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Causal association between serum 25-Hydroxyvitamin D levels and cutaneous melanoma: a two-sample Mendelian randomization study

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Background: Despite numerous observational studies on the association between serum 25-Hydroxyvitamin D levels and cutaneous melanoma, causal inferences remain ambiguous due to confounding and reverse causality. This study aimed to elucidate the causal relationship between serum 25-Hydroxyvitamin D levels and melanoma incidence using Mendelian randomization (MR).

Methods: A two-sample MR was conducted using genetic variants associated with serum 25-Hydroxyvitamin D levels as instrumental variables. Summary statistics for these variants were derived from genome-wide association studies, and those for melanoma risk were obtained from a comprehensive melanoma case-control study. Robustness of the results was assessed through sensitivity analyses, including the “leave-one-out” approach and tests for potential pleiotropy.

Results: The MR analysis provided substantial evidence of a positive causal relationship between serum 25-Hydroxyvitamin D levels and the incidence of cutaneous melanoma, suggesting that each unit increase in serum 25-Hydroxyvitamin D levels corresponds with an increased risk of melanoma. Tests for pleiotropy showed minimal effects, and the sensitivity analysis confirmed no disproportionate influence by any individual single nucleotide polymorphism (SNP).

Conclusion: The findings indicated a potentially causal positive association between serum 25-Hydroxyvitamin D levels and melanoma risk, challenging traditional beliefs about vitamin D’s role in melanoma. This emphasizes the need for a balanced and personalized approach to vitamin D supplementation and sun

exposure, particularly in high-risk populations. These results should be interpreted with caution due to potential unrecognized pleiotropy and confounding factors. Future research should focus on validating these findings in diverse populations and exploring underlying biological mechanisms.

KEYWORDS

serum 25-hydroxyvitamin D, cutaneous melanoma, Mendelian randomization, genetic variants, causal inference, sun exposure

1 Introduction

Cutaneous melanoma, a malignant neoplasm stemming from skin melanocytes (1), is a major worldwide health concern due to escalating incidence and high mortality rates (2). Over the past few decades, this aggressive skin cancer with a pronounced metastatic propensity has seen a marked increase in prevalence, underscoring the urgency of a thorough understanding of its etiology (3). The etiology of melanoma is multifactorial, involving a complex combination of environmental and genetic determinants (4). Exposure to ultraviolet (UV) radiation is a well-established risk factor, playing a pivotal role in the disease's onset and progression (5). However, our understanding of other potential modifiable risk factors, such as Vitamin D—which is predominantly generated through UV exposure—is less clear and warrants further investigation (6).

Despite significant advancements in early detection, prevention measures, and therapeutic strategies, melanoma presents considerable challenges (7). These challenges are largely due to its resistance to conventional treatments, advanced stages at diagnosis, and high metastatic potential (8, 9). Comprehensive insight into the disease's etiology, risk factors, and the specific determinants of pathogenesis is crucial for devising more effective prevention strategies, targeted therapies, and improving overall prognosis for patients. This further emphasizes the importance of examining genetic and environmental interactions, especially concerning potential modifiable factors such as Vitamin D (10).

Vitamin D, synthesized primarily through sunlight exposure and dietary intake, is crucial for multiple physiological functions including bone health, immune regulation, and cell differentiation and proliferation (11, 12). The regulation of vitamin D metabolism involves the significant action of several enzymes, particularly CYP27A1, CYP27B1, and CYP24A1, which are genes critical for the synthesis and degradation of this vitamin (13, 14). Primarily expressed in the liver, CYP27A1 initiates the conversion of vitamin D into its active form, calcitriol, through a process known as hydroxylation (15). This conversion is further catalyzed by CYP27B1, which is predominantly expressed in the kidneys (16, 17). Meanwhile, CYP24A1, largely found in the kidneys and various other tissues, oversees the breakdown of active vitamin D metabolites into inactive forms (18). This degradation process is integral for maintaining vitamin D homeostasis, emphasizing the crucial role of CYP24A1 in this biological regulatory system (19).

The primary circulating form, 25-Hydroxyvitamin D (25(OH)D), serves as a reliable biomarker of Vitamin D status (20). The potential protective role of Vitamin D, specifically serum 25(OH)D, against various cancers, including cutaneous melanoma - a highly aggressive skin cancer - has been a subject of significant research interest (10, 21). This interest is further amplified by the dual role of sunlight as a source of Vitamin D synthesis and a known risk factor for melanoma (22, 23).

However, the epidemiological evidence linking serum 25(OH)D levels and melanoma incidence has been inconsistent (24). Some studies indicate a protective role of higher serum 25(OH)D levels against melanoma (25–28), while others suggest no significant association or produce contradictory results (29–34). These discrepancies are thought to arise from confounding variables such as lifestyle, genetics, sunlight exposure, skin type, and the potential for reverse causation, thus complicating the inference of a causal relationship (35). Given these limitations inherent in observational studies, there is a pressing need for more robust research methodologies that can provide more valid causal inferences.

This research utilizes a two-sample Mendelian Randomization (MR) approach to investigate the potential causal link between serum 25-Hydroxyvitamin D levels and the incidence of cutaneous melanoma (36, 37). The MR methodology, which employs Single Nucleotide Polymorphisms (SNPs) as instrumental variables (IVs), offers an effective strategy to estimate causal relationships, mitigating bias from confounding factors and reverse causation that often confound traditional observational studies (38, 39). The study relies on three fundamental MR assumptions: relevance, independence, and exclusion restriction, to ensure that selected SNPs have a robust association with serum 25-Hydroxyvitamin D levels, are not associated with confounding variables, and affect melanoma risk exclusively through their impact on serum 25-Hydroxyvitamin D levels (40). The selection of SNPs and the outcome data were sourced from large-scale, publicly available genome-wide association study (GWAS) datasets (41).

Three key MR analysis methods were applied: Inverse Variance Weighted (IVW), weighted median, and MR-Egger regression (42). These techniques provide a comprehensive examination of the potential causal relationship while addressing varying conditions of instrument validity and pleiotropy. To verify the robustness and validity of the findings, a series of sensitivity analyses were conducted, including Cochran's Q Test, a Pleiotropy Test, and a "leave-one-out" analysis (43). The Radial MR method, an

innovative technique for outlier identification and exclusion, was also employed, thereby enhancing the reliability of the findings (44). The analysis of this study indicate a statistically significant causal association between serum 25-Hydroxyvitamin D levels and melanoma incidence.

Our findings not only shed light on the potential role of Vitamin D in melanoma pathogenesis but also underscore the possible implications for prevention and therapeutic strategies, particularly in regard to vitamin D optimization strategies. This study bridges a gap in the existing literature and sets the foundation for future research, although clinical decision-making should carefully consider the inherent limitations of MR studies, individual health considerations, and the multifaceted nature of melanoma etiology. Our findings point to new avenues for melanoma prevention, but further investigation is warranted to fully elucidate the precise biological implications and clinical applicability of serum 25-Hydroxyvitamin D levels in melanoma risk.

2 Materials and methods

2.1 Mendelian randomization study design

Our study was undertaken following the framework of a two-sample MR model utilizing preselected instrumental variables (37, 45). The schematic framework of the MR design is delineated in Figure 1. The validity of our research hinged on three pivotal assumptions (40): (1) Relevance Assumption: the Single Nucleotide Polymorphisms demonstrated a robust association with serum 25-Hydroxyvitamin D concentrations which were measured using a validated assay method; (2) Independence Assumption: SNPs were not linked to confounding variables which were identified based on established biological and epidemiological knowledge about potential confounders of the association between 25-Hydroxyvitamin D and melanoma; (3) Exclusion Restriction Assumption: SNPs influence melanoma outcomes solely through their potential impact on serum 25-Hydroxyvitamin D levels which required a comprehensive review of the existing literature to exclude other potential causal pathways (39). In adherence to MR analysis standards, we carefully selected SNPs that were previously reported to be strongly associated with serum 25-Hydroxyvitamin D concentrations. These were chosen as they were not linked to known confounding variables and their influence on melanoma was only due to their potential impact on serum 25-Hydroxyvitamin D levels, thereby satisfying the three key MR assumptions.

In order to ensure the robustness of our study, a comprehensive verification of these assumptions was performed through a thorough statistical analysis of SNP–exposure and SNP–outcome associations (46). This was crucial in order to meet the rigorous statistical requirements for a valid MR study and strengthen the credibility of our conclusions. To further substantiate our findings and strengthen the validity of our study, our analyses also accounted for potential bidirectional relationships, secondary pleiotropic effects, and population stratification, which may pose plausible threats to the validity of these assumptions.

The MR analyses were executed using R software (version 4.0.3), supplemented with the “TwoSampleMR” (version 0.5.6) and “RadialMR” (version 1.0) packages. The R environment was preferred due to its extensive statistical functionalities and capacity to handle large-scale genomic datasets, crucial for executing an MR study of this magnitude (47). The TwoSampleMR package enables the implementation of two-sample MR analysis by providing functions for data extraction, harmonization, and performing several statistical methods, while the RadialMR package, based on modified second-order weights, allows for the detection and subsequent exclusion of outliers (37).

2.2 Data acquisition for exposure and outcome

Genetic associations for the serum 25-Hydroxyvitamin D levels (ebi-a-GCST90000618) were sought in publicly available genome-wide association study datasets, which contained data for 496,946 samples and 6,896,093 SNPs (48). These large, heterogeneous datasets provide a valuable and diverse genetic background for assessing the association of SNPs with vitamin D levels (49). These large datasets were chosen to ensure adequate power to detect even small effect sizes and to allow for the inclusion of a large number of IVs (50). These datasets fulfilled the minimum criteria requisite for importation from the European Bioinformatics Institute (EBI) database of complete GWAS summary data (51). We pinpointed SNPs exhibiting robust associations with serum 25-Hydroxyvitamin D, establishing a stringent threshold for statistical significance ($P < 5 \times 10^{-9}$), linkage disequilibrium (LD) $r^2 < 0.001$, and LD distance $> 10,000$ kb. The F statistic was employed to rule out weak instrument bias that might contravene the first MR assumption, thereby evaluating the strength of the association between SNPs and serum 25-Hydroxyvitamin D levels (52, 53). This rigorous selection process ensures the minimization of false-positive results, enhancing the reliability of our IVs. The use of such large and comprehensive GWAS datasets ensures the robustness and external validity of our findings (54). The stringent criteria set for SNP selection help ensure the quality of IVs and the accuracy of subsequent analyses.

Regarding the outcome data, we obtained melanoma skin cancer GWAS data ieu-b-4969 from the ieu-b datasets, a summary data compilation generated by several consortia that were manually curated, initially created for MR-Base (55). This dataset was selected for its extensive coverage and high-quality data, ensuring that the subsequent analyses would be adequately powered and encompass a comprehensive range of genetic variations associated with melanoma (56). This melanoma data consisted of 375,767 samples, and included 11,396,019 SNPs which were all carefully checked for quality control measures including genotyping accuracy and Hardy-Weinberg equilibrium. We emphasized harmonization to minimize inconsistencies and discrepancies between the different datasets, which is a critical aspect when working with such large-scale genetic data. The data was harmonized for subsequent MR analysis including the alignment of the effect allele and standardization of the units of measurement for both the exposure and outcome variables. Moreover, in order to

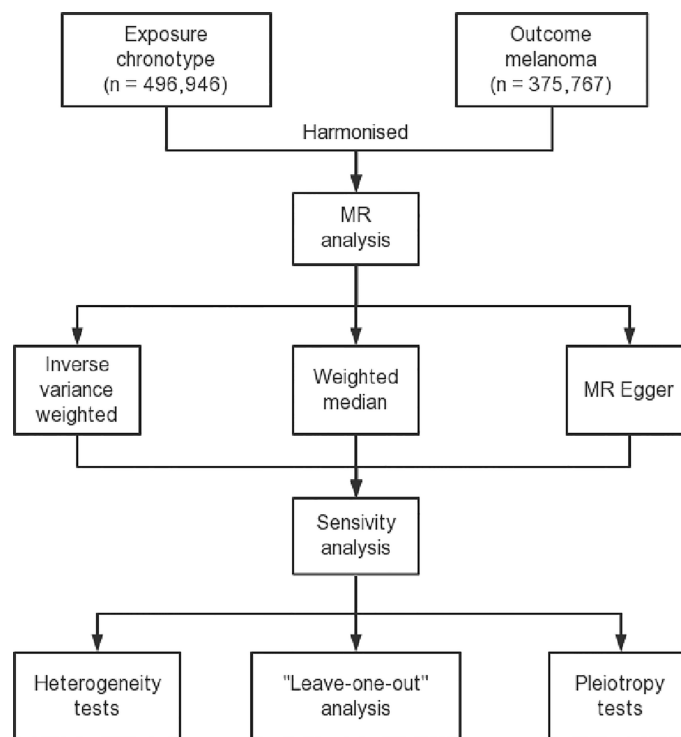


FIGURE 1

Flow diagram delineating the design process of a two-sample Mendelian randomization study. This figure includes a description of the selection of instrumental variables, identification of exposure and outcome datasets, and the methods used for the MR analysis and sensitivity analysis.

mitigate any potential bias, we strictly observed a minor allele frequency (MAF) cut-off of 0.01, thus ensuring that all included SNPs had sufficient population frequency to warrant their inclusion (57).

2.3 MR analysis

A two-sample MR analysis was performed employing three primary methods: inverse variance weighted median, weighted median, and MR-Egger, aiming to assess the potential causal relationship between serum 25-Hydroxyvitamin D levels and melanoma (37). These three methods each address different potential sources of bias in MR analyses, and thus together provide a robust and comprehensive evaluation of the causal relationship. IVW approach combines the strengths of different SNPs and their individual effects in an efficient manner to yield an overall estimate. Weighted median allows for more heterogeneity, enabling up to 50% of the genetic variants to be invalid instruments. Meanwhile, MR-Egger provides a measure of directional pleiotropy and is less prone to bias when the assumptions of the other two methods are violated. Each analysis was conducted using the corresponding two-sample MR packages in R, per developers' guidelines. The use of multiple methods provides a comprehensive and robust assessment of potential causal relationships, while also providing an opportunity for comparison and cross-validation of the results.

The IVW approach combined meta-analysis with Wald estimates for each SNP to yield an aggregate effect estimate for

melanoma. IVW results remain unbiased provided no horizontal pleiotropy is observed (58). The Wald ratio for each SNP was calculated as the ratio of the SNP-outcome association to the SNP-exposure association (59). Horizontal pleiotropy, where genetic variants affect the outcome through pathways other than the exposure, can introduce bias into the MR estimates. The assumption of no horizontal pleiotropy is critical as it ensures that the SNP's effect on melanoma is channeled solely through its influence on serum 25-Hydroxyvitamin D levels, thereby ensuring valid estimates (60).

While estimates from the random and fixed effects IVW models are identical, the variance in the random effects model is inflated to account for SNP heterogeneity. Consequently, the fixed-effect model was implemented in scenarios devoid of observed heterogeneity ($p > 0.05$) which assumes that the true effect size is the same for all SNPs and any variation is due to sampling error, thus providing a more conservative estimate. Adoption of the appropriate model as per the observed heterogeneity helps prevent inaccuracies that could arise due to the misapplication of a fixed or random effects model.

MR-Egger regression, grounded on the assumption of instrument strength independence from direct effect, enables the evaluation of pleiotropy presence via the intercept term (61). This intercept can be interpreted as an estimate of the average direct effect of the genetic variants on the outcome, not through the exposure. In other words, it provides a measure of the overall directional pleiotropy. An intercept value equal to zero suggests nonexistence of horizontal pleiotropy and MR-Egger regression

outcome consistency with IVW. This method also allows for the assessment of any potential directional pleiotropy - a significant deviation from zero indicates that the IVs may be affecting the outcome through pathways other than 25-Hydroxyvitamin D levels. Pleiotropy, if undetected, can introduce bias and misdirect our interpretations of the results.

The correlation LD between selected SNPs and potential confounding factors required careful assessment to ensure methodological robustness and conformity with the second MR assumption, as any correlation is unacceptable. In the context of an MR analysis, SNPs in LD could violate the Independence Assumption and confound the results. Therefore, a clumping procedure was undertaken to ensure the SNPs were in minimal LD with each other, thereby enhancing the validity of our study. This step is crucial as it reduces the possibility of SNP-SNP interaction, which can confound the results.

2.4 Sensitivity analysis

We utilized Cochran's Q Test and a Pleiotropy Test to assess the robustness of our findings (62). Cochran's Q statistics were employed to quantify the heterogeneity among the IVs (63). Heterogeneity among the IVs could reflect an invalid assumption of no horizontal pleiotropy or a violation of the Exclusion Restriction Assumption. This allowed us to understand if the individual SNP effects were more varied than what would be expected by chance alone. In addition, to pinpoint potentially heterogeneous SNPs, a "leave-one-out" analysis was carried out (64). This analysis evaluated the reliability of the relationship between SNPs and exposure, and assessed whether any particular SNP was contributing disproportionately to significant results. Evidence of heterogeneity suggests that certain genetic instruments may be invalid ($p < 0.05$). The leave-one-out analysis is a robust way to identify any single genetic instrument that may unduly influence the study results, ensuring the stability of our MR estimates. Such an analysis is invaluable in identifying and excluding potential outlier SNPs that may unduly influence the MR estimates.

Pleiotropy tests were carried out to investigate the influence of serum 25-Hydroxyvitamin D levels on melanoma risk within the context of MR analysis (65). A p-value less than 0.05 indicates an absence of horizontal pleiotropy among selected genetic

instruments and suggests the need for a more comprehensive modelling framework to identify outliers. Detecting pleiotropy early is vital for maintaining the integrity of the study, as unidentified pleiotropy can potentially bias the MR results. Any indication of pleiotropy prompted an in-depth exploration of the data and warranted a comprehensive examination of the outliers in our modelling framework. Figure 2 is the schematic representation of the comprehensive design of the analysis process for the Mendelian randomization study (66).

2.5 Radial MR analysis

Our study utilized an innovative methodology, employing modified second-order weights, to investigate potential outliers within MR analysis. This was facilitated through the use of the "RadialMR" package (version 1.0) in the R programming environment, permitting the identification of outliers that could distort the causal estimates and enabling subsequent reanalysis after their exclusion (44). The modified second-order weights calculated using RadialMR account for both the first and second moments of the error term. This is in contrast to the traditional MR-Egger regression that only considers the first moment. By considering both moments, RadialMR can detect influential outliers that might bias the MR estimates and remove them, thus providing a more robust and reliable estimate of the causal effect. The entire process was automated within the package, ensuring the standardization of the method across all the data.

Radial MR has been increasingly recognized for its capability to detect and adjust for potential outlier SNPs. By reweighting the SNP estimates and corresponding standard errors based on their deviance from the overall MR estimate, the Radial MR methodology can help limit undue influence from outlier SNPs. It offers another layer of robustness to our study and can contribute significantly to the precision of the estimates. In the course of the Radial MR analysis, SNPs identified as outliers were removed in a stepwise manner and the MR estimates were recalculated at each step. The iterative nature of the Radial MR analysis allowed us to examine the impact of each SNP and assess the stability of our results. If a particular SNP caused a significant deviation in the MR estimate, this might indicate a violation of the assumptions underlying MR analysis, such as pleiotropy or linkage with confounding factors.

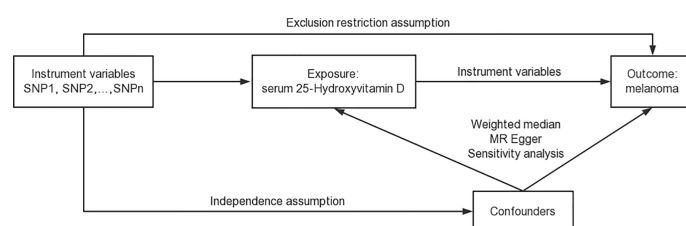


FIGURE 2

Schematic representation of the comprehensive design of the Mendelian randomization study. This figure provides a visual summary of our study design, illustrating the key steps in the data acquisition, analysis, and interpretation process.

3 Results

3.1 Causality between serum 25-Hydroxyvitamin D levels and melanoma incidence

A statistically significant causal association was inferred between serum 25-Hydroxyvitamin D levels and the incidence of melanoma, as determined by the Inverse Variance Weighted (IVW) Mendelian Randomization (MR) method ($\beta = 0.0022159$, $p = 0.0494391$) (Figure 3). This approach uses genetic variants as instrumental variables (IVs) to dissect causal associations in observational studies. With the avoidance of environmental confounding and bias due to reverse causation, which commonly plague conventional observational studies, the MR approach gives robust evidence of causality.

In the realm of MR, the β coefficient indicates the magnitude of the effect size or the estimated change in the outcome (melanoma incidence in this case) associated with a one unit increase in the exposure (serum 25-Hydroxyvitamin D levels). The p-value is indicative of the statistical significance of this association, with our $p = 0.0494391$ suggesting that this association is significant at the 5% level. The result indicates that for each unit increase in serum 25-Hydroxyvitamin D levels, the incidence of melanoma increases by 0.0022159 units. It's worth noting that this β coefficient, despite being relatively small, points towards a positive relationship, suggesting an increase in melanoma incidence with elevated serum 25-Hydroxyvitamin D levels. It's important to

acknowledge the fact that even a seemingly minuscule rise in melanoma risk can be of significant public health relevance, given the severity and escalating incidence of this form of skin cancer worldwide (67).

The scatter plot shown in Figure 3 visually portrays the individual genetic variants that contribute to the aggregate data point, representing the causal estimate. Each data point on the scatter plot signifies a genetic variant, with its position being determined by its association with both melanoma risk and vitamin D levels. The slope of the regression line captures the average causal effect of these genetic variants, providing a visual representation of the aggregate causal estimate determined by the IVW method.

In the context of MR studies, heterogeneity refers to the variability in the estimates of the causal effect derived from each individual genetic variant. The Cochran Q-test of heterogeneity, applied to the IVW method, indicated the absence of significant heterogeneity in the study ($Q = 95.46249$, $P = 0.1299563$) (Table 1). This Cochran Q-test result further bolsters the study's validity by demonstrating the homogeneity of the genetic instruments used (68). A non-significant Q statistic indicates that the variation across the different causal estimates is within what might be expected due to sampling variability, suggesting the lack of bias in the causal estimation. This Q statistic and associated p-value highlight that the individual effects sizes derived from the different SNPs included in the MR analysis do not significantly differ from each other, thereby confirming homogeneity among the studies and supporting the overall validity of the pooled causal estimate. However, the p-value

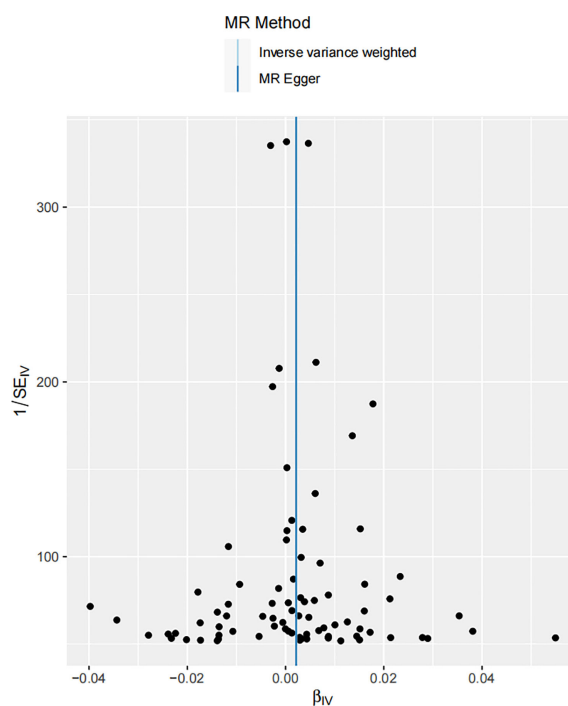


FIGURE 3

Scatter plot illustrating the associations between melanoma skin cancer (y-axis) and Serum 25-Hydroxyvitamin D levels (x-axis). The slope of the regression line serves as an estimate of the causal effect between these variables.

TABLE 1 Detailed MR findings of the causal association between serum 25-Hydroxyvitamin D levels and melanoma incidence.

Method	nSNP	β	Se	pval	Q_pval	Pleiotropy
Inverse variance weighted	82	0.0021413	0.0017640	0.2283738	0.1299563	0.9560984
MR Egger	82	0.0002823	0.0017567	0.8723171	0.1144536	
Weighted median	82	0.0022159	0.0011278	0.0494391		
Simple mode	82	0.0021478	0.0032797	0.5143997		
Weighted mode	82	0.0007570	0.0015899	0.6352514		

above 0.05 does not entirely exclude the possibility of minor heterogeneity among the included SNPs.

Table 1 presents the results from various Mendelian randomization analyses, employing different methodologies such as Inverse Variance Weighted, MR Egger, Weighted Median, Simple Mode, and Weighted Mode. Each row provides key metrics including the number of SNPs (nSNP), effect size (β), standard error (Se), p-value, Q p-value (a measure of heterogeneity), and a Pleiotropy test result, thus offering a comprehensive view of the genetic association and potential bias in each analysis method.

3.2 Robustness of the causal relationship between serum 25-Hydroxyvitamin D levels and melanoma

In the robustness assessment through a “leave-one-out” sensitivity analysis, the IVW estimates obtained after successively excluding each SNP approximated the IVW estimates from the complete set of SNPs. This consistency suggested that no individual SNP exerted a substantial influence on the estimated causal relationship (Figure 4). This analysis is pivotal in mitigating the potential of a single SNP disproportionately skewing the estimated

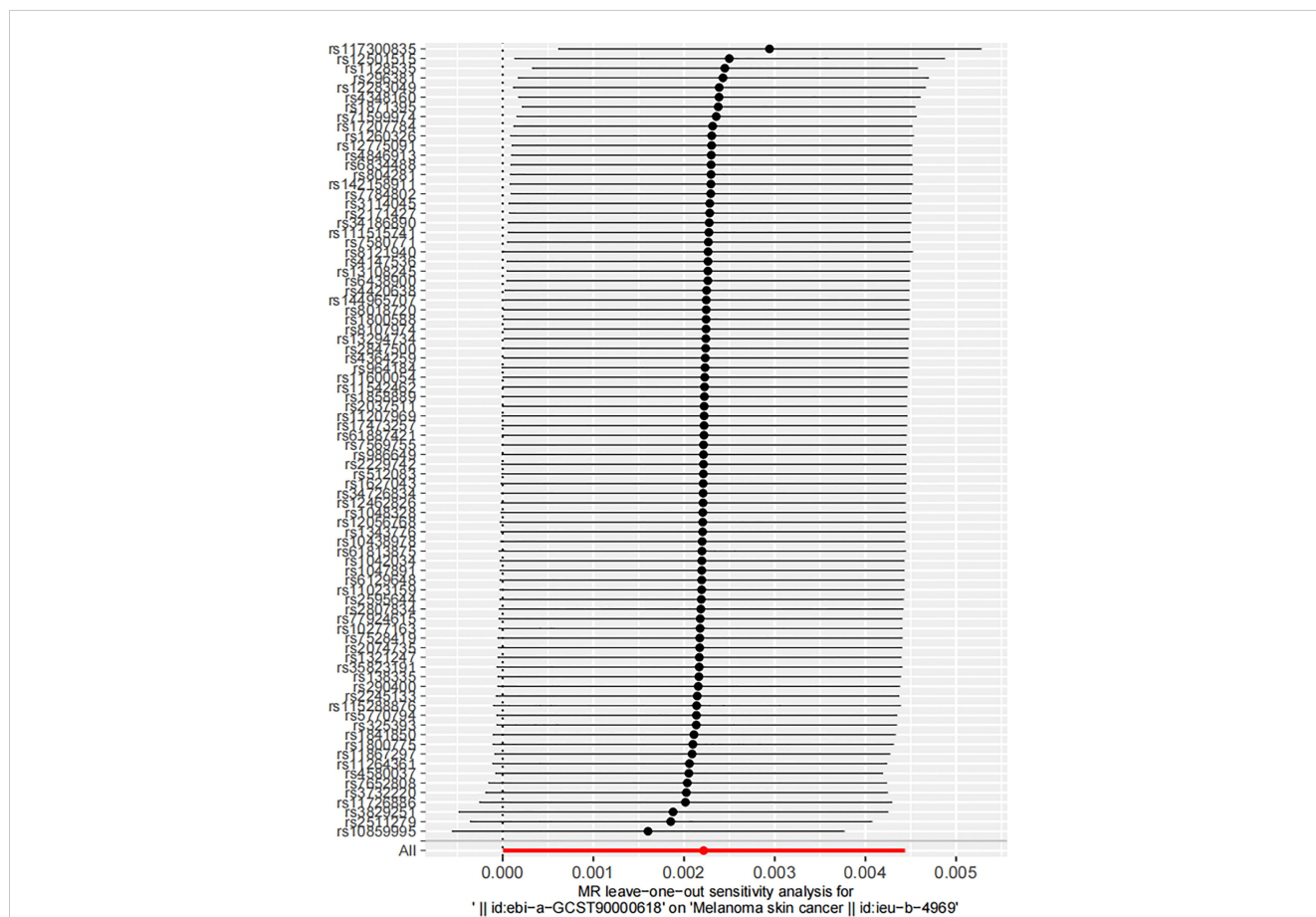


FIGURE 4 Leave-one-out sensitivity analysis of the impact of serum 25-Hydroxyvitamin D levels on melanoma skin cancer incidence. Each circle represents an estimate of the causal effect susceptibility between Serum 25-Hydroxyvitamin D levels and melanoma skin cancer, with the bars indicating the confidence interval (CI).

effect size. The consistent estimates from this analysis highlight the robustness of the IVW method, reducing the likelihood of overestimated or spurious causal inferences. If the revised estimates do not deviate significantly from the original, it implies that no single SNP disproportionately affects the results. It indicates that the identified correlation does not rely on any particular SNP, lending credibility to the inference that the effect is a genuine result of the overall genetic variation.

This figure visualizes the dispersion of the causal effect estimates upon successive omission of each SNP. The position of the circles along the y-axis represents the effect estimate, while the horizontal bars reflect the degree of uncertainty around these estimates. The tight clustering of these points along the y-axis reflects the consistency of the estimates, reinforcing the stability of the causal inference.

A detailed annotation of the SNPs illustrated on the ordinate of Figure 4 is provided in Supplementary Table 1 by searching in the PhenoScanner V2 database (69, 70). This table not only lists the gene symbols corresponding to each SNP, but it also highlights the traits associated with these genetic variations, thereby emphasizing potential phenotypic implications of these genomic discrepancies. This feature underscores the potential phenotypic ramifications inherent in these genetic variations, highlighting the complex interplay between genotype and phenotype. In interpreting these genetic associations, it is imperative to consider the broader genomic context in which these SNPs exist. Notably, while the SNPs' impact on 25-Hydroxyvitamin D levels might influence melanoma risk, they could also confer pleiotropic effects that

potentially influence other phenotypes. This data provides an exhaustive map of the genomic landscape surrounding the examined association, enabling a deeper understanding of the genetic underpinnings potentially influencing both Vitamin D levels and melanoma risk

3.3 Assessing pleiotropic impact on the causal link between serum 25-Hydroxyvitamin D levels and melanoma incidence

To further evaluate the robustness of the MR study, a pleiotropic test was conducted. Pleiotropy is a phenomenon where a single gene or genetic variant influences multiple traits. It can be a potential source of bias in MR studies, making its assessment vital for ensuring the accuracy of the results. In order for MR to maintain validity, it is essential that there is no violation of the exclusion restriction assumption - that is, genetic variants should not be directly associated with the outcome beyond their influence on the exposure. Pleiotropy, particularly horizontal pleiotropy, where the effect of a genetic variant on the outcome surpasses its impact on the exposure, can potentially bias the MR results. Our pleiotropic test indicated no substantial pleiotropic effect, reinforcing the validity of our findings (Figure 5). The genetic variants used as IVs in our MR analysis did not show signs of significantly influencing melanoma incidence through pathways other than their impact on serum 25-Hydroxyvitamin D levels.

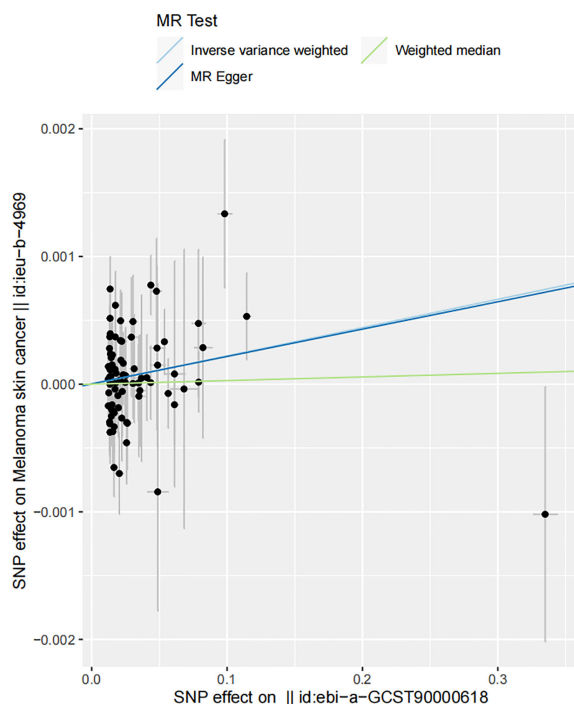


FIGURE 5

Funnel plot of the estimated causal effect of serum 25-Hydroxyvitamin D levels on melanoma skin cancer incidence. Each point symbolizes the estimated causal effect of each instrumental variable (IV). The dark blue vertical line represents the MR-Egger method-derived causal effect estimate, while the light blue line signifies the equivalent estimate derived via the IVW method.

This is a critical consideration, as ignoring potential pleiotropy could lead to erroneous interpretations of the causal relationship. The absence of horizontal pleiotropy is crucial in our MR framework as it upholds the direction and magnitude of the estimated causal relationship between the variables (60).

This funnel plot serves as a visual assessment of potential pleiotropic effects. The spread of the points provides an indication of the degree of heterogeneity across the causal estimates obtained from individual IVs. The symmetry of the plot around the causal effect line further reinforces the absence of substantial horizontal pleiotropy. The clustering of IVs around the vertical line, indicating the MR-Egger method-derived causal effect estimate, supports the notion of symmetric distribution, a key assumption in MR-Egger regression, thereby demonstrating the absence of substantial horizontal pleiotropy (71). The close alignment of the MR-Egger and IVW estimates further supports the argument that pleiotropy is unlikely to have significantly distorted our results.

3.4 Persistent correlation between serum 25-Hydroxyvitamin D levels and melanoma by the radial MR analysis

In the final stages of our study, the Radial MR method was harnessed to assess the outliers identified earlier. The outcome of this rigorous analysis revealed a positive correlation in MR results,

even upon outlier exclusion. This persistent correlation underscores the robustness of our findings against statistical anomalies (Figure 6). The Radial MR method, an advanced outlier-detection technique, provides another level of reliability by ensuring the robustness of the study's conclusions despite the presence of potential outliers. The persistent positive correlation indicates that the key findings of the study are not overly reliant on a small number of influential data points. Outliers in genetic association studies can often be a consequence of various factors such as genetic heterogeneity, population stratification, or genotyping errors (72). By conducting a Radial MR analysis, which excludes potential outliers, we have ensured that the observed correlation is not an artifact of a few extreme observations, further strengthening the robustness of our conclusions. Despite the presence of outliers, as indicated by the yellow portions of the plot, the overall shape and pattern of the plot underscores the consistent association between Vitamin D levels and melanoma risk.

In summation, our Mendelian randomization study provides evidence for a causal relationship between serum 25-Hydroxyvitamin D levels and the risk of melanoma. This relationship has been reinforced through a series of rigorous validations including sensitivity, pleiotropic, and outlier analyses. Our results propose a positive causal effect of serum 25-Hydroxyvitamin D levels on melanoma incidence.

However, it is pertinent to emphasize that MR results should not be interpreted in isolation, but need to be considered within the context of the broader body of evidence. While MR analyses provide

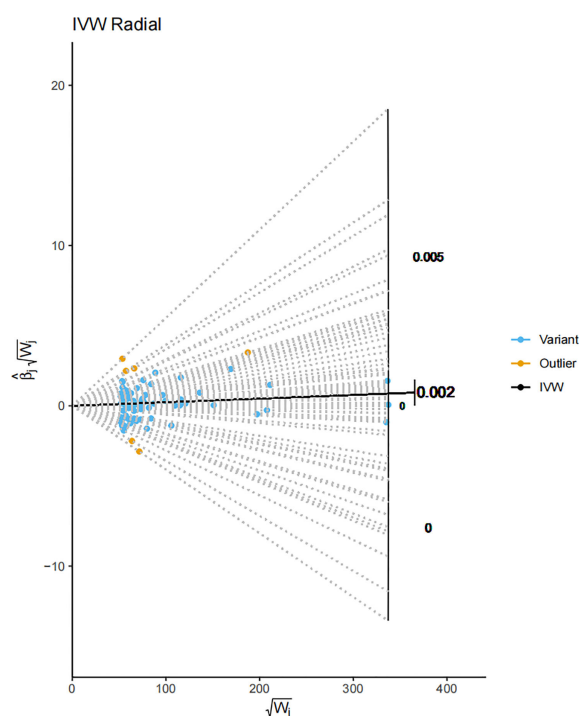


FIGURE 6

A Radial MR plot detailing the IVW radial of SNPs. The blue portions of the plot denote variant SNPs, while the yellow portions signify the identified outliers. This plot gives a visual representation of the impact of each SNP on the overall result, distinguishing between regular genetic variants and the outliers. The IVW radial plot indicates that the influence of the outliers on the overall outcome is minimal, supporting the conclusion that our results are robust.

evidence for causal relationships in an observational setting, validation through experimental or interventional studies is necessary to gain insight into the actual biological mechanisms that underpin this relationship. The ideal way to pursue this would be through prospective cohort studies or randomized controlled trials. Moreover, any conclusions should be interpreted keeping in view the overall health benefits of vitamin D, and the complex interplay between vitamin D physiology, skin cancer biology, and genetics.

4 Discussion

Our two-sample MR study, using the IVW method and leveraging genetic variants as IVs, provided substantial evidence for a causal association between serum 25-Hydroxyvitamin D levels and the incidence of cutaneous melanoma. This approach circumvented the environmental confounding and reverse causation bias, commonly encountered in traditional observational studies. Our study yielded a β coefficient of 0.0022159, indicating a significant positive correlation between increased serum 25-Hydroxyvitamin D levels and melanoma incidence. This relationship was consistently shown to be statistically significant, albeit with a small β coefficient, indicating that each unit increase in serum 25-Hydroxyvitamin D levels corresponds with an increased risk of melanoma. This is notable considering the increasing global incidence of this skin cancer.

We assessed the robustness of our results through multiple measures. The lack of significant heterogeneity among the genetic variants used reinforced the validity of our pooled causal estimate. Further, the “leave-one-out” sensitivity analysis affirmed that no individual SNP exerted a disproportionate influence on the estimated causal relationship. Tests for potential pleiotropy found minimal effects, which further validated the causality inferred. The Radial MR analysis, even after outlier exclusion, consistently demonstrated a persistent positive correlation between serum 25-Hydroxyvitamin D levels and melanoma incidence.

The study's results have profound public health implications, suggesting that, contrary to traditional beliefs, elevated serum 25-Hydroxyvitamin D levels may play a contributory role in the development of melanoma. This provides a nuanced perspective on melanoma etiology, which can guide future investigations and potentially inform prevention strategies. Despite the generally beneficial health effects attributed to serum 25-Hydroxyvitamin D (73), its potential influence on melanoma development should not be overlooked.

This study presents a unique perspective, in contrast to some existing literature, by revealing a positive causal relationship between increased serum 25-Hydroxyvitamin D levels and melanoma risk (27, 34). This finding diverges from previous observational studies that have often suggested an inverse or null association, potentially due to limitations inherent in such studies such as confounding and reverse causality (27, 74–76). The divergence from observational studies reflects the intricate nature of Vitamin D metabolism, immune modulation, skin carcinogenesis, and the multifaceted role of UV radiation (77,

78). It further emphasizes the importance of nuanced, context-specific investigations and robust methodologies to decipher this complex relationship (28, 34, 79).

These divergent findings underscore the complex interplay between Vitamin D physiology, sun exposure necessary for Vitamin D synthesis, and the risk of skin cancer (80, 81). On one hand, Vitamin D is acknowledged for its beneficial roles in health (82), while on the other hand, sun exposure, being a significant source of Vitamin D, is also a primary risk factor for melanoma due to potential DNA damage from ultraviolet radiation (83). This dynamic highlights the intricate balance between the potential benefits and hazards of sun exposure. UV radiation is a shared risk factor for melanoma and a primary source of Vitamin D synthesis, creating a complex interplay between the potential benefits and hazards of sun exposure and Vitamin D's generally protective effects (32).

The research findings necessitate a reassessment of current recommendations concerning sun exposure and vitamin D supplementation, especially for high-risk populations (84). The study questions the perception of vitamin D as an exclusively beneficial agent, highlighting the potential risks associated with its excessive intake. This calls for a nuanced understanding of vitamin D's role in melanoma pathogenesis and advocates a careful risk-benefit assessment regarding vitamin D supplementation and sun exposure. A key insight from the study is the critical need for personalized medicine strategies that take into account individuals' genetic susceptibility when deciding about Vitamin D supplementation.

The study underscores the utility of Mendelian randomization as a robust tool in biomedical research, capable of identifying causal relationships that might be overlooked in traditional observational studies. This strengthens the opportunity to delve deeper into the complex interplay between vitamin D metabolism, genetics, and skin cancer biology (85). However, despite the potential association between elevated vitamin D levels and melanoma, the study reaffirms the established health benefits of maintaining adequate vitamin D levels. It emphasizes the importance of a balanced approach to vitamin D supplementation and sun exposure, alongside careful monitoring of serum vitamin D levels, especially in populations at high risk of melanoma.

From a policy perspective, these findings prompt a thorough reevaluation of vitamin D supplementation guidelines, particularly for high-risk populations (86). Regarding biomedical research, the discovery of this relationship presents opportunities to probe the underlying biological mechanisms that may explain how elevated serum 25-Hydroxyvitamin D levels contribute to increased melanoma risk (87). Nonetheless, given the intricate nature of vitamin D metabolism and its various health benefits, the study's findings should be interpreted with caution.

While the study provides valuable insights, it does possess a few inherent limitations. Firstly, the study assumes that the instrumental variables, in this case, genetic variants, impact the outcome solely via their effect on the exposure, a requirement known as the exclusion restriction criterion. However, despite our rigorous tests, we cannot definitively exclude the possibility of unrecognized pleiotropy, where a gene could affect multiple traits, or unknown confounding factors influencing our findings. Secondly, our analysis does not provide detailed insights into the biological mechanisms connecting serum

25-Hydroxyvitamin D levels and melanoma risk. The identified causal relationship does not fully unravel the complex biology of Vitamin D and its role in melanoma incidence. Further experimental validation or prospective cohort studies are necessary to comprehend these mechanisms.

Our study also acknowledges limitations related to the generalizability of the findings. The genetic instruments used were primarily identified in populations of European ancestry, potentially limiting the application of our results to other ethnic groups. Additionally, our study did not account for individual-level confounders, nor did it explore potential non-linear relationships between serum 25-Hydroxyvitamin D levels and melanoma risk. The study also overlooked the modulatory roles of factors like age, sex, and environmental UV exposure, given its reliance on summarized population-level data. Moreover, our analysis assumes a linear relationship between exposure and outcome, which may oversimplify the biological reality.

Future research should adopt an integrative approach that validates initial findings, investigates underlying biological mechanisms, expands the scope of genetic studies, replicates results in diverse populations, and explores potential confounding factors. Studies should focus on establishing the optimal range of vitamin D levels that balance the potential risks and benefits, the health implications of vitamin D in skin cancer prevention, and the association of vitamin D with other forms of skin cancer.

5 Conclusion

The comprehensive MR study evidenced a positive causal relationship between serum 25-Hydroxyvitamin D levels and melanoma incidence, holding significant implications for public health policies, clinical guidelines, and cancer prevention strategies. However, given the intricacy of vitamin D metabolism, skin cancer biology, and the broader health benefits of vitamin D, these findings necessitate cautious interpretation and further exploration. Continued research is needed to consolidate these findings, unravel the complex interplay between genetics, environment, and biology, and fully understand the biological mechanisms underlying this association. Despite the inherent limitations, Mendelian randomization proves a valuable tool in biomedical research, resolving causal ambiguities and enhancing context-specific, evidence-based health interventions. Future research should focus on corroborating these findings, dissecting all contributing factors to melanoma risk, and illuminating novel therapeutic targets. Ultimately, any health policy or strategy modulating vitamin D levels must balance these findings with the broader health benefits of vitamin D.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

BC, QL and BW made substantial contributions to the conception and design of the study. BC, QL, RK and XS performed data acquisition and analysis. BC, JY and XN made substantial contributions to drafting the article and graphs. XL and BW reviewed and marked the complete manuscript. All the authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2023.1154107/full#supplementary-material>

SUPPLEMENTARY TABLE 1

Gene symbols and traits corresponding to the SNPs listed in the ordinate of .

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