IVW) to assess the association between serum 25(OH) D levels and melanoma risk.

Materials and Methods

Study participants

Summary data was obtained from a large (12,874 cases and 23,203 controls) meta-analysis of melanoma risk GWAS [24]. This population was confined to those of European ethnicity. Detailed descriptions of the characteristics of study participants and quality control measures have been previously reported [25]. Approval to undertake this study was obtained from the Human Research Ethics Committee of the QIMR Berghofer Medical Research Institute. All studies contributing to the initial meta-analysis conformed to the Declaration of Helsinki protocols, with participants giving their informed consent [24].

Genetic SNP instruments

We constructed our genetic instrument using five SNPs associated with 25(OH) D levels at the level of genome–wide significance (Table 1). Four were from a study conducted by the SUNLIGHT consortium [25]. The SNPs were rs12785878 (near the *DHCR7* gene), rs10741657 (*CYP2R1*), rs2282679 (*GC*), and rs6013897 (*CYP24A1*) [25](Table 1). The fifth SNP, rs116970203 (*CYP2R1*), was obtained from a recent meta-analysis of 39,655 individuals conducted by Manousaki and colleagues (Table 1). This SNP (rs116970203) is a rare genetic variant (Minor allele frequency = 2.5%) with a large effect on 25(OH) D levels and independent from the *CYP2R1* SNP rs10741657 identified by Wang and colleagues (Table1) [26].

The five instrument SNPs are in or near genes involved in the vitamin D metabolic pathway (Figure 1). *DHCR7* is responsible for the conversion of pro-vitamin D3 (7-dehydrocholesterol) to cholesterol, while *GC* mediates the transport of pro-vitamin D, vitamin D to the liver/tissues. *CYP2R1* and *CYP24A1* are involved in the conversion of cholecalciferol to

25(OH) D (calcidiol) and 24, 25(OH) 2D [27]. When combined as an IV these five SNPs account for 3.6% of the variance in 25(OH) D levels (Table 1).

Initially we considered the possibility to include rs10745742 *(AMDHD1)* and rs8018720 (*SEC23A)*, newly identified SNPs associated with 25(OH) D concentrations from a recent large scale GWAS by Jiang et al.,[28] to increase the variance explained by genetic instruments. However, these two SNPs were shown to be a source of potential violation of the independence assumption via confounding effects on both skin cancer and vitamin D production. SNP rs10745742 (*AMDHD1*), is in eQTL for the *HAL* gene, which encodes the histidine ammonia-lyase enzyme. This enzyme catalyses conversion of the histidine into urocanic acid and ammonia. Urocanic acid is a UVR absorbent phytochemical, sometimes termed a "Natural sunscreen", and may modify skin cancer risk directly via UVR induced immunosuppression [29,30]. rs8018720 (*SEC23A*) gene encodes for a component of vesicle transport. Vesicle transport is critical in melanosome formation during melanin production and distribution, and may interact with UVR absorption [31].

Further, these novel SNPs rs10745742 *(AMDHD1) and* rs8018720 (*SEC23A)* were significantly associated with melanoma risk ($P = 1.6 \times 10^{-4}$, $P = 2 \times 10^{-3}$) respectively. As such did not include the SNPs which are potentially associated with vitamin D, UVR and pigmentation in our analysis.

Evaluating the validity of MR assumptions in our study

Strong instrument assumption

All of the SNPs selected for use as instrumental variables were genome-wide significantly *(*$P < 5 \times 10^{-8}$) associated with 25(OH) D levels. Although SNPs at lower thresholds (e.g. $P < 1 \times$

10^{-6}) exceed the traditional F statistic > 10 for strong instruments in MR, such SNPs may represent false positives in a GWAS context and hence were not used here [32]. All 5 SNPs we selected as IVs are associated with genes which encode the proteins/enzymes which catalyse the vitamin D metabolic reactions (See Figure 1) supporting their role as useful IVs. Finally, rather than using single IV, we have used a combined IV of 5 independent genetic variants. This combined IV explains more variance of vitamin D levels than a single IV and provides more power to assess causality.

Independence assumption and exclusion restriction assumption

Population Stratification

When performing MR, differences in allele frequencies in sub populations that have different disease prevalence (population stratification), may lead to false associations, resulting in incorrect inference. In the GWAS where we extracted the IV data, potential population stratification had been sufficiently controlled by principal components analysis (for more information see Law et al., Wang et al., [24,25])

Pleiotropy

The association of a genetic variant with multiple exposures (pleiotropy) can introduce bias in the MR framework if these additional exposures are independent from the exposure of interest (25(OH) D levels) and are associated with the outcome (melanoma). In this scenario, the exclusion restriction assumption and independence assumption are violated as these genetic variants would thus have independent associations to the outcome (melanoma) other than via the risk factor (25(OH) D levels).

First we included SNPs instruments which have not shown to be associated with either pigmentation or UVR response in published literature, as these traits are known risk factors for melanoma [33].

Then, our selected genetic instruments were tested for potential violations of these assumptions by checking for associations with possible confounding exposures for melanoma and vitamin D (height, BMI, waist circumference, smoking, and alcohol intake frequency) by examining previous GWAS results. Further we included phenotypic traits which are associated with melanoma risk (hair colour, skin colour, facial aging, propensity to sunburn) as potential confounding factors (Supplementary Tables 1-11). To screen SNPs were first considered if each SNP was associated with the potential confounder, using a Bonferroni corrected *P*-value of 0.0009 (corrected for 11 traits and 5 SNPs). We found no significant associations for any of the traits, except SNP rs10741657. Although the P-value for this SNP is < 0.05, the fact that it only reaches $P = 8.80 \times 10^{-4}$ in a very large sample size (N>300,000) means that the effect of this SNP on hair colour is negligible, making it very unlikely that it represents a meaningful pleiotropic effect.

As we discussed above, the SNPs we used as IVs are in or near the genes that encode the rate limiting enzymes of vitamin D metabolic pathway. Hence, it is highly likely that each included IV SNP has a direct role in the vitamin D metabolism rather than via a confounding factor.

Statistical analysis

Two sample summary data MR analyses were conducted using Mendelian randomization v0.3.0, a comprehensive MR package made for R [34]. For the main analysis we used the IVW method to infer the causal odds ratios [35]. When we use IVW method for multiple genetic variants, the final MR estimate was calculated by weighting the causal odds ratio of each genetic variant according to its variance, assuming that genetic variants are uncorrelated [23]. Additionally, we computed causal odds ratio estimates using the weighted median method, maximum-likelihood method and MR-Egger regression methods as a sensitivity analysis, as implemented in the Mendelian randomization v0.3.0 package (Supplementary figures 2 - 4). Finally, we used meta-analysis to integrate our MR results with those from a recent MR study examining the relation between vitamin D and melanoma using UK biobank data [36].

Results

Following confirmation that there were no significant association between the IV SNPs and potential confounding factors which would violate MR assumptions (Supplementary Tables 1 - 11) we performed MR analysis. Results were scaled to represent a 20 nmol/L change in 25(OH) D. 20 nmol/L represents a large change in serum levels, equivalent to moving a person from 66th percentile to the 33rd percentile on the raw nmol/L scale [37]. At this level of change in 25(OH) D concentration, the effect on melanoma risk was close to null (OR = 1.06, 95% CI = 0.95 – 1.19) (Table 1, Supplementary Table 13). The causal odds ratios for individual SNPs, and the combined genetic instrument, are shown in Table 1. The overall result moved closer toward the null, with narrower confidence intervals, when we conducted a meta-analysis combining our study results with the UK Biobank melanoma vitamin D causal odds ratio estimate (OR = 1.02, 95% CI = 0.92 – 1.13).

Sensitivity analyses

The validity of the causal estimates obtained from IVW method depends on the validity of the instrumental variables we used. Hence, we obtained causal odds ratios for the association of vitamin D and melanoma from alternative MR approaches, the weighted median method (OR = 1.11, 95% CI = 0.95 – 1.29) and the maximum-likelihood method (OR = 1.06, 95% CI = 0.94 – 1.19) (Supplementary Table 13). The weighted median method provides accurate casual estimates when at least 50% the weights are from valid instrumental variables [38]. A bivariate normal relationship between exposure and the outcome and similar causal effect estimate for each genetic variant is assumed in maximum-likelihood method [23]. Finally, egger regression was applied to provide a more accurate estimate when the assumptions of MR are violated (e.g. pleiotropy leading to violation of the independence and exclusion restriction assumptions) [39]. Hence, we performed egger regression in case our IVs had undetected directional pleiotropy, but the results were similar to the other methods (OR = 1.08, 95% CI = 0.86 – 1.37) [39] (Supplementary Table 13, Supplementary Figure 4). Further, the egger regression intercept value was almost equal to zero (-0.004, *P*-value = 0.85) suggesting that directional pleiotropy does not exist. The graphical representations of comparison of effect estimates from different methods are illustrated in Supplementary Figure 2.

Discussion

In this large MR study we found no evidence for a causal association between genetically determined vitamin D levels and melanoma risk. However, we need to consider the substantial measurement errors of vitamin D and the variance explained by our IVs. As such, we cannot exclude the possibility of having a causal relationship of vitamin D and melanoma

and further replication studies are warranted. Our findings based on MR are different to those from some observational studies, likely due to limitations of the observational study design. A case control study conducted by Millen and colleagues in 2004 reported that dietary vitamin D was associated with a halving of the risk for melanoma (OR = 0.52, CI 0.32 – 0.86 comparing high versus low quintiles) [40]. The positive findings in Millen et al., may be due to confounding which is difficult to control for in the case-control setting. Additionally the Millen et al., (2004) study was conducted using food frequency questionnaires which are prone to recall bias. Interestingly, an Australian study published in 2013 reported that the serum 25(OH) D concentrations above 75 nmol/ L were associated with increased risk for melanoma when compared to below 75 nmol/L (OR=2.71, CI: 0.98 - 7.48)[22] – in that study high vitamin D levels were associated with greater sun exposure, with the additional sun exposure likely explaining the increase in melanoma risk. Even if they have adjusted and controlled for confounding from time spent outdoors using a multivariate regression model it is questionable whether that would have adequately controlled for the confounding from additional sun exposure. Furthermore, we noted that this prospective cohort study follow up period was 11 years and depended on the self-reported sun exposure history which could be variable in that long period of time. Another limitation is that they have relied on serum vitamin D levels measured at a single time. A large prospective study followed up 68,611 participants for 10 years and examined the dietary and supplemental intake of vitamin D and melanoma risk. They found no association between increased vitamin D intake and melanoma risk (OR = 1.05, 95% CI = 0.79 – 1.40) [21].

Strengths

A major strength of our study is the large sample size, which allowed us to place narrow confidence intervals in our estimates of the causal odds ratio. For valid inference using MR it is necessary for the SNP instruments to only affect melanoma risk via their effect on 25 (OH)D levels - in our case this is likely because all our chosen SNP instruments have a well-established functional role in the vitamin D metabolic pathway [41]. Previous observational studies on vitamin D and melanoma used UVR exposure or dietary consumption of vitamin D as the proxy to determine the vitamin D levels in humans. These proxies may not represent the correct measurement of vitamin D status due to measurement error, reverse causality (cases spending less time outdoors) or recall bias. Another strength of our study is that we use a measure of vitamin D level which is derived from large genome-wide association studies, resulting in an estimate of vitamin D levels which is determined at birth and which represents lifelong status. Using genetic variants as proxy measures of vitamin D status is largely free from confounding, especially that due to sun exposure.

Limitations

Our individual SNP instruments generally explained a small amount of vitamin D variance. All the SNPs, except rs10741657 and rs2282679, accounted for less than 1% variance. However, this limitation was alleviated by combining all the SNPs into an instrument explaining 3.6% of trait variance. We have not included a (post-hoc) power calculation for this study because all information about the ability of our study to make a clear statement about the relationship between vitamin D and melanoma risk is contained in the confidence intervals on our causal odds ratio estimates [42]. We have carefully selected the instrumental

variables, avoiding possible pleiotropic associations. However, we cannot rule out the residual confounding which may introduce false positive associations.

Conclusion

In summary, we found no evidence for a causal association between genetic determinants of vitamin D concentrations and melanoma risk using a methodically robust MR method which is unlikely to be affected by confounding and reverse causation. Our work does not provide support for the use of vitamin D supplementation as a means to reduce melanoma risk.

Acknowledgements

We acknowledge Australian National Health and Medical Research Council funding (1123248). Stuart Macgregor is supported by Australian Research Council Fellowship and National Health and Medical Research Council fellowships. For acknowledgements for the melanoma meta-analysis please see the supplementary document.

Table 1: Mendelian randomization results: 25(OH) D and melanoma

Chr	BP	SNP	Gene	EA/NEA	β	*P*-value	% VE	OR	95%CI
4	72608383	rs2282679	*GC*	G/T	-4.67	3.4×10^{-302}	1.5	1.10	0.94 - 1.29
11	14876718	rs116970203	*CYP2R1*	A/G	-8.0	2.0×10^{-90}	0.4	0.96	0.70 - 1.31
11	14914878	rs10741657	*CYP2R1*	G/A	-1.72	6.5×10^{-81}	1.0	1.15	0.78 - 1.70
11	71167449	rs12785878	*DHCR7*	G/T	-2.11	6.4×10^{-129}	0.4	0.84	0.56 - 1.24
20	52742479	rs6013897	*CYP24A1*	A/T	-0.98	3.4×10^{-17}	0.3	1.06	0.43 - 2.64
		Combined					3.6	1.06	0.95 - 1.19

Chr - Chromosome, BP – chromosome position (hg19), SNP - Single nucleotide polymorphism, Gene – gene symbol, EA - Effect Allele, NEA - Non effect allele, β - Magnitude of association between SNP and 25(OH)D (nmol/L)in raw scale, % VE per allele - Variation in 25(OH)D concentrations explained per effect allele. β effect size for 25(OH)D change on the raw nmol/L scale. OR for melanoma is for a 20nmol/L change of 25(OH) D on the raw scale. For rs116970203 the raw nmol/L scale is taken from vitamin D all-cancer Ong et al.,[36]; all others are from Dimitrakopoulou et al.,[43].

Figure Legends

Figure 1: Vitamin D metabolic pathway

Footnotes:
24,25(OH)2D - 24,25 dihydroxyvitamin D
1,25(OH)2D – 1,25 dihydroxyvitamin D
25(OH)2D – 25 hydroxyvitamin D

Figure 2: Directed Acyclic Graph (DAG) depiction of our study of vitamin D levels and the risk of melanoma

Supporting Information

Please see the additional document.

References

1 Dou R, Ng K, Giovannucci EL, *et al.* Vitamin D and colorectal cancer: molecular, epidemiological and clinical evidence. *Br J Nutr* 2016; **115**:1643–60.

2 Nair R, Maseeh A. Vitamin D: The 'sunshine' vitamin. *J Pharmacol Pharmacother* 2012; **3**:118–26.

3 Grant WB. A Review of the Evidence Supporting the Vitamin D-Cancer Prevention Hypothesis in 2017. *Anticancer Res* 2018; **38**:1121–36.

4 Mizoue T. Ecological study of solar radiation and cancer mortality in Japan. *Health Phys* 2004; **87**:532–8.

5 Rossi M, McLaughlin JK, Lagiou P, *et al.* Vitamin D intake and breast cancer risk: a case–control study in Italy. *Ann Oncol* 2009; **20**:374–8.

6 Abbas S, Linseisen J, Slanger T, *et al.* Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--results of a large case-control study. Carcinogenesis. 2007; **29**:93–9.

7 Ahonen MH, Tenkanen L, Teppo L, *et al.* Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control* 2000; **11**:847–52.

8 Ma Y, Zhang P, Wang F, *et al.* Association between vitamin D and risk of colorectal cancer: a systematic review of prospective studies. *J Clin Oncol* 2011; **29**:3775–82.

9 Waterhouse M, Risch HA, Bosetti C, *et al.* Vitamin D and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Case-Control Consortium. *Ann Oncol* 2016; **27**:208.

10 Yin L, Grandi N, Raum E, *et al.* Meta-analysis: serum vitamin D and breast cancer risk. *Eur J Cancer* 2010; **46**:2196–205.

11 Ahn J, Peters U, Albanes D, *et al.* Serum Vitamin D Concentration and Prostate Cancer Risk: A Nested Case–Control Study. *J Natl Cancer Inst* 2008; **100**:796–804.

12 Ashmore JH, Gallagher CJ, Lesko SM, *et al.* No Association Between Vitamin D Intake, VDR Polymorphisms, and Colorectal Cancer in a Population-Based Case-Control Study. Cancer Epidemiology Biomarkers & Prevention. 2015; **24**:1635–7.

13 Osborne JE, Hutchinson PE. Vitamin D and systemic cancer: is this relevant to malignant melanoma? *Br J Dermatol* 2002; **147**:197–213.

14 Veierod MB, Adami HO, Lund E, *et al.* Sun and solarium exposure and melanoma risk: effects of age, pigmentary characteristics, and nevi. *Cancer Epidemiol Biomarkers Prev* 2010; **19**:111–20.

15 Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. *Dermatoendocrinol* 2013; **5**:51–108.

16 Moukayed M, Grant WB. Molecular link between vitamin D and cancer prevention. *Nutrients* 2013; **5**:3993–4021.

17 Brożyna AA, Jóźwicki W, Slominski AT. Decreased VDR expression in cutaneous melanomas as marker of tumor progression: new data and analyses. *Anticancer Res* 2014; **34**:2735–43.

18 Newton-Bishop JA, Beswick S, Randerson-Moor J, *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.

19 Lindqvist PG. The Winding Path Towards an Inverse Relationship Between Sun Exposure and All-cause Mortality. *Anticancer Res* 2018; **38**:1173–8.

20 Vinceti M, Malagoli C, Fiorentini C, *et al.* Inverse association between dietary vitamin D and risk of cutaneous melanoma in a northern Italy population. *Nutr Cancer* 2011; **63**:506–13.

21 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.

22 van der Pols JC, Russell A, Bauer U, *et al.* Vitamin D status and skin cancer risk independent of time outdoors: 11-year prospective study in an Australian community. *J Invest Dermatol* 2013; **133**:637–41.

23 Burgess S, Butterworth A, Thompson SG. Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data. *Genet Epidemiol* 2013; **37**:658–65.

24 Law MH, Bishop DT, Lee JE, *et al.* Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. *Nat Genet* 2015; **47**:987–95.

25 Wang TJ, Zhang F, Richards JB, *et al.* Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet* 2010; **376**:180–8.

26 Manousaki D, Dudding T, Haworth S, *et al.* Low-Frequency Synonymous Coding Variation in

CYP2R1 Has Large Effects on Vitamin D Levels and Risk of Multiple Sclerosis. *Am J Hum Genet* 2018; **103**:1053.

27 Berry D, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. Current Opinion in Nephrology and Hypertension. 2011; **20**:331–6.

28 Jiang X, O'Reilly PF, Aschard H, *et al.* Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat Commun* 2018; **9**:260.

29 Walterscheid JP, Nghiem DX, Kazimi N, *et al.* Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor. Proceedings of the National Academy of Sciences. 2006; **103**:17420–5.

30 Welsh MM, Karagas MR, Applebaum KM, *et al.* A role for ultraviolet radiation immunosuppression in non-melanoma skin cancer as evidenced by gene-environment interactions. *Carcinogenesis* 2008; **29**:1950–4.

31 Sitaram A, Marks MS. Mechanisms of protein delivery to melanosomes in pigment cells. *Physiology* 2012; **27**:85–99.

32 Burgess S, Thompson SG, CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. International Journal of Epidemiology. 2011; **40**:755–64.

33 Buniello A, MacArthur JAL, Cerezo M, *et al.* The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Research. 2019; **47**:D1005–12.

34 Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. International Journal of Epidemiology. 2017; **46**:1734–9.

35 Burgess S, Scott RA, Timpson NJ, *et al.* Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol* 2015; **30**:543–52.

36 Ong J-S, Gharahkhani P, An J, *et al.* Vitamin D and overall cancer risk and cancer mortality: a Mendelian randomization study. *Hum Mol Genet* 2018; **27**:4315–22.

37 Ong J-S, Cuellar-Partida G, Lu Y, *et al.* Association of vitamin D levels and risk of ovarian cancer: a Mendelian randomization study. *Int J Epidemiol* 2016; **45**:1619–30.

38 Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016; **40**:304–14.

39 Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015; **44**:512–25.

40 Millen AE, Tucker MA, Hartge P, *et al.* Diet and melanoma in a case-control study. *Cancer Epidemiol Biomarkers Prev* 2004; **13**:1042–51.

41 Clendenen TV, Ge W, Koenig KL, *et al.* Genetic Polymorphisms in Vitamin D Metabolism and Signaling Genes and Risk of Breast Cancer: A Nested Case-Control Study. *PLoS One* 2015;

10:e0140478.

42 Colegrave N. Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. Behavioral Ecology. 2003; **14**:446–7.

43 Dimitrakopoulou VI, Tsilidis KK, Haycock PC, *et al.* Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study. *BMJ* 2017; **359**:j4761.