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Peer reviewed



Mendelian randomization

## Association of vitamin D levels and risk of ovarian cancer: a Mendelian randomization study

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## Abstract

**Background:** *In vitro* and observational epidemiological studies suggest that vitamin D may play a role in cancer prevention. However, the relationship between vitamin D and ovarian cancer is uncertain, with observational studies generating conflicting findings. A potential limitation of observational studies is inadequate control of confounding. To overcome this problem, we used Mendelian randomization (MR) to evaluate the association between single nucleotide polymorphisms (SNPs) associated with circulating 25-hydroxyvitamin D [25(OH)D] concentration and risk of ovarian cancer.

**Methods:** We employed SNPs with well-established associations with 25(OH)D concentration as instrumental variables for MR: rs7944926 (*DHCR7*), rs12794714 (*CYP2R1*) and rs2282679 (*GC*). We included 31 719 women of European ancestry (10 065 cases, 21 654 controls) from the Ovarian Cancer Association Consortium, who were genotyped using customized Illumina Infinium iSelect (iCOGS) arrays. A two-sample (summary data) MR approach was used and analyses were performed separately for all ovarian cancer (10 065 cases) and for high-grade serous ovarian cancer (4121 cases).

**Results:** The odds ratio for epithelial ovarian cancer risk (10 065 cases) estimated by combining the individual SNP associations using inverse variance weighting was 1.27 (95% confidence interval: 1.06 to 1.51) per 20 nmol/L decrease in 25(OH)D concentration. The estimated odds ratio for high-grade serous epithelial ovarian cancer (4121 cases) was 1.54 (1.19, 2.01).

**Conclusions:** Genetically lowered 25-hydroxyvitamin D concentrations were associated with higher ovarian cancer susceptibility in Europeans. These findings suggest that increasing plasma vitamin D levels may reduce risk of ovarian cancer.

### Key words:

#### Key Messages

- Previous observational studies have reported conflicting findings on the association between serum 25(OH)D concentration and ovarian cancer.
- Results from this study suggest that lower 25(OH)D concentration associates with higher susceptibility to ovarian cancer.
- Among different ovarian cancer subtypes, the magnitude of association was the highest for high-grade serous ovarian cancer.

## Introduction

Ovarian cancer is one of the most fatal cancers among women.<sup>1</sup> Survival following diagnosis is poor (less than 50% at 5 years post-diagnosis) with a mortality rate of 152 000 per year worldwide.<sup>2,3</sup> The most common histological subtype is serous carcinoma (further classified into high-grade serous and low-grade serous); other subtypes include mucinous, clear cell and endometrioid carcinomas.<sup>4</sup> Higher parity and oral contraceptive use reduce risk whereas established risk factors include a history of endometriosis, obesity and family history of ovarian or

breast cancer.<sup>5</sup> Several recent studies have examined whether or not serum 25-hydroxyvitamin D [25(OH)D] concentrations are associated with ovarian cancer risk or mortality.<sup>6–12</sup>

Vitamin D is produced in the skin when 7-dehydrocholesterol is exposed to Ultraviolet B. It is transported to the liver, where it is hydroxylated to become 25(OH)D. It then undergoes a second hydroxylation step, primarily in the liver, to become the active form, 1,25-dihydroxyvitamin D (calcitriol). Whereas 25(OH)D is relatively inactive, it has a long half-life and its production is loosely regulated,

making it a useful indicator of vitamin D status. *In vitro* and animal studies suggest that calcitriol has a variety of anti-cancer effects, including the prevention of cell disjunction,<sup>13–16</sup> preventing overgrowth and exerting multiple anti-proliferative and anti-inflammatory effects.<sup>17</sup>

The association between vitamin D and ovarian cancer is controversial. Most recent observational studies found no strong evidence for an association between circulating 25(OH)D and risk for this cancer.<sup>7,8,10,18–20</sup> One limitation of these studies is that their findings may only be generalized for specific populations because of the latitudes in which they were conducted. Furthermore, the variety of different 25(OH)D measurement techniques as well as the different subtype distribution of ovarian cancers used in the various studies might have also affected the results.<sup>8</sup> More fundamentally, a limitation of observational studies is that confounding and reverse causation can make it difficult to interpret the results. For example, affected individuals may have altered vitamin D levels due to their disease status. Randomized clinical trials (RCT) are an attractive alternative to observational studies, as these remove biases from confounding and reverse causation. However, RCTs are costly and logistically cumbersome, and there are no published RCTs assessing the relationship between 25(OH)D levels and risk of epithelial ovarian cancer.

Mendelian randomization (MR) is an approach for evaluating associations of an exposure with a disease.<sup>21,22</sup> This technique utilizes the fact that allelic variants are assigned at random during meiosis, making them potentially robust and unbiased (free from confounding effects) instruments to gauge the effect of an exposure (e.g. low vitamin D) on a trait (e.g. cancer).<sup>22</sup> An instrumental variable (SNP) used in a MR study also has to satisfy the following assumptions<sup>21,22</sup>: (i) the instrumental variable is associated with the exposure of interest, (ii) the instrumental variable is independent of confounding factors that might confound the association of the exposure with the outcome and (iii) the instrumental variable is only associated with the outcome through the exposure (Figure 1). Two key determin-

ants of the power of an MR study are the variance in the modifiable exposure explained by the genetic variants (SNPs) and the sample size of the study associating the relevant SNPs with the trait of interest. To date, SNPs associated with vitamin D level explain only a very small proportion (approximately 1–4%) of the trait variance. Therefore, for MR to be informative for vitamin D concentrations, large sample sizes are needed. Here we use large-scale data from the Ovarian Cancer Association Consortium (OCAC) in an MR framework to assess whether or not SNPs associated with 25(OH)D concentration are related to risk of ovarian cancer.

## Methods

### Data sources

Individual-level genetic data from the OCAC were used in this study. Participants from 43 studies from around the world were genotyped using the Illumina Infinium iSelect (iCOGS) array.<sup>23</sup> Quality control was as per previous work, with related individuals and ancestry outliers removed.<sup>4</sup> We excluded 13 studies of individuals of non-European ancestry<sup>4</sup>; the remaining studies that contributed to our analysis are listed in Supplementary Table 4 (available as Supplementary data at *IJE* online). For examination of all histotypes of ovarian cancer combined, we had 10 065 cases and 21 654 controls for analysis. The distribution of histological subtypes is shown in Table 1. For high-grade serous ovarian cancer, 4121 cases were available. We also performed MR analysis on the other subtypes individually, although sample sizes were much smaller than for high-grade serous cancer.

### SNP selection criteria

Several SNPs have been observed in association with 25(OH)D concentrations: rs6013897 in the *Cytochrome P450, family 24, subfamily A, polypeptide 1* (CYP24A1) gene; rs2282679 and rs7041 in the *Group-Specific*

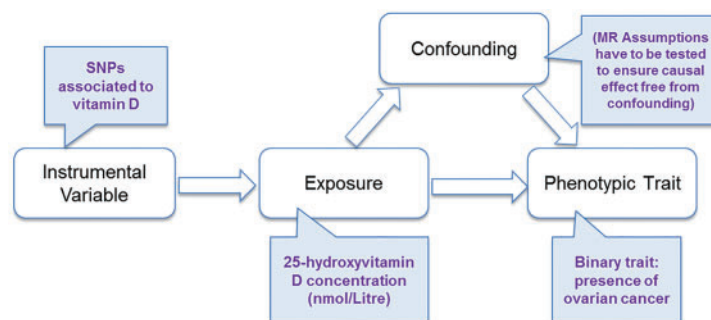


Figure 1. Schematic of the Mendelian randomization framework in our study using vitamin D SNPs as instrumental variables.

**Table 1.** Distribution of cases based on epithelial ovarian carcinoma subtypes

EOC subtypes	Number of cases
High-grade serous	4121
Low-grade serous	363
Serous of unknown grade	1344
Mucinous	662
Clear cell	621
Endometrioid	1350
Others	1604

*Component (GC) gene*; rs12800438 and rs7944926 near the *7-Dehydrocholesterol Reductase (DHCR7)* gene; and rs10741657 and rs12794714 in the *Cytochrome P450, family 2, subfamily R, polypeptide 1 (CYP2R1)* gene.<sup>24–30</sup> The iCOGs array directly genotyped rs12794714 and rs2282679; rs7944926 was the best imputed DHCR7 SNPs (imputation quality score of 0.92) described by previous study.<sup>31</sup> We were unable to include rs6013897 in CYP24A1, as there were no SNPs in adequate linkage disequilibrium (>0.3) genotyped on our arrays. These SNPs are potential instrumental variables with respect to 25(OH)D concentrations. To ensure that these SNPs instruments can be applied to the MR via summary statistics approach, we first required accurate 25(OH)D association estimates for each of the SNP—the most accurate estimates available were those from Afzal *et al.*<sup>31</sup> for the SNPs within/near DHCR7 and CYP2R1, whereas the estimates for the GC SNP is only available in Mokry *et al.*<sup>26</sup> [the effect of the GC SNP on 25(OH)D was only estimated based on 2347 individuals<sup>26</sup> whereas the estimates for DHCR7 and CYP2R1 were derived based on 30 792 individuals<sup>31</sup>]. We then examined their associations with various potential confounders using publicly available GWAS datasets (the complete list of potential confounders that were investigated is available in Supplementary Table 1, available as Supplementary data at *IJE* online).

### Statistical analyses

MR operates by comparing the estimated magnitude of the association of the SNPs on the modifiable risk factor [25(OH)D concentration] with the magnitude of the association of the SNP on the outcome of interest (ovarian cancer). Estimates of the association of the relevant SNPs with ovarian cancer status were derived using logistic regressions using SNPTEST.<sup>32</sup> We adjusted for intra-ethnic (i.e. within Europeans) population differences by incorporating the first six principal components and indicators for study number as covariates in the SNP-outcome regressions. To check for evidence of residual population stratification, we

computed the genomic control lambda value from 195 183 directly genotyped autosomal SNPs genome-wide. Additional confounding variables such as time spent outdoors, socio-economic status and body mass index (BMI) were not adjusted in our model, as this information was not available on all individuals in our dataset. Instead, samples with available confounder data ( $n < 26\ 000$ ) were retained for subsequent sensitivity analysis (see the 'Discussion' section).

In the absence of information on 25(OH)D concentration levels in the OCAC dataset, we applied a two-sample approach that uses only summary data to assess indirect associations<sup>33</sup> where estimates for the SNP-outcome associations are from a different sample than the SNP-exposure associations. Here, we obtain 25(OH)D association estimates from GWAS summary statistics for SNP instruments that passed the selection criteria mentioned above. Combining these magnitudes of association, the association of 25(OH)D concentration levels on ovarian cancer, the weighted estimate can be computed using the Wald-type ratio estimator.<sup>21</sup> The weighted model that was used to obtain the instrumental variable estimates are shown in the Supplementary section (available as Supplementary data at *IJE* online). Analyses were performed for all epithelial ovarian cancers irrespective of histological subtype and separately for high-grade serous epithelial ovarian cancer. To be compatible with previous studies,<sup>31,34</sup> estimates were scaled to a 20-nmol/L change in 25(OH)D level; 20 nmol/L is approximately the inter-tertile range (66th percentile to 33rd percentile) observed in a large European study.<sup>31</sup>

## Results

### Validation of instrument strength

We examined each of the MR assumptions in turn. To satisfy the first MR assumption, our SNPs must be clearly associated with 25(OH)D concentrations; typically, an F-statistic > 10 is a commonly used threshold for a strong instrument. We specifically chose SNPs from DHCR7, CYP2R1 and GC, which have been clearly shown to be associated with 25(OH)D concentrations. In Afzal *et al.*,<sup>31</sup> the SNPs we use are very strongly associated where the F-statistics for each SNP is >90. For the GC SNP, the association of this variant with log-transformed 25(OH)D were adequate with an F-statistic of 13.38. The SNPs combined explain about 1.3% of the variance in 25(OH)D concentration. It is important to note that these studies were among few of the many studies linking these SNPs to 25(OH)D concentrations.<sup>24,26,28,29,34</sup> This evidence

combined suggests that the SNPs we used are valid instruments (i.e. weak instrument bias is not a problem in our study).

### Assessment for pleiotropy

Next we assessed possible pleiotropy. Of the known ovarian cancer risk factors, some have an established genetic component, with large GWASs conducted. Examining these GWAS findings, we found no evidence for association between the SNPs in *DHCR7* and *CYP2R1* and potential confounders such as smoking behaviour (Supplementary Table 1), hence satisfying the second MR assumption. We found that neither the lead SNPs nor any SNPs correlated with them were associated with the possible confounders after Bonferroni corrections. For the other ovarian cancer risk factors (OC use, parity), large-scale GWASs have not been conducted because inherited genetic factors are unlikely to play a major role. The third MR assumption can be difficult to test directly although the vitamin D metabolism pathway is well understood and there is substantial evidence that *DHCR7* and *CYP2R1* play roles in determining or modulating 25(OH)D concentration.<sup>24,25,34</sup>

### Population stratification

MR analyses are unbiased when they reflect the true relationship between genotype and phenotype (rather than e.g. artefactual associations from unmodelled population structure). Our estimated genomic control lambda value (rescaled to 1000 cases and controls) was  $\lambda_{1000} = 1.005$ , implying no major effects of population structure. Principal component analysis showed that the OCAC cases and controls were well matched for ancestry

(Supplementary Figures 2 and 3, available as Supplementary data at *IJE* online).

### Association of SNPs to 25(OH)D concentration

To estimate the association of the chosen SNPs on 25(OH)D concentrations, we used SNP-25(OH)D association estimates from both published studies<sup>26,31</sup> that were corrected for seasonal variation. It was shown that the variant rs7944926 near *DHCR7* reduced 25(OH)D concentration levels by 2.0 nmol/L per risk allele (A) and the variant rs12794714 in *CYP2R1* reduced 25(OH)D concentration levels by 3.0 nmol/L per risk allele (A). Upon performing conversion of the 25(OH)D estimates from the natural logarithm scale,<sup>26</sup> the variant rs2282679 near *GC* was shown to reduce 25(OH)D levels by approximately 2.5 nmol/L per 25(OH)D decreasing allele (C).

### MR analysis for all ovarian cancer subtypes

We determined the associations between the 25(OH)D associated SNPs (rs7944926 and rs12794714) and risk of ovarian cancer in Table 2. rs12794714 and rs2282679 was directly genotyped in our dataset, whereas rs7944926 was well imputed (imputation quality score 0.92). For all epithelial ovarian cancer subtypes combined, the estimated magnitude of association for a 1.0-nmol/L change in 25(OH)D level was  $-0.0076$  [standard error (SE) = 0.0109] for the MR analysis performed via rs7944926 in *DHCR7*. This translates into an odds ratio (OR) of 1.17 (0.76–1.78) per 20-nmol/L decrease in 25(OH)D levels. Similarly, the magnitude of association was  $-0.0137$ , SE = 0.0063 for rs12794714 in *CYP2R1*, with corresponding OR of 1.31 (1.03–1.69) per 20-nmol/L decrease in 25(OH)D and the magnitude of association is  $-0.0110$ , SE = 0.0082 with OR of 1.25(0.90–1.71) for

**Table 2.** Mendelian randomization results: 25(OH)D concentration and ovarian cancer

SNPs	EA/NEA	25(OH)D per 25(OH)D decreasing allele (nmol/L)			All epithelial ovarian subtype ( $n = 10\,065$ cases)				Only high-grade serous epithelial ovarian subtype ( $n = 4\,121$ cases)			
		$\beta_{zx}$	$\sigma_{zx}$	$R^2$	$\beta_{zy}$	$\sigma_{zy}$	$\beta_{IVW}$	$\sigma_{IVW}$	$\beta_{zy}$	$\sigma_{zy}$	$\beta_{IVW}$	$\sigma_{IVW}$
rs7944926	A/G	-2	0.19	0.40%	0.0153	0.0217	-0.0076	0.0109	0.0418	0.0309	-0.0209	0.0154
rs12794714	A/G	-3	0.22	0.60%	0.0412	0.0189	-0.0137	0.0063	0.0772	0.0270	-0.0257	0.0091
rs2282679	C/A	-2.5	0.70	0.30%	0.0276	0.0205	-0.0110	0.0082	0.0432	0.0292	-0.0173	0.0117
Combined	-	-	-	1.30%	-	-	-0.0118	0.0045	-	-	-0.0218	0.0067

EA/NEA refers to the Effect Allele and Non-Effect Allele.  $\beta_{zy}$  denotes the magnitude of association of the SNP-outcome estimate.  $\sigma_{zx}$  is the standard error of the SNP-exposure estimate.  $\beta_{zx}$  denotes the magnitude of association of Z, the SNP instrument on X, the modifiable exposure level (25(OH)D).  $\sigma_{zy}$  is the standard error of  $\beta_{zy}$ .  $R^2$  is the proportion of variance in 25(OH)D explained by the SNP(s).  $\beta_{IVW}$  is the estimate and  $\sigma_{IVW}$  its standard deviation.  $\beta_{zy}$  is presented on the log(OR) scale.  $\beta_{IVW}$  is presented on the log(OR) scale for a single unit (1-nmol/L) change in 25(OH)D—see text for OR scale changes for a 20-unit (nmol/L) change in 25(OH)D. Note: the  $\beta_{zx}$  estimate for rs2282679 is obtained from Mokry *et al.* and transformed to natural scale (from natural logarithm) using an intercept at  $e^4$  (~54.59) nmol/L of 25(OH)D. Standard errors for these estimates were calculated from F-statistics. The variance explained ( $R^2$ ) for rs12794714 and rs7944926 were obtained directly from Afzal *et al.*, whereas the  $R^2$  for rs2282679 was computed from Mokry *et al.*



rs2282679 in GC. Since all these SNPs are independent, a more accurate estimate will be obtained from the combined associations of the three SNPs. The combined weighted magnitude of association is  $-0.0118$ , with a SE of  $0.0045$ . The resultant OR per 20-nmol/L change in 25(OH)D on all epithelial ovarian cancer subtypes combined is  $1.27$  ( $1.06$ – $1.51$ ).

### MR analysis for high-grade serous ovarian cancer

Similar associations were observed between SNPs for 25(OH)D concentration and high-grade serous epithelial ovarian cancer. We obtained a magnitude of association estimate of  $-0.0209$  ( $SE = 0.0154$ ) and  $-0.0257$  ( $SE = 0.0091$ ) and  $-0.0173$  ( $SE = 0.0117$ ) for rs7944926, rs12794714 and rs2282679, respectively. This resulted in an OR of  $1.51$  ( $0.83$ – $2.78$ ) using rs7944926,  $1.67$  ( $1.18$ – $2.38$ ) using rs12794714 and  $1.41$  ( $0.89$ – $2.23$ ) per 20-nmol/L decrease in 25(OH)D. Weighting across all SNP instruments yielded an estimated magnitude of  $-0.0218$  ( $SE = 0.0067$ ). Hence, a 20-nmol/L decrease in 25(OH)D corresponds to an OR of  $1.54$  ( $1.19$ – $2.01$ ) for high-grade serous ovarian cancer (see Figures 2 and 3).

### Discussion

Even though the SNPs chosen in our study only explain a small fraction ( $\sim 1.3\%$ ) of the variance of 25(OH)D concentration, because our case–control sample was so large, we were able to demonstrate associations with ovarian cancer risk. A genetically scored decrease of 20 nmol/L of serum 25(OH)D concentration levels increased the risk of epithelial ovarian cancer by about 30% in European-ancestry women, with a larger association seen in high-grade serous disease.

### Comparison with previous findings

A recent Danish study<sup>31</sup> used MR to show that low circulating 25(OH)D concentrations were associated with cancer mortality among Europeans. That study did not separate the associations of risk and mortality and was underpowered to draw conclusions on any specific cancer type. Here, for the first time, we demonstrate that, for epithelial ovarian cancer, there is a causal effect of low 25(OH)D concentrations on risk.

Our results are inconsistent with some previous studies that have reported no associations between 25(OH)D and ovarian cancer status. The recent meta-analysis<sup>8</sup> of 10 individual cohort studies (884 cases and 1605 controls) found no association between 25(OH)D concentration and development of ovarian cancer. Findings from epidemiologic

studies may differ from our MR-based results because observational studies can be affected by confounding and reverse causation, though cohort studies such as Yin *et al.*<sup>8</sup> would be expected to be less affected.

### Strength and limitations

A strength of our study is that the mechanism through which our chosen SNPs influence 25(OH)D levels is well understood. *DHCR7* encodes the enzyme 7-dehydrocholesterol reductase, which is responsible for the conversion of 7-dehydrocholesterol to cholesterol. Reduced activities of 7-dehydrocholesterol reductase, leading to low cholesterol and accumulation of 7-dehydrocholesterol, are partially attributable to *DHCR7* variants.<sup>24,25,29</sup> Although rs7944926 lies outside *DHCR7*, this variant modulates expression of *DHCR7*.<sup>35</sup> *CYP2R1* is an enzyme which converts vitamin D<sub>3</sub> to 25(OH)D in the liver,<sup>36</sup> with rs12794714 unambiguously associated with 25(OH)D concentrations via GWAS.<sup>29</sup> The GC gene has a primary role in vitamin D transport. Previous studies shown that the rs2282679 variant in particular were also strongly associated ( $P = 4.0 \times 10^{42}$ ) with serum vitamin D binding protein (DBP) based on the study performed on 1674 individuals in the Twins UK cohort.<sup>29</sup> The GC variants were also hypothesized to affect bioavailability of vitamin D through variation in circulating DBP. In view of evidence for its association towards vitamin D, the rs2282679 SNP is among one of the most associated variants with 25(OH)D ( $P = 1.9 \times 10^{-109}$ ) in the SUNLIGHT GWAS.<sup>29</sup> These variants (rs7944926, rs12794714 and rs2282679) thus affect 25(OH)D levels through varying vitamin D metabolism, bioavailability or transport, rendering them appropriate instrumental variables for use in MR.<sup>26,27,31,34</sup>

One limitation is that our two-sample MR analysis assumes that the standard error of the exposure [SNP to 25(OH)D] estimates is negligibly small.<sup>33,37</sup>—given the large sample size in the Danish study,<sup>31</sup> this is a reasonable assumption. In addition, the MR framework assumes a linear relationship in the association of the SNP instruments on the underlying exposure. Although our MR estimates indicate that a decrease of 20 nmol/L in 25(OH)D concentration is associated with a 30% increased risk of epithelial ovarian cancer, this estimated effect size is derived from a larger sample size of women with a range of 25(OH)D concentrations. Previous studies using MR to examine 25(OH)D concentrations with different outcomes have dealt with this in various ways. For example, the published study that we used<sup>31</sup> assumed linearity of change across raw 25(OH)D values. In contrast, the study by Mokry *et al.*<sup>26</sup> on vitamin D and multiple sclerosis (MS)

**Causal OR for 20nmol/Liter change in 25(OH)D on risk of all ovarian cancer and high grade serous subtype**

All subtypes (N=10 065)	EA/NEA	OR (95% CI.)
rs12794714	A/G	1.31(1.03,1.69)
rs7944926	A/G	1.17(0.76,1.78)
rs2282679	C/A	1.25(0.90,1.71)
<b>Combined</b>	<b>-</b>	<b>1.27(1.06,1.51)</b>

HG serous (N=4 121)	EA/NEA	OR (95% CI.)
rs12794714	A/G	1.67(1.18,2.38)
rs7944926	A/G	1.51(0.83,2.78)
rs2282679	C/A	1.41(0.89,2.23)
<b>Combined</b>	<b>-</b>	<b>1.54(1.19,2.01)</b>

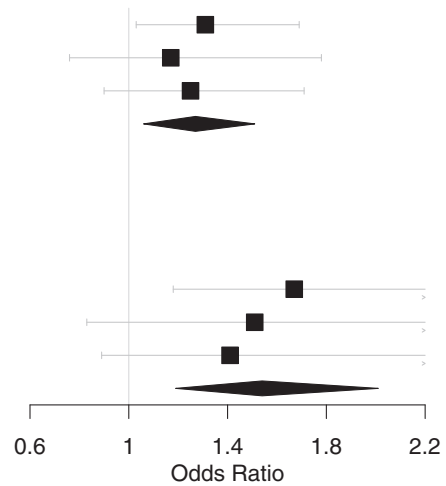


Figure 2. Causal OR of 25(OH)D on all ovarian cancer and high grade serous ovarian cancer.

**Causal OR for 20nmol/Liter change in 25(OH)D towards risk of ovarian cancer by subtypes**

Subtypes	Cases	OR (95% CI.)
Mucinous	662	1.00(0.70, 1.43)
Clear cell	621	1.27(0.72, 2.24)
Endometrioid	1 350	1.20(0.81, 1.78)
Serous	5 828	1.21(0.84, 1.76)
Others	1 604	1.10(0.76, 1.60)
<b>All cases</b>	<b>10 065</b>	<b>1.27(1.06, 1.51)</b>

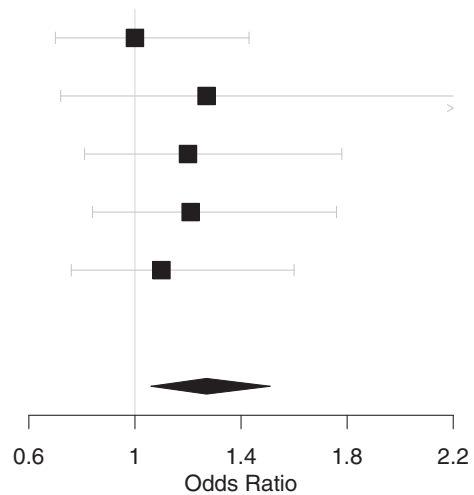


Figure 3. Causal OR of 25(OH)D on individual ovarian cancer subtypes.

considered the association to be linear on log-transformed 25(OH)D.

We examined the implications of these approaches by re-computing our findings based on exposure estimates on

the original scale (from the Danish study<sup>31</sup>) and on the log scale (from MR study on MS<sup>26</sup>) (see Supplementary Table 2, available as Supplementary data at *IJE* online). We note that, in addition to the scale differences, the estimates of

the magnitude of association of each SNP on 25(OH)D differed due to random sampling error (with estimates from the Danish study<sup>31</sup> derived from a much larger sample size than those in the MS study<sup>26</sup>). We hence repeated our analysis by adopting SNP-exposure estimates used by the MS study<sup>26</sup> for the SNP rs12785878 (LD to rs7944926 with  $r^2 = 1.0$ ) in the *DHCR7* gene. Although our result was robust to differences in scaling [log-transformed or non-transformed 25(OH)D concentrations, see Supplementary Table 2, available as Supplementary data at *IJE* online], in practice, a 20-nmol/L increase is more likely to make an impact on women with low 25(OH)D concentrations than those whose concentration is already high.

In our main analysis, there were concerns that the effect of the GC SNP on 25(OH)D was not estimated with high accuracy (GC SNP estimates were based on 2347 individuals<sup>26</sup> whereas the estimates for *DHCR7* and *CYP2R1* were derived based on 30 792 individuals<sup>31</sup>), as well as concerns that the GC SNP may not influence 25-hydroxyvitamin D's biological activity in a predictable way.<sup>31,38,39</sup> Nonetheless, we conducted a sensitivity analyses to examine the effect of excluding this SNP. When the GC SNP was excluded, our results were unchanged (the association with ovarian cancer of the combined effect of the three SNPs was very similar to that obtained using just two SNPs; see Supplementary Table 5, available as Supplementary data at *IJE* online).

Another potential limitation of our analysis is residual pleiotropy. We found no evidence for SNP–confounder association based on the subset of participants with available confounder information (Supplementary Table 6, available as Supplementary data at *IJE* online), although we cannot rule out associations with unmeasured confounders. An approach such as Egger regression<sup>40</sup> can potentially be applied to further test the MR assumptions but these require more SNPs than the three employed here.

### Interpretation of findings

Observation of a larger magnitude of association (OR = 1.54) with high-grade serous cancer for lower 25(OH)D concentration suggests that the association of circulating 25(OH)D with risk of ovarian cancer may be confined to the high-grade serous type, although the confidence limits of the two ORs are overlapping and high-grade serous cancer is contained within all ovarian cancer. The results for histological subtypes other than high-grade serous carcinoma are shown in Figure 3 (for association of each individual SNP, see Supplementary Table 3, available as Supplementary data at *IJE* online) and there is no evidence for association for non-serous disease. For all non-

high-grade serous cancers combined, the OR was 1.12 (0.89–1.41).

The association of lower circulating vitamin D [25(OH)D] levels to risk of epithelial ovarian cancer appear to be consistent with a recent MR study<sup>31</sup> looking at all-cancer mortality. Vitamin D activating enzymes and vitamin D receptors are present in many tissues, with the regulation of 1–3% of gene expression in these tissues attributable to vitamin D.<sup>35</sup> Studies have also shown that vitamin D is involved in the regulation of cell processes (proliferation, differentiation and apoptosis) in several cell types that are central to the development of cancer.<sup>14,41–43</sup> Thus, our findings warrant further investigations on the biological role of vitamin D [specifically 25(OH)D] in mortality as well as risk of ovarian cancer.

In conclusion, we demonstrate an association between low 25(OH)D concentration and risk of ovarian cancer in women of European ancestry, with our MR approach providing estimates which are unaffected by the confounding or biases present in observational studies. Whilst our results cannot guarantee causality, placed in the context of other epidemiological studies, they provide additional evidence supportive of a causal link between vitamin D and risk of ovarian cancer.

### Supplementary Data

Supplementary Data are available at *IJE* online.

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