Circulating 25-hydroxyvitamin D and survival outcomes of colorectal cancer: evidence from population-based prospective cohorts and Mendelian randomisation

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Circulating 25-hydroxyvitamin D and survival outcomes of colorectal cancer: evidence from population-based prospective cohorts and Mendelian randomisation

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BACKGROUND: To investigate the association between circulating 25-hydroxyvitamin D (25-OHD) and colorectal cancer (CRC) survival outcomes.

METHODS: We conducted analyses among the Study of Colorectal Cancer in Scotland (SOCeCS) and the UK Biobank (UKBB). Both cancer-specific survival (CSS) and overall survival (OS) outcomes were examined. The 25-OHD levels were categorised into three groups, and multi-variable Cox-proportional hazard models were applied to estimate hazard ratios (HRs). We performed individual-level Mendelian randomisation (MR) through the generated polygenic risk scores (PRS) of 25-OHD and summary-level MR using the inverse-variance weighted (IVW) method.

RESULTS: We observed significantly poorer CSS (HR = 0.65, 95% CI = 0.55–0.76, P = 1.03 × 10^{-7}) and OS (HR = 0.66, 95% CI = 0.58–0.75, P = 8.15 × 10^{-11}) in patients with the lowest compared to those with the highest 25-OHD after adjusting for covariates. These associations remained across patients with varied tumour sites and stages. However, we found no significant association between 25-OHD PRS and either CSS (HR = 0.98, 95% CI = 0.80–1.19, P = 0.83) or OS (HR = 1.07, 95% CI = 0.91–1.25, P = 0.42). Furthermore, we found no evidence for causal effects by conducting summary-level MR analysis for either CSS (IVW: HR = 1.04, 95% CI = 0.85–1.28, P = 0.70) or OS (IVW: HR = 1.10, 95% CI = 0.93–1.31, P = 0.25).

CONCLUSION: This study supports the observed association between lower circulating 25-OHD and poorer survival outcomes for CRC patients. Whilst the genotype-specific association between better outcomes and higher 25-OHD is intriguing, we found no support for causality using MR approaches.

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INTRODUCTION

Epidemiological evidence supports an association between 25-hydroxyvitamin D (25-OHD) and CRC risk [1], but not causality [1, 2]. There is very little work has addressed any potential association between 25-OHD and CRC survival, especially in assessing causality. Previous observational evidence yielded inconsistent findings for the association between 25-OHD concentration and CRC survival [3, 4, 5, 6]. These studies were mostly small in sample size (N < 1000) and meta-analyses revealed significant between-study heterogeneity [3]. The uncertainty in the evidence could, in part, be attributed to the influence of confounding factors, such as anatomical subsites [7, 8].

In addition to observational evidence, the latest SUNSHINE trial failed to establish causality by detecting a marginal, yet non-significantly improved median progression-free survival among stage IV patients with high-dose versus standard-dose supplementation (8000 IU/d vs 400 IU/d) of vitamin D3 [9]. Another trial among elders from the Finnish population suggested vitamin D3 supplementation (1600 IU/d or 3200 IU/d) did not reduce either CRC incidence or mortality [10]. However, previous meta-analyses demonstrated a clinically meaningful beneficial effect of vitamin D supplementation on CRC survival [11, 12].

Mendelian randomisation study (MR) is an instrumental variable method, which can avoid the influence of confounders, and provide new insights into the potential causal association. The latest genome-wide association study (GWAS) has contributed a wealth of genetic risk variants for 25-OHD and enabled the generation of polygenic risk score (PRS) which has been established as an informative prediction measure of heritable traits [13]. Therefore, in the current study, we first conducted...
observational studies in the Study of Colorectal Cancer in Scotland (SOCCS) and the UK Biobank (UKBB) controlling for potential confounding effects. We then conducted both individual- and summary-level MR analyses to identify any causality.

**METHODS**

The SOCCS is a prospective, population-based case-control study aiming to investigate genetic and environmental factors associated with the risk and survival of colorectal cancer cases across Scotland. The UKBB is a large-scale prospective cohort with in-depth genetic and health information from the general population of the UK. The circulating 25-OHD level was May-standardised [4, 14] and the rank-based inverse-normal transformation was applied to normalise the distribution of May-standardised 25-OHD levels. Further descriptions of the two cohorts and the measurement of 25-OHD concentration can be found in supplementary methods and in our previous publications [15, 16]. We have considered two up-to-date GWASs [15, 16]. We have considered two up-to-date GWASs for our genetic instruments [17, 18]. Consequently, a total of 133 genetic variants associated with circulating 25-OHD concentration at \( p < 5 \times 10^{-8} \) were selected from the GWAS with 417,580 Europeans from the UKBB and these variants could explain from 5.7% to 10.5% of the variance for the 25-OHD level [17]. At last, 113 variants in UKBB and 107 variants in SOCCS were selected to generate the 25-OHD genetic instrument (Fig. S1). We generated a PRS by adding the weighted dosages of risk alleles for each of the variants. The list of genetic variants for 25-OHD is presented in Tables S1 & S2.

**Statistical analyses**

We plotted the predicted hazard ratio of CRC by 25-OHD and no disparity from linearity was observed (Fig S2). To reduce survivor bias, we restricted the observational analysis to incident cases in UKBB (Fig S3). Finally, 2936 and 3181 CRC patients who have both 25-OHD levels and survival records from SOCCS and UKBB, respectively, were included in the observational analysis. We categorised 25-OHD levels into three groups using clinical cutoffs defined by the Institute of Medicine [19] (Group 1: <25 nmol/L, Group 2: 25–50 nmol/L and Group 3: >50 nmol/L). We then analysed the associations between circulating 25-OHD and other covariates by one-way analysis of variance for continuous variables or \( \chi^2 \) test for categorical variables. Survival estimates for patients in each vitamin D group were estimated using the Kaplan-Meier approach. To account for the effects of covariates, we fitted three multivariable Cox proportional hazard models to estimate hazard ratios (HRs) of 25-OHD concentrations taking the group with the lowest 25-OHD level as a reference. The proportional hazards assumption was evaluated using Schoenfeld residuals based on model 3 including all covariates [20]. When any deviations were found, we re-estimated the HRs after stratifying for this covariate. We also investigated the potential gene-environment interaction between 25-OHD and the status of vitamin D receptor (VDR) polymorphism rs11568820 as suggested by previous findings [5]. In addition, we conducted stratified analysis by tumour site and stages based on the data availability.

We performed MR analyses in 5675 and 5847 CRC patients for whom genotype data and survival records were available in SOCCS and UKBB, respectively. An F-statistic less than ten indicates the presence of weak instrument effects [21]. The statistical power was estimated using a non-centrality parameter approach [22]. To perform individual-level MR, the association between PRS and CRC survival was assessed by proportional hazards models, adjusting for age and sex. AJCC stage was adjusted only in SOCCS (unavailable in the UKBB). Whereas for summary-level MR, the same genotype data and survival records were available in SOCCS and UKBB, and meta-analysis yielded an HR of 0.65 (95%CI = 0.55–0.76, \( P = 1.03 \times 10^{-7} \)) for CSS and 0.66 (95%CI = 0.58–0.75, \( P = 8.15 \times 10^{-11} \)) for OS. Consistent improvement in CSS and OS were observed and MR studies was set at 0.05 (two-sided). We applied a Bonferroni corrected significance threshold (\( P < 0.004 \)) to correct false positive rates from multiple tests in the subgroup analyses. All statistical analyses were performed on R v3.6.3. MR analyses were performed using the package ‘MendelianRandomization’ [32], ‘MRPRESSO’ [25], and ‘mr.raps’ [26].

**RESULTS**

**Observational studies**

A total of 2936 and 3181 CRC patients with 20,336 and 19,675 total person-years of follow-up from the SOCCS and UKBB respectively were included in this study. The basic characteristics of CRC patients are presented in Table 1. At the time of sampling, 29.56% and 12.54% of patients fulfilled the criteria for vitamin D deficiency (<25 nmol/L), and 47.10% and 47.72% were at insufficient levels (25–50 nmol/L) for SOCCS and UKBB respectively. Distributions of covariates for each 25-OHD tertile can be broadly consistent time-dependent effects for the covariates (Tables S5 and S6). The Kaplan-Meier survival estimates of cancer-specific (CSS) and overall survival (OS) under three 25-OHD groups were presented in Fig. 1. Cox models were employed to estimate the effect sizes of 25-OHD on CRC survival outcomes. As shown in Table 2 and Fig. 2, we observed significantly poorer CSS and OS for patients with the lowest 25-OHD in both SOCCS and UKBB, and meta-analysis yielded an HR of 0.65 (95%CI = 0.55–0.76, \( P = 1.03 \times 10^{-7} \)) for CSS and 0.66 (95%CI = 0.58–0.75, \( P = 8.15 \times 10^{-11} \)) for OS. Consistent improvement in CSS and OS were found in Tables S3 and S4.
Fig. 1 Kaplan–Meier survival estimates of colorectal cancer-specific and overall survival in different circulating 25-OHD groups. Survival estimates of colorectal cancer-specific survival in SOCCS and UKBB, and overall survival in SOCCS and UKBB, within different circulating 25-OHD groups. (Group 1: 25-OHD < 25 nmol/L, Group 2: 25-OHD = 25–50 nmol/L, Group 3: 25-OHD > 50 nmol/L). The HR and the corresponding P value were estimated in Model 1 by comparing the colorectal cancer-specific survival or overall survival difference between patients in Group 2/3 and in Group 1.

Table 2. Results of Cox regression models for the effect of circulating 25-OHD on colorectal cancer survival.

<table>
<thead>
<tr>
<th>Strata</th>
<th>Group 1a</th>
<th>Group 2a</th>
<th>Group 3a</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
<td>P</td>
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<tr>
<td>CRC death-SOCCS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>Ref</td>
<td>0.77</td>
<td>0.65–0.90</td>
</tr>
<tr>
<td>Model 2</td>
<td>Ref</td>
<td>0.75</td>
<td>0.64–0.89</td>
</tr>
<tr>
<td>Model 3</td>
<td>Ref</td>
<td>0.81</td>
<td>0.66–0.99</td>
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<tr>
<td>All cause of death-SOCCS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>Ref</td>
<td>0.85</td>
<td>0.74–0.97</td>
</tr>
<tr>
<td>Model 2</td>
<td>Ref</td>
<td>0.84</td>
<td>0.73–0.96</td>
</tr>
<tr>
<td>Model 3</td>
<td>Ref</td>
<td>0.90</td>
<td>0.76–1.06</td>
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<tr>
<td>CRC death-UKBB</td>
<td></td>
<td></td>
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<tr>
<td>Model 1</td>
<td>Ref</td>
<td>0.83</td>
<td>0.67–1.03</td>
</tr>
<tr>
<td>Model 2</td>
<td>Ref</td>
<td>0.84</td>
<td>0.68–1.03</td>
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<td>Model 3</td>
<td>Ref</td>
<td>0.85</td>
<td>0.69–1.06</td>
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<tr>
<td>All cause of death-UKBB</td>
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<tr>
<td>Model 1</td>
<td>Ref</td>
<td>0.73</td>
<td>0.62–0.87</td>
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<tr>
<td>Model 2</td>
<td>Ref</td>
<td>0.74</td>
<td>0.62–0.87</td>
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<tr>
<td>Model 3</td>
<td>Ref</td>
<td>0.74</td>
<td>0.63–0.88</td>
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Model 1: Adjusted for age and sex; Model 2: Adjusted for age, sex, tumour site (colon, rectum and colorectum), and season of blood sampling; Model 3 in SOCCS: age, sex, tumour site (colon, rectum and colorectum), the season of blood sampling and AJCC stages; Model 3 in UKBB: age, sex, tumour site (colon, rectum and colorectum), the season of blood sampling and BMI; P trend was tested in Model 1 by using the continuous rank-based inverse-normal transformed 25-OHD.

SOCCS Study of Colorectal Cancer in Scotland, UKBB UK Biobank, HR hazard ratio, CI confidence interval.

was identified in SOCCS when comparing patients in Group 2 with those in Group 1, although significant benefit was only found for OS in UKBB (Table 2).

The basic characteristics of CRC patients in subgroups are presented in Table S7. For colon cancer, the lower 25-OHD level was associated with poorer CSS (\( P = 6.82 \times 10^{-8} \)) and OS in SOCCS (\( P = 2.87 \times 10^{-10} \)) but not in UKBB (Table S8). For rectal cancer, the lower 25-OHD level was associated with poorer CSS in SOCCS (\( P = 2.00 \times 10^{-8} \)) and OS in both SOCCS (\( P = 2.00 \times 10^{-8} \)) and UKBB (\( P = 0.002 \)) (Table S8). The lower 25-OHD levels were strongly associated with poorer CSS (stage II: \( P = 0.002 \); stage III: \( P = 4.79 \times 10^{-5} \)) and OS (stage II: \( P = 5.75 \times 10^{-7} \); stage III: \( P = 1.78 \times 10^{-5} \)) among stage II and stage III patients in SOCCS (Table S8). Additionally, we observed a significant effect difference between GG and AA/AG genotype carriers of rs11568820 in SOCCS but not in UKBB (Table S9). In the interaction analysis of SOCCS, the interaction term had an HR of 0.82 (95%CI = 0.69–0.96, \( P = 0.014 \)) for CCS and 0.84 (95%CI = 0.73–0.95, \( P = 0.008 \)) for OS, taking AA/AG genotypes as the reference.

**Mendelian randomisation study**

A total of 5675 and 5847 CRC patients who have genotype data with 38,238 and 62,422 person-years of follow-up in SOCCS and UKBB respectively were included in the MR analyses (Table S10). The PRS was significantly correlated with an increased vitamin D level among both CRC cases (\( P = 1.60 \times 10^{-8} \) in SOCCS and \( P < 2 \times 10^{-16} \) in UKBB) and non-CRC participants (\( P = 2.61 \times 10^{-8} \) in SOCCS and \( P < 2 \times 10^{-16} \) in UKBB; Table S11). By applying these 113 or 107 SNPs, we generated strong genetic instruments with F-statistics exceeding 343 for SOCCS and 353 for UKBB respectively (Table S12). In the meta-analysis of these two studies, our statistical power is 80% to detect a minimum HR of 0.81 for CSS and 0.84 for OS.

The Kaplan-Meier survival estimates of CSS and OS by comparing tertiles of PRS are presented in Fig. 3. No significant causal association was observed between 25-OHD and CRC survival among SOCCS, UKBB or combined (Fig. 2 and Table S13). By using PRS, the HRs (95%CI) were 0.98 (0.80, 1.19) and 1.07 (0.91, 1.25) for CSS and OS respectively per unit increase in 25-OHD PRS after performing a meta-analysis of SOCCS and UKBB estimations. In the summary-level MR, similar effect estimates, namely null causal associations, were observed in the IVW model. The HRs (95%CI) were 1.04 (0.85, 1.28) and 1.10 (0.93, 1.31) for CSS and OS respectively per standard deviation increase of the rank-based inverse-normal transformed 25-OHD by adjusting age, sex and stages in SOCCS, and age and sex in UKBB.

**DISCUSSION**

Based on the two prospective cohorts, we observed robust associations between lower circulating 25-OHD and poorer CRC-CSS and -OS. The association was consistent across patients with tumours of various stages and anatomical sites. However, although we created a strengthened genetic instrument to perform both individual- and summary-level MR analyses, the results did not support a causal association between 25-OHD and CRC survival outcomes. We categorised patients using clinical cutouts of 25-OHD and findings were consistent with our previous...
Survival estimates of colorectal cancer-specific and overall survival in the top and bottom tertiles of 25-OHD PRS. Survival estimates of colorectal cancer-specific survival a in SOCCS and c in UKBB, and overall survival b in SOCCS and d in UKBB, in the top (Tertile 3) and bottom (Tertile 1) tertiles of 25-OHD PRS. The HR and the corresponding P value were estimated by comparing the colorectal cancer-specific survival or overall survival difference between patients in tertile 3 and in tertile 1 of 25-OHD PRS, adjusting for age and sex.

Our results indicated the effect of 25-OHD on CRC survival outcomes may be stronger in patients with later stages and those who carry the GG genotype of Cdx-2 VDR polymorphism. Evidence has supported a stronger effect in more advanced stages [3, 34]. In the current study, 25-OHD effects were observed strongly in stage II and stage III, and marginally in stage IV, but not in stage I patients. In addition, stage I patients had the highest median 25-OHD level while stage IV patients had the lowest. It is possible that participants with higher 25-OHD tend to undergo CRC screening more frequently which results in the earlier detection of CRC. VDR is an important factor in the regulation of calcium absorption functioned by the active form of vitamin D (1,25-dihydroxyvitamin D3). In the current study, we have replicated the interacting effect of VDR polymorphism in the enlarged SOCCS cohort while failing to replicate them in UKBB. This could be due to the difference between studies, which merits further investigation into diverse datasets.

25-OHD is the precursor to the steroid hormone calcitriol which is related to numerous mechanisms of oncogenesis [35]. For example, calcitriol is related to the induction of colonic epithelial cell differentiation in colon cancer patients through mechanisms such as the regulation of the β-catenin and transforming growth factor-β [36–38]. Calcitriol exhibits anti-inflammatory effects on tumour cells through pathways such as suppression of prostaglandin action [39] and effects related to tumour growth, invasion and angiogenesis through inhibiting the expression of tenascin-C [40]. However, we did not find a causal association between circulating 25-OHD and CRC survival by conducting MR analyses, even though, the strong genetic instrument has significantly increased the statistical power of this study.

The results of published RCTs were inconclusive. The SUNSHINE trial with 139 CRC patients receiving mFolfox6 and bevacizumab therapy plus high versus standard-dose vitamin D supplement (8000 IU/d vs 400 IU/d), found a non-significant improvement in median progression-free survival for patients with high dosage vitamin D supplement [9]. Another trial (AMATERASU) from Japan did not identify significantly improved relapse-free survival for resected epithelial carcinomas in the digestive tract (200 CRC patients) with supplementation of vitamin D (2000 IU/d) [41]. Our MR analysis added evidence that vitamin D supplementation may lead to limited benefits in terms of survival outcomes for CRC patients. It could also be possible that the lack of evidence from RCTs was due to insufficient study sample size, study duration, and dose of supplementation [42, 43]; the lack of evidence from MR studies could be due to the variance of vitamin D leveraged by genetic instruments was generally low [44]. Our previous MR
analyses targeting CRC risk have challenged the protective effect of 25-OHD against carcinogenesis, which implied the effect on cancer progression could also be small [2, 45]. Given the biological plausibility and limited sample size of our MR study, further efforts are warranted to explore potential small to modest causal effects, and also effects in more targeted sub-populations of CRC patients. Circulating 25-OHD is determined by the joint effect from genetics and environmental factors such as vitamin D supplements intake [46] which could potentially confound the observed association between 25-OHD and survival. However, our analyses were performed based on the measured concentrations of 25-OHD, and we were unable to further dissect this effect due to unavailable data. Consequently, caution is warranted in the interpretation of the results and future research is needed to investigate the role of vitamin D supplementation in this association.

Strengths and limitations
One of the major strengths of the current study is the large sample size of the two prospective studies which increases the statistical power of this study and allows comprehensive subgroup analyses. Another strength of this study is that we tested both observational and casual associations. To test the potential analyses. Another strength of this study is that we tested both statistical power of this study and allows comprehensive subgroup sample size of the two prospective studies which increases the Strengths and limitations between genetic instruments and confounders. Finally, two cohorts of the current study were based in the UK, which could limit the generalisability of our results.

CONCLUSION
In summary, in this large observational and MR study, we found a lower level of circulating 25-OHD is associated with worse CRC-specific and overall survival. The association is retained across different tumour stages and sites. The MR analyses did not support causal associations between circulating 25-OHD and CRC survival. Our findings suggest that vitamin D may serve as a prognostic biomarker, but it is less likely a possible therapeutic target for CRC patients.

DATA AVAILABILITY
The individual-level datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. All summary statistics used in this study can be found in supplementary materials.

REFERENCES
27. Burgess S, Foley CN, Allara E, Staley JR, Howson JMM. A robust and ef-


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AUTHOR CONTRIBUTIONS

MGD and ET conceived this study. XZ and YH designed the methodology and conducted data analysis with support from XL, RS, LZ, LW and PV-S. XZ and YH drafted the manuscript and conducted data interpretation with ET, MT, LZ, MGD, XL and PV-S. YH, MT, XZ, WX, SMF, HC, ET and MGD generated the initial colorectal cancer survival GWAS data. All authors have contributed to the manuscript drafting and revision.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS STATEMENT

Study cohorts used were approved by the ethical review board at respective study centres in accordance with the tenets of the Declaration of Helsinki (details: 02/0/097 [NSCCG], 01/0/5 [SOCCS], 05/S1401/89 [GS&FHS], LREC/1998/4/183 [LBC1921], 2003/2/29 [LBC1936], 17/5C/0079 [CORGI] and 07/S0703/136 [SCOT]). The UK Biobank was approved by the North West Multi-centre Research Ethics Committee (11/NW/0382).

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41416-024-02643-5.

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