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ORIGINAL RESEARCH ARTICLE

The mediating role of Vitamin D in cancer-related fatigue associated with hepatocellular carcinoma: A Mendelian randomization study

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Abstract

Introduction: Cancer-related fatigue is a debilitating symptom among hepatocellular carcinoma (HCC) patients, significantly impacting their quality of life. Emerging evidence suggests that Vitamin D may help alleviate fatigue; however, its causal role remains unclear.

Objective: This study aims to investigate the causal relationship between HCC, Vitamin D, and fatigue by assessing the mediating role of Vitamin D using Mendelian randomization (MR) analysis.

Methods: A two-sample MR analysis was conducted using genome-wide association study (GWAS) data. Genetic instruments for "HCC," "Vitamin D," and "fatigue" were obtained from the Integrative Epidemiology Unit OpenGWAS database. Inverse variance weighting, MR-Egger regression, weighted median, simple mode, and weighted mode methods, as well as sensitivity analyses such as the MR-Egger intercept, Cochran's *Q* test, leave-one-out analysis, and MR Pleiotropy RESidual Sum and Outlier, were employed to ensure robustness. Finally, a two-step MR analysis was performed to quantify Vitamin D's mediation effect.

Results: HCC showed a significant causal effect on increased fatigue risk (odds ratio = 1.78; 95% confidence interval: 1.16–2.74; $p<0.05$) and decreased Vitamin D levels ($\beta = -12.61$; $p<0.05$). Higher Vitamin D levels were associated with reduced fatigue severity ($\beta = -0.01$; $p<0.05$). Mediation analysis indicated that 21.74% of the effect of HCC on fatigue was mediated by Vitamin D. Sensitivity analyses confirmed the robustness of these findings.

Conclusion: Vitamin D may partially mediate the HCC–fatigue relationship inferred from genetic proxies for chronic fatigue syndrome, highlighting its potential, albeit preliminary, role as a therapeutic target for cancer-related fatigue management. Future clinical trials should evaluate the efficacy of Vitamin D supplementation in alleviating fatigue among HCC patients.

Keywords: Hepatocellular carcinoma; Cancer-related fatigue; Vitamin D; Mendelian randomization; Mediation analysis

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1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor worldwide, with China accounting for approximately 50% of global cases, thereby facing a substantial disease burden.[1,2] Cancer-related fatigue (CRF) is characterized by persistent physical, emotional, and cognitive exhaustion that is not relieved by rest and often worsens over time.[3] This pervasive and debilitating symptom significantly reduces the quality of life among HCC patients.[4] Recent studies have highlighted the multifactorial pathophysiology of CRF, involving neuroinflammation, mitochondrial dysfunction, and dysregulation of the kynurenine pathway, which may serve as potential therapeutic targets.[5,6]

Despite its high prevalence, CRF remains underdiagnosed and undertreated in clinical oncology settings, highlighting the urgent need for mechanistic insights and evidence-based interventions.[7] Surgery, transarterial chemoembolization, and systemic therapies such as targeted drugs and immunotherapy are common treatment modalities for HCC and have achieved significant progress in HCC control. However, no definitive clinical protocol currently exists for the management of CRF. The complexity of CRF, which encompasses skeletal muscle metabolism,[8] hypothalamic–pituitary–adrenal axis regulation,[9] immune–inflammatory responses,[10] and central nervous system dysfunction,[11] further complicates its management.

Emerging evidence highlights the role of Vitamin D in alleviating fatigue,[12] particularly among cancer patients.[13-15] Studies have indicated that Vitamin D supplementation can reduce CRF severity, particularly in breast cancer patients, potentially through immunomodulatory and antioxidant mechanisms.[13,16] In addition, previous research has shown that higher serum levels of 25-hydroxyvitamin D (25[OH] D)-3 are associated with improved survival outcomes.[17]

Vitamin D deficiency is common in HCC patients and may exacerbate systemic inflammation and impair energy metabolism, thereby contributing to fatigue.[18,19] Large-scale epidemiological evidence further supports the protective role of sufficient Vitamin D against HCC development. For instance, a prospective analysis of 447,028 United Kingdom Biobank participants demonstrated that higher serum 25(OH)D levels were associated with up to a 48% reduction in HCC risk, with a 12% risk decrease per 10 nmol/L increment. Moreover, individuals with both high Vitamin D levels and low genetic risk exhibited a 78% lower HCC incidence. Complementing this, a recent United States cohort study of over 2.4 million matched patients confirmed that Vitamin D deficiency independently increases the risks of cirrhosis, HCC, and mortality, and that achieving sufficiency through supplementation reduces mortality and normalizes HCC risk, with optimal protective effects observed at serum levels of 40–60 ng/mL.[20,21] However, it remains unclear whether Vitamin D plays a mediating role in CRF among HCC patients. Specifically, whether reduced Vitamin D levels contribute to the onset or exacerbation of fatigue symptoms, as well as the underlying molecular mechanisms, has not yet been investigated or reported in the literature.

Therefore, the present study employs a Mendelian randomization (MR) approach to investigate the causal relationships between HCC and fatigue, with Vitamin D serving as a potential mediator. The MR analysis strengthens causal inference by minimizing the impact of confounding factors and reducing reverse causality, thereby addressing key limitations inherent to traditional observational studies. Using a two-step MR framework, this study quantifies the mediating effect of Vitamin D in the HCC–fatigue pathway, providing novel insights into potential therapeutic targets for managing CRF in patients with HCC.

However, it should be acknowledged that, due to the absence of a large-scale genome-wide association study (GWAS) specifically addressing CRF in HCC patients, this study utilizes genetic instruments derived from chronic fatigue syndrome (CFS) as a proxy. While CFS and CRF share overlapping symptomatology, they arise from distinct etiologies—a limitation that constrains the direct generalizability of the findings to the oncology context.

2. Materials and methods

2.1. Data source

This MR study was conducted using publicly available GWAS datasets. Data were extracted from the Integrative Epidemiology Unit (IEU) OpenGWAS project database (https://gwas.mrcieu.ac.uk/), encompassing genetic associations with HCC, Vitamin D levels (measured as serum 25[OH]D3), and fatigue. The fatigue GWAS was derived from a study of CFS rather than CRF. As no large-scale GWAS for CRF currently exists, CFS was used as the best available proxy, although this introduces potential etiological discrepancies that must be considered when interpreting the results. The study design adhered to the Strengthening the Reporting of Observational Studies in Epidemiology–MR guidelines to ensure transparency and reproducibility.[22] As this study involved secondary analyses of previously collected and disseminated data, additional ethical approval was not required.

2.2. Selection of instrumental variables (IVs)

To ensure robust and credible results, the IVs selected for a two-sample MR study must satisfy three core

assumptions: (i) The IVs must exhibit a strong association with the exposure of interest; (ii) the IVs should be independent of confounding factors that could bias the relationship between the exposure and outcome; and (iii) the IVs should influence the outcome solely through their effect on the exposure, with no alternative pathways.[23] Figure 1 illustrates these assumptions and the role of selected single-nucleotide polymorphisms (SNPs) as IVs in relation to the exposure (HCC), mediator (Vitamin D), and outcome (fatigue), while avoiding confounders and reverse causality.

SNPs associated with the exposure were initially identified from GWAS using the following criteria: A genome-wide significance threshold of $p<5 \times 10^{-6}$,[24] a genetic distance of 10,000 kb, and a linkage disequilibrium measure of $r^2 < 0.001$. To satisfy the exclusion restriction assumption, we excluded SNPs with a significant association with the outcome at a threshold of $p<5 \times 10^{-5}$.[25] To further evaluate the validity of these SNPs as IVs, we calculated *F*-statistics for each variant to minimize weak instrument bias, excluding SNPs with an *F*-statistic <10.[26] PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) was used to determine whether the selected SNPs exhibited genome-wide significant associations with other traits, thereby preventing potential confounding.[27] Finally, to ensure consistency between exposure and outcome SNPs, we harmonized alleles and excluded ambiguous palindromic SNPs and incompatible SNPs.[28]

2.3. MR methods

The primary MR analysis was conducted using univariable two-sample MR with the inverse variance weighting method. To assess the robustness of the inverse variance weighting estimates, alternative MR methods, including MR-Egger regression, weighted median, simple mode, and weighted mode tests, were employed. These analyses were conducted to minimize the potential bias caused by genetic IVs operating through unidentified pathways and to address inconsistencies in the strength of IV effects. Sensitivity analyses aimed at detecting and adjusting for potential pleiotropy and heterogeneity were conducted to ensure the robustness and reliability of the MR findings. Specifically, the MR-Egger intercept was employed to identify directional pleiotropy. If the intercept term was very close to 0, then the MR-Egger model was approximately equivalent to inverse variance weighting.

$p>0.05$ indicated that the possibility of pleiotropy was minimal or non-existent, suggesting that the SNPs were associated only with the exposure and not with other confounding variables. Heterogeneity was assessed using Cochran's *Q* test and a "leave-one-out" analysis, which sequentially excludes individual IVs to evaluate their impact, and the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) was also applied for outlier detection and adjustment.

All statistical analyses were performed using R software version 4.2.3 (Core Team, Austria) with the "TwoSampleMR" and "MR-PRESSO" packages. Visualization of MR results was achieved through forest plots generated by GraphPad Prism software version 9.0 (GraphPad Software Inc., USA). A statistical significance threshold of $p<0.05$ was applied throughout the analyses.

2.4. Mediation MR analysis

A two-step MR approach was performed to estimate the mediating effect of the mediator (Vitamin D) on the causal association between HCC and fatigue. In the first step,

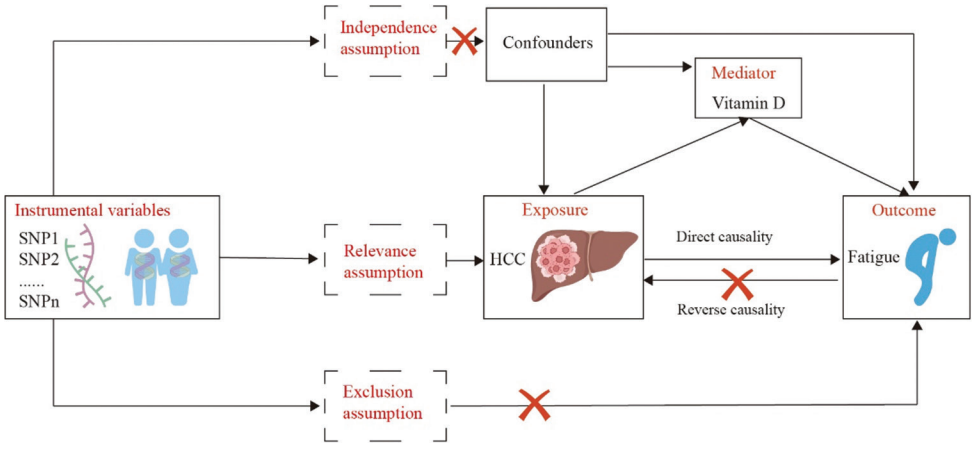

Figure 1. Illustration of the assumptions for Mendelian randomization analysis and the mediation model
Abbreviations: HCC: Hepatocellular carcinoma; SNP: Single-nucleotide polymorphism.

univariable two-sample MR was used to determine the causal effect (β_1) of HCC on Vitamin D and the total effect (β_0) of HCC on fatigue. In the second step, the direct effect (β_2) of the mediator (Vitamin D) on fatigue was calculated by adjusting for the effect of HCC on fatigue via the inverse variance weighting method. The mediation effect was then quantified by calculating the proportion mediated as follows[29]:

$$\text{Proportion mediated} = \frac{\beta_1 \times \beta_2}{\beta_0} \quad (1)$$

This process enabled the quantification of Vitamin D's role in mediating the causality between HCC and fatigue. All statistical analyses were conducted using R software version 4.2.3 and visualized through forest plots generated by GraphPad Prism software version 9.0. The flowchart for the MR analysis is shown in Figure 2.

3. Results

3.1. IVs included in the analysis

The study utilized genetic variants from GWAS for HCC, Vitamin D levels, and CFS to construct the IVs. The GWAS for HCC (GWAS ID: ieu-b-4953) was derived from the United Kingdom Biobank and included 168 cases and 372,016 controls, with a total sample size of 372,184 individuals of European ancestry. Genetic associations for Vitamin D levels were obtained from the Medical Research Council IEU GWAS (GWAS ID: ukb-b-18593), including a sample of 64,979 participants of European descent. The GWAS for CFS (GWAS ID: ebi-a-GCST90038694) contained data from 2092 cases and 482,506 controls, amounting to a total sample size of 484,598 individuals. The detailed characteristics of the included GWAS datasets are presented in Table 1.

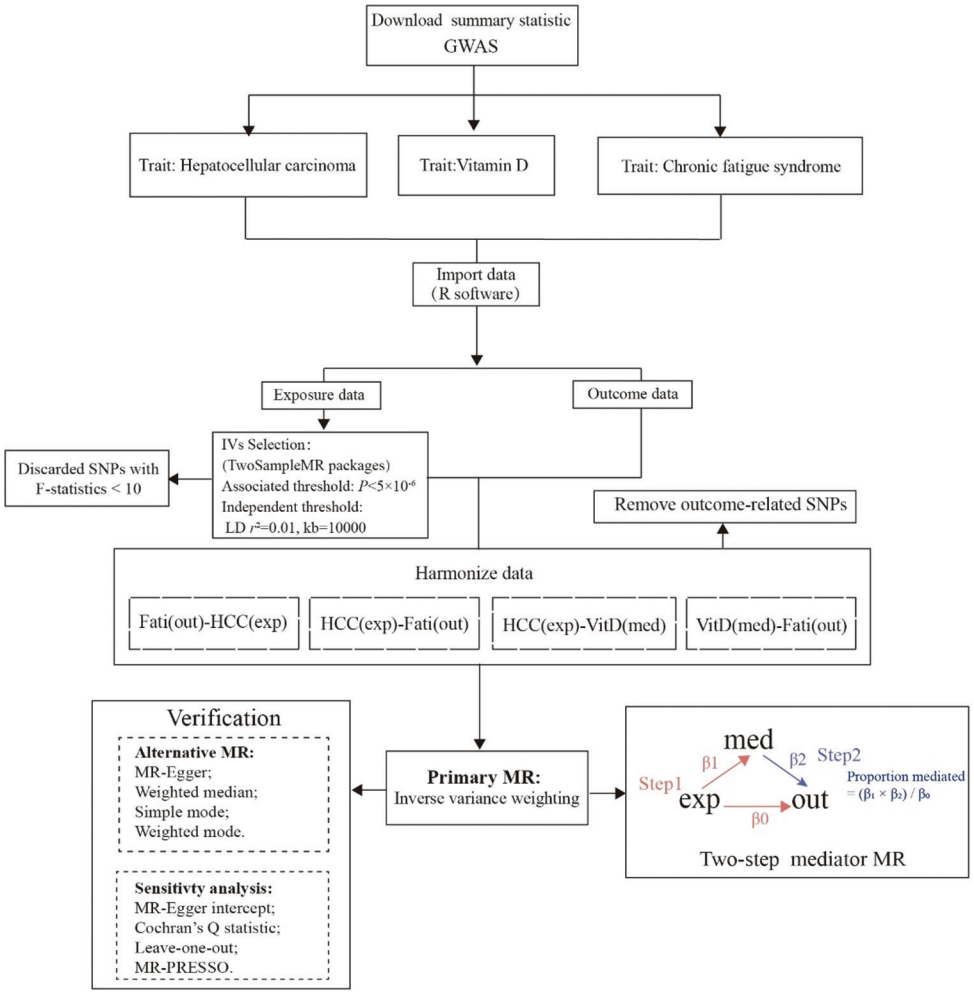

Figure 2. Flowchart of the MR analysis examining the causal relationships among HCC, Vitamin D levels, and fatigue-related phenotypes
Abbreviations: GWAS: Genome-wide association study; HCC: Hepatocellular carcinoma; IV: Instrumental variable; MR: Mendelian randomization; MR-PRESSO: Mendelian randomization Pleiotropy RESidual Sum and Outlier; SNP: Single-nucleotide polymorphism.

3.2. Effects of HCC on fatigue

The findings revealed a positive association between HCC and fatigue (odds ratio [OR] = 1.78; 95% confidence interval [CI]: 1.16–2.74; p=0.008). To validate these findings, additional analyses were conducted using the MR-Egger (OR = 2.22; 95% CI: 0.42–11.73; p=0.391), weighted median (OR = 1.73; 95% CI: 1.01–2.98; p=0.047), simple mode (OR = 1.70; 95% CI: 0.08–3.60; p=0.214), and weighted mode (OR = 1.74; 95% CI: 0.81–3.75; p=0.204). Across all methods, the ORs consistently suggested a potential effect of HCC on fatigue, with β-values ranging from 0.53 to 0.80 (Figure 3A). Cochran's Q test indicated no evidence of heterogeneity (p=0.984), and the MR-Egger intercept analysis revealed no evidence of horizontal pleiotropy (p=0.80) (Table 2). MR-PRESSO analysis confirmed that no SNPs introduced bias.

The leave-one-out sensitivity analysis examined the robustness of the results by iteratively excluding each SNP. The analysis revealed that no single SNP disproportionately influenced the overall association, thereby supporting the stability and reliability of the findings (Figure 3B). Figure 3C illustrates the scatter plot of SNP effects on HCC versus their effects on fatigue. Lines representing different MR methods indicated a consistent direction of association, reinforcing the causal relationship. The inverse variance weighting method demonstrated the strongest association, with other methods aligning closely, providing robust evidence of a causal link between HCC and fatigue. These results collectively highlight that HCC is a potential contributor to fatigue.

3.3. Effects of Vitamin D on fatigue

Figure 4 illustrates the MR analysis of the causal relationship between Vitamin D levels and fatigue.

Table 1. Details of the instrumental variables included in the Mendelian randomization analyses

Phenotype	GWAS ID	Consortium	Population	Gender	Sample size (n)	Control group (n)	Objective group (n)	Number of SNPs	Year	PMID
HCC	ieu-b-4953	UK Biobank	European	Male and female	372,184	372,016	168	6,304,034	2021	-
Chronic fatigue syndrome	ebi-a-GCST90038694	NA	NA	NA	484,598	482,506	2,092	9,587,836	2021	33959723
Vitamin D	ukb-b-18593	MRC-IEU	European	Male and female	64,979	NA	NA	9,851,867	2018	NA

Abbreviations: GWAS: Genome-wide association study; HCC: Hepatocellular carcinoma; MRC-IEU: Medical Research Council Integrative Epidemiology Unit; NA: Not available; PMID: PubMed identifier; SNP: Single nucleotide polymorphism; UK: United Kingdom.

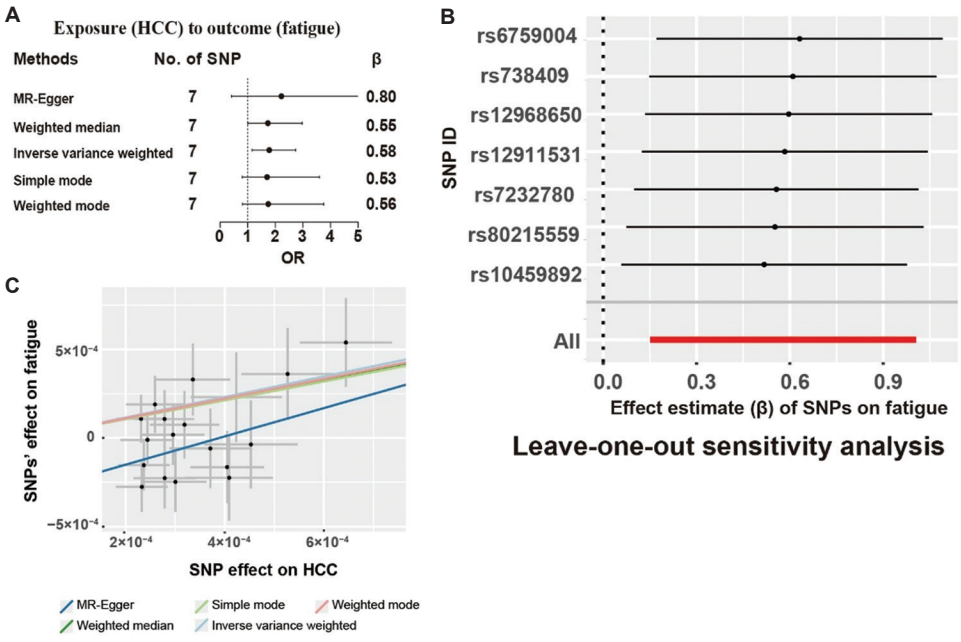

Figure 3. MR results and sensitivity analyses of the effects of HCC on fatigue: (A) Forest plot of MR analyses; (B) leave-one-out sensitivity analysis; and (C) scatterplot results across five MR methods

Abbreviations: HCC: Hepatocellular carcinoma; MR: Mendelian randomization; OR: Odds ratio; SNP: Single-nucleotide polymorphism.

Table 2. Heterogeneity and pleiotropy analyses

Exposure	Outcome	Heterogeneity			Pleiotropy	
		Method	Cochran's *Q*	*p*-value	MR-Egger intercept	*p*-value
HCC	Fatigue	Inverse variance weighting	1.037	0.984	-7.11×10^{-5}	0.800
Vitamin D	Fatigue	Inverse variance weighting	80.436	5.18e-08	5.33×10^{-5}	0.756
HCC	Vitamin D	Inverse variance weighting	12.513	0.768	0.005	0.413

Abbreviations: HCC: Hepatocellular carcinoma; MR: Mendelian randomization.

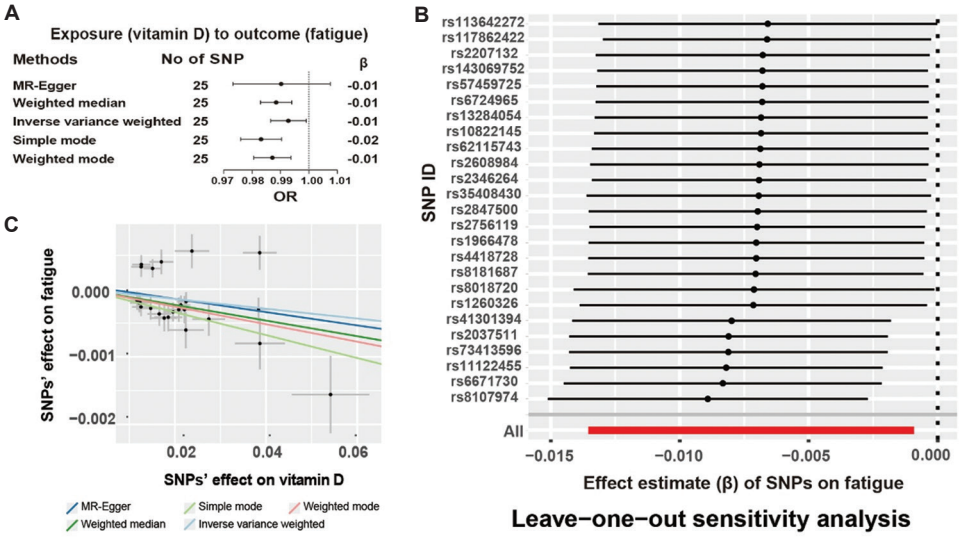

Figure 4. MR results and sensitivity analyses of the effects of vitamin D on fatigue: (A) Forest plot of MR analyses; (B) leave-one-out sensitivity analysis; and (C) scatterplot results across five MR analyses
Abbreviations: MR: Mendelian randomization; OR: Odds ratio; SNP: Single-nucleotide polymorphism.

Although the direct causal effect of Vitamin D on fatigue was modest, a consistent negative association was observed. Following outlier correction using MR-PRESSO, all MR methods yielded ORs close to 1, with β-values ranging from −0.01 to −0.02, suggesting a small and potentially fragile role of Vitamin D in alleviating fatigue (Figure 4A).

Although Cochran's *Q* test detected significant heterogeneity among the IVs ($p=5.18\times10^{-8}$), suggesting potential violations of the "no horizontal pleiotropy" assumption or variability in instrument strength, horizontal pleiotropy was not detected by the MR-Egger intercept ($p=0.76$; Table 2). This pattern, significant heterogeneity without directional pleiotropy, may reflect context-dependent effects of Vitamin D on fatigue or the influence of weak instruments and therefore warrants cautious interpretation of the causal estimate. Leave-one-out sensitivity analysis confirmed that no SNPs influenced the overall findings (Figure 4B). The scatter plot further supported the consistency across MR methods, showing similar directional effects and linear relationships between SNP impacts on Vitamin D and fatigue (Figure 4C). This consistency supports a negative correlation between Vitamin D and fatigue, though the relationship remains modest.

3.4. Effects of HCC on Vitamin D

Given that our earlier findings demonstrated associations between HCC and fatigue, as well as between Vitamin D and fatigue, we postulated that Vitamin D may mediate the relationship between HCC and fatigue. Figure 5 supports this hypothesis, demonstrating a causal effect of genetically predicted HCC on Vitamin D levels using the inverse variance weighting method (OR = 9.48×10^{-9}; 95% CI: 9.74×10^{-14}–9.23×10^{-4}; $p=0.002$). Sensitivity analyses were also performed to reinforce this conclusion.

As shown in Figure 5A, MR-Egger (β = −27.75, $p>0.05$), weighted median (β = −10.16, $p>0.05$), simple mode (β = −10.24, $p>0.05$), and weighted mode (β = −10.74, $p>0.05$) all yielded consistent negative associations between HCC and Vitamin D. MR-PRESSO detected no outlier SNPs. Cochran's *Q* test indicated no evidence of heterogeneity ($p=0.768$; Table 2), and

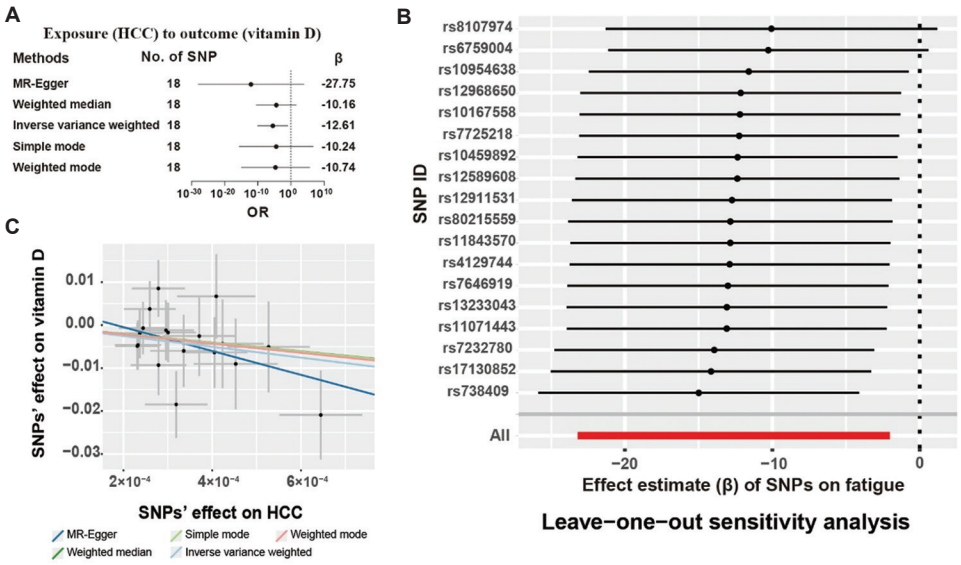

Figure 5. MR results and sensitivity analyses of the effects of HCC on vitamin D: (A) Forest plot of MR analyses; (B) leave-one-out sensitivity analyses; and (C) scatterplot results across five MR analyses
Abbreviations: HCC: Hepatocellular carcinoma; MR: Mendelian randomization; OR: Odds ratio; SNP: Single-nucleotide polymorphism.

the MR-Egger intercept analysis showed no evidence of horizontal pleiotropy (p=0.413; Table 2). Leave-one-out sensitivity analysis further confirmed that no single SNP disproportionately affected the overall association between HCC and Vitamin D (Figure 5B). Furthermore, scatterplots consistently illustrated a negative relationship between HCC and Vitamin D levels across different methods (Figure 5C). These findings collectively support the hypothesis that Vitamin D may play a mediating role in the association between HCC and fatigue, although further research is needed to elucidate the underlying mechanisms.

3.5. The mediation effect of Vitamin D in the causal association between HCC and fatigue

Using the two-step MR approach, we evaluated the mediating role of Vitamin D in the causal pathway linking HCC to fatigue. In the first step, a significant causal association between HCC and Vitamin D levels was identified ($\beta_1 = -12.61$, $p<0.05$), indicating that HCC negatively affects Vitamin D levels. In the second step, we established a causal relationship between Vitamin D levels and fatigue risk ($\beta_2 = -0.01$, $p<0.05$), suggesting that reduced Vitamin D levels increase the risk of fatigue. The direct causal effect of HCC on fatigue was also significant ($\beta_0 = 0.58$, $p<0.05$).

To quantify the mediation effect of Vitamin D, we calculated the indirect effect as:

$$\beta_{indirect} = \beta 1 \times \beta 2 = (-12.61) \times (-0.01) = 0.126 \quad (2)$$

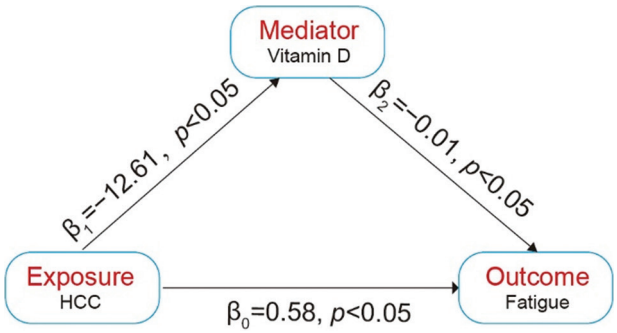

Figure 6. The mediating effect of the mediator (Vitamin D) on the causal association between HCC and fatigue
Abbreviation: HCC: Hepatocellular carcinoma.

and the proportion mediated by Vitamin D as:

$$\text{Proportion mediated} = \beta_{indirect}/\beta_{total} = \frac{\beta_1 \times \beta_2}{\beta_0} = \frac{0.126}{0.58} = 21.74\% \quad (3)$$

These results indicate that approximately 21.74% of the total effect of HCC on fatigue is mediated through Vitamin D. These findings suggest that Vitamin D plays a partial but significant mediating role in the HCC–fatigue pathway, highlighting its potential as a protective factor in CRF. These results highlight the importance of targeting Vitamin D pathways in therapeutic strategies for mitigating fatigue in HCC patients. Detailed results are presented in Figure 6.

4. Discussion

HCC patients frequently experience fatigue due to both disease-related factors and treatment-induced side effects. CRF may interfere with treatment efficacy and significantly impair patients' quality of life. Currently, no specific therapeutic strategies are available for CRF management. Emerging evidence suggests that low Vitamin D levels are associated with HCC progression.[20] Moreover, as a key micronutrient involved in metabolic regulation, Vitamin D has been implicated in the development of CRF. Therefore, MR analysis was conducted to investigate whether Vitamin D plays a mediating role in the association between HCC and fatigue, with the goal of elucidating the potential of Vitamin D supplementation as a therapeutic approach for managing fatigue in HCC patients.

In this study, we first identified a positive association between HCC and fatigue, suggesting that HCC contributes to fatigue. For the causal relationship between Vitamin D levels and fatigue, our findings indicated that although the direct causal effect of Vitamin D on fatigue was modest, a consistent potential role for Vitamin D in alleviating fatigue was observed. Furthermore, a negative relationship between HCC and Vitamin D levels was demonstrated across multiple analytical methods. To precisely calculate the proportion mediated role of Vitamin D, a two-step MR analysis was conducted, which demonstrated that approximately 21.74% of the total effect of HCC on fatigue is mediated through Vitamin D. This modest proportion underscores Vitamin D's partial but significant role in the HCC–fatigue pathway.

However, this estimate must be interpreted in light of the significant heterogeneity observed in the Vitamin D–fatigue pathway ($p<0.001$), which suggests that the causal effect of Vitamin D on fatigue may not be uniform across all genetic instruments. This heterogeneity could arise from biological context-dependency (e.g., effects modified by inflammation or metabolic status), measurement error in the proxy fatigue phenotype, or residual pleiotropy, all of which introduce uncertainty into the mediation proportion and highlight the need for replication in larger, more homogeneous datasets.

The liver plays a central role in Vitamin D metabolism, where 25-hydroxylases (e.g., CYP2R1 and CYP27A1) catalyze the conversion of Vitamin D into 25(OH)D, the primary circulating form of vitamin D.[30] In HCC, liver dysfunction caused by cirrhosis and other factors often impairs 25-hydroxylase activity, leading to reduced 25(OH)D synthesis.[31] In addition, the liver produces Vitamin D-binding protein, which is crucial for Vitamin D transport, and its levels are decreased in HCC patients, potentially further exacerbating Vitamin D deficiency.[32,33]

The hallmark features of HCC, including chronic inflammation and oxidative stress, further aggravate Vitamin D deficiency.[30] Elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin 6, in HCC patients have been reported to suppress Vitamin D synthesis and metabolism.[34,35] Moreover, oxidative stress damages hepatic mitochondria and cellular components,[36] potentially disrupting Vitamin D metabolism and interfering with Vitamin D receptor signaling pathways.[37]

Given the extensive role of Vitamin D in immune and metabolic regulation, its deficiency may contribute significantly to the development of fatigue in HCC patients. Genetic factors may also influence Vitamin D metabolism in HCC patients. The SNP rs8107974 appears in both the HCC–Vitamin D and Vitamin D–fatigue pathways, suggesting a potential regulatory role. Located in the intronic region of the *SUGP1* gene, rs8107974 may influence the splicing of *SUGP1*, a key regulator of mRNA processing. Studies indicate that abnormal splicing of *SUGP1* may affect the expression of genes involved in energy metabolism and inflammation regulation, contributing to metabolic alterations and fatigue severity in HCC patients.[38,39] Furthermore, the chronic inflammatory environment in HCC may interact with *SUGP1* function,[40] thereby exacerbating metabolic dysregulation and fatigue symptoms.

These findings establish Vitamin D as a key mediator in the HCC–fatigue axis, with potential involvement of rs8107974-mediated regulation of *SUGP1*. MR and mediation analyses support the mediating role of Vitamin D in HCC-related fatigue, suggesting its potential as a biomarker and a therapeutic target for precise interventions in HCC-associated fatigue.

This study employed the MR technique to provide robust causal evidence, minimizing confounding and reverse causality inherent in observational designs. Sensitivity analyses enhanced the reliability of results, whereas the mediation framework quantified Vitamin D's role in the HCC–fatigue pathway. However, several limitations warrant consideration. First, while MR minimizes confounding, its validity relies heavily on the availability of large, well-powered GWAS datasets. The GWAS for fatigue included only 2092 cases—a relatively small sample size that may reduce statistical power, increase standard errors, and limit the precision of causal estimates. This limitation is particularly relevant for the Vitamin D–fatigue pathway, where significant heterogeneity was observed, suggesting potential violations of MR assumptions or the influence of weak instruments.

Furthermore, small case numbers increase susceptibility to the winner's curse and may inflate effect sizes in discovery

GWAS, which could propagate into downstream MR analyses. Although sensitivity analyses (e.g., MR-PRESSO and leave-one-out) were employed to mitigate bias, the modest effect sizes and partial consistency across methods underscore the need for cautious interpretation.

Another fundamental limitation is the use of the CFS GWAS dataset rather than CRF-specific GWAS data. While both conditions manifest as persistent, debilitating fatigue, their underlying pathophysiologies differ substantially. CRF is driven by tumor burden, systemic inflammation, metabolic dysregulation, and treatment toxicity, whereas CFS is often associated with post-viral triggers, autonomic dysfunction, and immune dysregulation without malignancy. Consequently, the genetic architecture captured by the CFS GWAS may not fully reflect the biological pathways specific to CRF in HCC patients. This mismatch weakens the direct clinical translatability of our findings and suggests that the observed mediation effect, while statistically significant, may not precisely mirror the HCC–CRF–Vitamin D axis in oncology populations. Future MR studies employing CRF-specific GWAS when available are essential to validate and refine these results.

Other limitations include the lack of CRF-specific GWAS, potential population bias due to reliance on European-ancestry datasets, and the complex, multifactorial nature of CRF. Future research should validate these findings in diverse populations, assess the clinical efficacy of Vitamin D supplementation through randomized controlled trials, and explore underlying molecular mechanisms, including metabolic and inflammatory pathways, to further elucidate the biological link between Vitamin D, HCC, and fatigue.

5. Conclusion

This study provides preliminary evidence that Vitamin D may play a modest role in mediating the relationship between HCC and CRF. By integrating MR and mediation analysis, we provide preliminary, hypothesis-generating evidence for Vitamin D's protective effects against CRF, offering a foundation for future interventional and mechanistic studies, which should be further validated in CRF-specific cohorts. Targeting Vitamin D deficiency in HCC patients may represent a promising therapeutic strategy to mitigate fatigue and improve overall clinical outcomes.

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Conflict of interest

The authors declare they have no competing interests.

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Investigation: Tianxiao Zheng
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Writing–review & editing: Shuang Xiang, Wanfu Lin

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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