

1 Association Of Vitamin D Levels And Risk Of Ovarian Cancer:

2 A Mendelian Randomization Study

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3 186 **Abstract**
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7 187 **Background**
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10 188 *In vitro* and observational epidemiological studies suggest that vitamin D may play a role in
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12 189 cancer prevention. However, the relationship between vitamin D and ovarian cancer is
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14 190 uncertain, with observational studies generating conflicting findings. A potential limitation
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16 191 of observational studies is inadequate control of confounding. To overcome this problem,
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18 192 we used Mendelian randomization (MR) to evaluate the association between single
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20 193 nucleotide polymorphisms (SNPs) associated with circulating 25-hydroxyvitamin D
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22 194 (25(OH)D) concentration and risk of ovarian cancer.
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30 195 **Methods**
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33 196 We employed SNPs with well-established associations with 25(OH)D concentration as
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35 197 instrumental variables for MR: rs7944926 (*DHCR7*), rs12794714 (*CYP2R1*) and rs2282679
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37 198 (*GC*). We included 31 719 women of European ancestry (10 065 cases, 21 654 controls) from
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39 199 the Ovarian Cancer Association Consortium, who were genotyped using customized Illumina
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41 200 Infinium iSelect (iCOGS) arrays. A two-sample (summary data) Mendelian randomization
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43 201 approach was used, and analyses were performed separately for all ovarian cancer (10 065
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45 202 cases) and for high-grade serous ovarian cancer (4 121 cases).
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54 203 **Results**
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3 204 The odds ratio for epithelial ovarian cancer risk (10 065 cases) estimated by combining the
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6 205 individual SNP associations using inverse variance weighting was 1.27 (95% confidence
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9 206 interval: 1.06 to 1.51) per 20nmol/L decrease in 25(OH)D concentration. The estimated odds
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12 207 ratio for high-grade serous epithelial ovarian cancer (4 121 cases) was 1.54 (1.19, 2.01).

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15 208 **Conclusions**

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18 209 Genetically lowered 25-hydroxyvitamin D concentrations were associated with higher
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21 210 ovarian cancer susceptibility in Europeans. These findings suggest that increasing plasma
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24 211 vitamin D levels may reduce risk of ovarian cancer.

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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.

213 Introduction

214 Ovarian cancer is one of the most fatal cancers among women [1]. Survival following
215 diagnosis is poor (less than 50% at 5 years post-diagnosis) with a mortality rate of 152 000
216 per year worldwide [2, 3]. The most common histological subtype is serous carcinoma
217 (further classified into high grade serous and low grade serous); other subtypes include
218 mucinous, clear cell and endometrioid carcinomas [4]. Higher parity and oral contraceptive
219 use reduce risk while established risk factors include a history of endometriosis, obesity and
220 family history of ovarian or breast cancer [5]. Several recent studies have examined whether
221 or not serum 25-hydroxyvitamin D (25(OH)D) concentrations are associated with ovarian
222 cancer risk or mortality [6-12].

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224 Vitamin D is produced in the skin when 7-dehydrocholesterol is exposed to UVB. It is
225 transported to the liver where it is hydroxylated to become 25(OH)D. It then undergoes a
226 second hydroxylation step, primarily in the liver, to become the active form, 1,25-
227 dihydroxyvitaminD (calcitriol). While 25(OH)D is relatively inactive, it has a long half-life and
228 its production is loosely regulated, making it a useful indicator of vitamin D status. *In vitro*
229 and animal studies suggest that calcitriol has a variety of anti-cancer effects, including the
230 prevention of cell disjunction [13-16], preventing overgrowth and exerting multiple anti-
231 proliferative and anti-inflammatory effects [17].

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233 The association between vitamin D and ovarian cancer is controversial. Most recent
234 observational studies found no strong evidence for an association between circulating
235 25(OH)D and risk for this cancer [7, 8, 10, 18-20]. One limitation of these studies is that their
236 findings may only be generalized for specific populations because of the latitudes in which
237 they were conducted. Furthermore, the variety of different 25(OH)D measurement
238 techniques as well as the different subtype distribution of ovarian cancers used in the
239 various studies might have also affected the results [8]. More fundamentally, a limitation of
240 observational studies is that confounding and reverse causation can make it difficult to
241 interpret the results. For example, affected individuals may have altered vitamin D levels
242 due to their disease status. Randomized clinical trials (RCT) are an attractive alternative to
243 observational studies as these remove biases from confounding and reverse causation.
244 However, RCTs are costly and logistically cumbersome, and there are no published RCTs
245 assessing the relationship between 25(OH)D levels and risk of epithelial ovarian cancer.

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247 Mendelian randomization (MR) is an approach for evaluating associations of an
248 exposure with a disease [21, 22]. This technique utilises the fact that allelic variants are
249 assigned at random during meiosis, making them potentially robust and unbiased (free from
250 confounding effects) instruments to gauge the effect of an exposure (e.g., low vitamin D) on

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3 251 a trait (e.g., cancer) [22]. An instrumental variable (SNP) used in a MR study also has to
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6 252 satisfy the following assumptions [21, 22]: 1) the instrumental variable is associated with
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9 253 the exposure of interest; 2) the instrumental variable is independent of confounding factors
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12 254 that might confound the association of the exposure with the outcome; and 3) the
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15 255 instrumental variable is only associated with the outcome through the exposure (Fig 1). Two
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18 256 key determinants of the power of an MR study are the variance in the modifiable exposure
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21 257 explained by the genetic variants (SNPs) and the sample size of the study associating the
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24 258 relevant SNPs with the trait of interest. To date, SNPs associated with vitamin D level
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27 259 explain only a very small proportion (approximately 1-4%) of the trait variance. Therefore,
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30 260 for MR to be informative for vitamin D concentrations, large sample sizes are needed. Here
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33 261 we use large-scale data from the Ovarian Cancer Association Consortium (OCAC) in an MR
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36 262 framework to assess whether or not SNPs associated with 25(OH)D concentration are
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42 264 (Fig 1 here: title - Schematic of the Mendelian randomization framework in our study using
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45 265 vitamin D SNPs as instrumental variables.)
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3 267 **Methods**
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8 268 **Data sources**
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10 269 Individual level genetic data from the Ovarian Cancer Association Consortium (OCAC) were
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12 270 used in this study. Participants from 43 studies from around the world were genotyped
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15 271 using the Illumina Infinium iSelect (iCOGS) array [23]. Quality control was as per previous
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17 272 work, with related individuals and ancestry outliers removed [4]. We excluded 13 studies of
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19 273 individuals of non-European ancestry [4], the remaining studies that contributed to our
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21 274 analysis were listed in Supplementary Table 4. For examination of all histotypes of ovarian
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23 275 cancer combined, we had 10 065 cases and 21 654 controls for analysis. The distribution of
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25 276 histological subtypes is shown in Table 1. For high-grade serous ovarian cancer, 4 121 cases
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27 277 were available. We also performed MR analysis on the other subtypes individually, although
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29 278 sample sizes were much smaller than for high grade serous cancer.
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46 281 **SNP selection criteria**
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51 283 the *Cytochrome P450, family 24, subfamily A, polypeptide 1* (CYP24A1) gene; rs2282679 and
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53 284 rs7041 in the *Group-Specific Component* (GC) gene ; rs12800438 and rs7944926 near the 7-
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3 285 *Dehydrocholesterol Reductase (DHCR7)* gene; and rs10741657 and rs12794714 in the
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6 286 *Cytochrome P450, family 2, subfamily R, polypeptide 1 (CYP2R1)* gene [24-30]. The iCOGs
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9 287 array directly genotyped rs12794714 and rs2282679; rs7944926 was the best imputed
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12 288 DHCR7 SNPs (imputation quality score of 0.92) described by previous study [31]. We were
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15 289 unable to include rs6013897 in CYP24A1 as there were no SNPs in adequate linkage
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18 290 disequilibrium ($r^2 > 0.3$) genotyped on our arrays. These SNPs are potential instrumental
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21 291 variables with respect to 25(OH)D concentrations. To ensure that these SNPs instruments
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24 292 can be applied to the MR via summary statistics approach, we first required accurate
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27 293 25(OH)D association estimates for each of the SNP – the most accurate estimates available
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30 294 were those from Afzal et al. [31] for the SNPs within/near DHCR7 and CYP2R1, whereas the
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33 295 estimates for the GC SNP is only available in Mokry et al. [26]. (the effect of the GC SNP on
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36 296 25(OH)D was only estimated based on 2 347 individuals [26] whereas the estimates for
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39 297 DHCR7 and CYP2R1 were derived based on 30 792 individuals [31]). We then examined their
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42 298 associations with various potential confounders using publicly available GWAS datasets (The
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45 299 complete list of potential confounders that were investigated is available in Supplementary
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48 300 Table 1).

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3 302 **Statistical analyses**
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6 303 MR operates by comparing the estimated magnitude of the association of the SNPs on the
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9 304 modifiable risk factor (25(OH)D concentration) with the magnitude of the association of the
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12 305 SNP on the outcome of interest (ovarian cancer). Estimates of the association of the
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15 306 relevant SNPs with ovarian cancer status were derived using logistic regressions using
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18 307 SNPTTEST [32]. We adjusted for intra-ethnic (i.e. within Europeans) population differences by
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21 308 incorporating the first six principal components and indicators for study number as
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24 309 covariates in the SNP-outcome regressions. To check for evidence of residual population
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27 310 stratification, we computed the genomic control lambda value from 195,183 directly
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30 311 genotyped autosomal SNPs genome-wide. Additional confounding variables such as time
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33 312 spent outdoors, socio-economic status and BMI were not adjusted in our model as these
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36 313 information were not available on all individuals in our dataset. Instead, samples with
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39 314 available confounder data ($n < 26\ 000$) were retained for subsequent sensitivity analysis
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42 315 (See Discussion).
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48 317 In the absence of information on 25(OH)D concentration levels in the OCAC dataset,
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51 318 we applied a two-sample approach that uses only summary data to assess indirect
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54 319 associations [33] where estimates for the SNP-outcome associations are from a different
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57 320 sample than the SNP-exposure associations. Here we obtain 25(OH)D association estimates
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3 321 from GWAS summary statistics for SNP instruments that passed the selection criteria
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6 322 mentioned above. Combining these magnitudes of association, the association of 25(OH)D
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9 323 concentration levels on ovarian cancer, the weighted estimate can be computed using the
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12 324 Wald-type ratio estimator [21]. The weighted model that was used to obtain the
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15 325 instrumental variable estimates are shown in the supplementary section. Analyses were
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18 326 performed for all epithelial ovarian cancers irrespective of histological subtype and
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21 327 separately for high-grade serous epithelial ovarian cancer. To be compatible with previous
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24 328 studies [31, 34], estimates were scaled to a 20nmol/Liter change in 25(OH)D level;
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27 329 20nmol/Liter is approximately the inter-tertile range (66th percentile to 33rd percentile)
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30 330 observed in a large European study [31].
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332 **Results**

333 **Validation of instrument strength**

334 We examined each of the MR assumptions in turn. To satisfy the 1st MR assumption our
335 SNPs must be clearly associated with 25(OH)D concentrations; typically an F-statistic >10 is a
336 commonly used threshold for a strong instrument. We specifically chose SNPs from DHCR7,
337 CYP2R1 and GC which have been clearly shown to be associated with 25(OH)D
338 concentrations. In Afzal et al. [31], the SNPs we use are very strongly associated where the F

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3 339 statistics for each SNP is >90. For the GC SNP, the association of this variant with log-
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6 340 transformed 25(OH)D were adequate with a F-statistic of 13.38. The SNPs combined explain
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9 341 about 1.3% of the variance in 25(OH)D concentration. It is important to note that these
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12 342 studies were among few of the many studies linking these SNPs to 25(OH)D concentrations
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15 343 [24, 26, 28, 29, 34]. This evidence combined suggests that the SNPs we used are valid
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18 344 instruments (i.e. weak instrument bias is not a problem in our study).
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22 23 24 346 **Assessment for pleiotropy**

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27 347 Next we assessed possible pleiotropy. Of the known ovarian cancer risk factors, some have
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30 348 an established genetic component, with large GWASs conducted. Examining these GWAS
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33 349 findings, we found no evidence for association between the SNPs in *DHCR7* and *CYP2R1* and
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36 350 potential confounders such as smoking behaviour (Supplementary Table 1), hence satisfying
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39 351 the 2nd MR assumption. We found that neither the lead SNPs, nor any SNPs correlated with
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42 352 them, were associated with the possible confounders after Bonferroni corrections. For the
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45 353 other ovarian cancer risk factors (OC use, parity), large scale GWASs have not been
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48 354 conducted because inherited genetic factors are unlikely to play a major role. The 3rd MR
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51 355 assumption can be difficult to test directly although the vitamin D metabolism pathway is
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54 356 well understood and there is substantial evidence that *DHCR7* and *CYP2R1* play roles in
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57 357 determining or modulating 25(OH)D concentration [24, 25, 34].
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67 **Population stratification**
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10 360 MR analyses are unbiased when they reflect the true relationship between genotype and
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12 361 phenotype (rather than for example artifactual associations from unmodeled population
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14 362 structure). Our estimated genomic control lambda value (rescaled to 1 000 cases and
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16 363 controls) was $\lambda_{1000} = 1.005$, implying no major effects of population structure. Principal
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18 364 component analysis showed that the OCAC cases and controls were well matched for
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24 365 ancestry (Supplementary Figure 2 and 3 in Supplementary material).
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31 **Association of SNPs to 25(OH)D concentration**
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34 368 To estimate the association of the chosen SNPs on 25(OH)D concentrations, we used SNP-
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36 369 25(OH)D association estimates from both published study [26, 31] that were corrected for
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38 370 seasonal variation. It was shown that the variant rs7944926 near DHCR7 reduced 25(OH)D
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40 371 concentration levels by 2.0 nmol/Liter per risk allele (A) and the variant rs12794714 in
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42 372 CYP2R1 reduced 25(OH)D concentration levels by 3.0 nmol/Liter per risk allele (A). Upon
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44 373 performing conversion of the 25(OH)D estimates from the natural logarithm scale [26], the
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46 374 variant rs2282679 near GC was shown to reduce 25(OH)D levels by approximately 2.5
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54 375 nmol/Liter per 25(OH)D decreasing allele (C).
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56 377 **Mendelian randomization analysis for all ovarian cancer subtypes**
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9 378 We determined the associations between the 25(OH)D associated SNPs (rs7944926 and
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11 379 rs12794714) and risk of ovarian cancer in Table 2. rs12794714 and rs2282679 was directly
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14 380 genotyped in our dataset, whereas rs7944926 was well imputed (imputation quality score
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17 381 0.92). For all epithelial ovarian cancer subtypes combined, the estimated magnitude of
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20 382 association for a 1.0 nmol/Liter change in 25(OH)D level was -0.0076 (standard error (S.E.)=
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23 383 0.0109) for the MR analysis performed via rs7944926 in *DHCR7*. This translates into an odds
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26 384 ratio (OR) of 1.17(0.76-1.78) per 20nmol/Liter decrease in 25(OH)D levels. Similarly, the
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29 385 magnitude of association was -0.0137 , S.E.= 0.0063 for rs12794714 in *CYP2R1*, with
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32 386 corresponding OR of 1.31(1.03-1.69) per 20 nmol/Liter decrease in 25(OH)D and the
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35 387 magnitude of association is -0.0110 , S.E.= 0.0082 with OR of 1.25(0.90-1.71) for rs2282679
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38 388 in GC. Since all these SNPs are independent, a more accurate estimate will be obtained from
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41 389 the combined associations of the three SNPs. The combined weighted magnitude of
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44 390 association is -0.0118 , with a S.E. of 0.0045. The resultant OR per 20nmol/Liter change in
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47 391 25(OH)D on all epithelial ovarian cancer subtypes combined is 1.27 (1.06-1.51).
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50 392 (Table 2 here)

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394 **Mendelian Randomization analysis for high grade serous ovarian cancer**

395 Similar associations were observed between SNPs for 25(OH)D concentration and high
396 grade serous epithelial ovarian cancer. We obtained a magnitude of association estimate of
397 -0.0209 (S.E.= 0.0154) and -0.0257 (S.E.= 0.0091) and -0.0173 (S.E.= 0.0117) for
398 rs7944926, rs12794714 and rs2282679 respectively. This resulted in an OR of 1.51(0.83-
399 2.78) using rs7944926, 1.67(1.18-2.38) using rs12794714, and 1.41(0.89-2.23) per 20
400 nmol/Liter decrease in 25(OH)D. Weighting across all SNP instruments yielded an estimated
401 magnitude of -0.0218 (S.E.= 0.0067). Hence a 20 nmol/Liter decrease in 25(OH)D
402 corresponds to an OR of 1.54(1.19-2.01) for high grade serous ovarian cancer.

403 (Figure 2 here)

404 (Figure 3 here)

405 **Discussion**

406 Even though the SNPs chosen in our study only explain a small fraction ($\sim 1.3\%$) of the
407 variance of 25(OH)D concentration, because our case-control sample was so large, we were
408 able to demonstrate associations with ovarian cancer risk. A genetically scored decrease of
409 20nmol/Liter of serum 25(OH)D concentration levels, increased the risk of epithelial ovarian
410 cancer by about 30% in European ancestry women, with a larger association seen in high

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10 413 **Comparison with previous findings**
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12 414 A recent Danish study [31] used MR to show that low circulating 25(OH)D concentrations
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14 415 were associated with cancer mortality among Europeans. That study did not separate the
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16 416 associations of risk and mortality and was underpowered to draw conclusions on any
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18 417 specific cancer type. Here, for the first time, we demonstrate that for epithelial ovarian
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20 418 cancer, there is a causal effect of low 25(OH)D concentrations on risk.
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30 420 Our results are inconsistent with some previous studies that have reported no
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32 421 associations between 25(OH)D and ovarian cancer status. The recent meta-analysis [8] of 10
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34 422 individual cohort studies (884 cases and 1 605 controls) found no association between
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36 423 25(OH)D concentration and development of ovarian cancer. Findings from epidemiologic
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38 424 studies may differ from our MR based results because observational studies can be affected
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40 425 by confounding and reverse causation, though cohort studies such as [8] would be expected
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42 426 to be less affected.
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3 428 **Strength and limitations**
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6 429 A strength of our study is that the mechanism through which our chosen SNPs influence
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9 430 25(OH)D levels is well understood. *DHCR7* encodes the enzyme 7-dehydrocholesterol
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12 431 reductase, which is responsible for the conversion of 7-dehydrocholesterol to cholesterol.
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15 432 Reduced activities of 7-dehydrocholesterol reductase, leading to low cholesterol and
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18 433 accumulation of 7-dehydrocholesterol, are partially attributable to *DHCR7* variants [24, 25,
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21 434 29]. Although rs7944926 lies outside *DHCR7*, this variant modulates expression of *DHCR7*
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24 435 [35]. *CYP2R1* is an enzyme which converts vitamin D₃ to 25(OH)D in the liver [36], with
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27 436 rs12794714 unambiguously associated with 25(OH)D concentrations via GWAS [29]. The GC
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30 437 gene has a primary role in vitamin D transport. Previous studies shown that the rs2282679
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33 438 variant in particular were also strongly associated ($P=4.0\times 10^{42}$) with serum vitamin D
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36 439 binding protein (DBP) based on the study performed on 1 674 individuals in the Twins UK
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39 440 cohort [29]. The GC variants were also hypothesized to affect bioavailability of vitamin D
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42 441 through variation in circulating DBP. In view of evidence for its association towards vitamin
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45 442 D, the rs2282679 SNP is among one of the most associated variant with 25(OH)D ($P=1.9\times 10^{-$
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48 443 109) in the SUNLIGHT GWAS [29]. These variants (rs7944926, rs12794714 and rs2282679)
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51 444 thus affect 25(OH)D levels through varying vitamin D metabolism, bioavailability or
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54 445 transport, rendering them appropriate instrumental variables for use in MR [26, 27, 31, 34].
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3 447 One limitation is that our two-sample MR analysis assumes that the standard error
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6 448 of the exposure (SNP to 25(OH)D) estimates is negligibly small [33, 37] – given the large
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9 449 sample size in the Danish study [31], this is a reasonable assumption. In addition, the MR
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12 450 framework assumes a linear relationship in the association of the SNP instruments on the
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15 451 underlying exposure. Although our MR estimates indicate that a decrease of 20nmol/Liter in
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18 452 25(OH)D concentration is associated with a 30% increased risk of epithelial ovarian cancer,
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21 453 this estimated effect size is derived from a larger sample size of women with a range of
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24 454 25(OH)D concentrations. Previous studies using MR to examine 25(OH)D concentrations
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27 455 with different outcomes have dealt with this in various ways. For example, the published
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30 456 study that we used [31] assumed linearity of change across raw 25(OH)D values. In contrast,
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33 457 the study by Mokry et al. [26] on vitamin D and multiple sclerosis (MS) considered the
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36 458 association to be linear on log transformed 25(OH)D.

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41 460 We examined the implications of these approaches by re-computing our findings
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44 461 based on exposure estimates on the original scale (from the Danish study [31]) and on the
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47 462 log scale (from MR study on MS [26]) (see Supplementary Table 2). We note that in addition
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50 463 to the scale differences, the estimates of the magnitude of association of each SNP on
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53 464 25(OH)D differed due to random sampling error (with estimates from the Danish study [31]
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56 465 derived from a much larger sample size than those in the MS study [26]). We hence

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3 466 repeated our analysis by adopting SNP-exposure estimates used by the MS study [26] for
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6 467 the SNP rs12785878 (LD to rs7944926 with $r^2 = 1.0$) in the *DHCR7* gene. Although our result
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9 468 was robust to differences in scaling (log transformed or non-transformed 25(OH)D
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12 469 concentrations, see Supplementary Table 2), in practice a 20nmol/Liter increase is more
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15 470 likely to make an impact on women with low 25(OH)D concentrations than those whose
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18 471 concentration is already high.

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24 473 In our main analysis, there were concerns that the effect of the GC SNP on 25(OH)D
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27 474 was not estimated with high accuracy (GC SNP estimates were based on 2 347 individuals
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30 475 [26] whereas the estimates for *DHCR7* and *CYP2R1* were derived based on 30 792
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33 476 individuals [31]), as well as concerns that the GC SNP may not influence in 25-
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36 477 hydroxyvitamin D's biological activity in a predictable way [31, 38, 39]. Nonetheless, we
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39 478 conducted a sensitivity analyses to examine the effect of excluding this SNP. When the GC
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42 479 SNP was excluded, our results were unchanged (the association with ovarian cancer of the
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45 480 combined effect of the 3 SNPs was very similar to that obtained using just 2 SNPs, see
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48 481 Supplementary Table 5).

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53 483 Another potential limitation of our analysis is residual pleiotropy. We found no
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56 484 evidence for SNP-confounder association based on the subset of participants with available
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3 485 confounder information (Supplementary Table 6) although we cannot rule out associations
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6 486 with unmeasured confounders. Approach such as Egger regression [40] can potentially be
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9 487 applied to further test the MR assumptions but these require more SNPs than the two
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12 488 employed here.

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17 490 **Interpretation of findings**

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20 491 Observation of a larger magnitude of association (OR=1.54) with high grade serous cancer
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23 492 for lower 25(OH) concentration suggests that the association of circulating 25(OH)D with
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26 493 risk of ovarian cancer may be confined to the high grade serous type, although the
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29 494 confidence limits of the two ORs are overlapping and high-grade serous cancer is contained
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32 495 within all ovarian cancer. The results for histological subtypes other than high grade serous
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35 496 carcinoma are shown in Figure 3 (for association of each individual SNP, see Supplementary
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38 497 Table 3), and there is no evidence for association for non-serous disease. For all non high-
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41 498 grade serous cancers combined, the odds ratio was 1.12 (0.89-1.41).

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47 500 The association of lower circulating vitamin D (25(OH)D) levels to risk of epithelial
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50 501 ovarian cancer appear to be consistent with a recent MR study [31] looking at all-cancer
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53 502 mortality. Vitamin D activating enzymes and vitamin D receptors are present in many
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56 503 tissues, with the regulation of 1-3% of gene expression in these tissues attributable to

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3 504 vitamin D [35]. Studies have also shown that vitamin D is involved in the regulation of cell
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6 505 processes (proliferation, differentiation and apoptosis) in several cell types that are central
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9 506 to the development of cancer [14, 41-43]. Thus, our findings warrant further investigations
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12 507 on the biological role of vitamin D (specifically, 25(OH)D) in mortality as well as risk of
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15 508 ovarian cancer.

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21 510 In conclusion, we demonstrate an association between low 25(OH)D concentration
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24 511 and risk of ovarian cancer in women of European ancestry, with our MR approach providing
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27 512 estimates which are unaffected by the confounding or biases present in observational
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30 513 studies. Whilst our results cannot guarantee causality, placed in the context of other
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33 514 epidemiological studies, they provide additional evidence supportive of a causal link
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36 515 between vitamin D and risk of ovarian cancer.

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42 43 44 45 46 518 **References**

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53 520 1. Sung PL, Chang YH, Chao KC, Chuang CM, Task Force on Systematic R, Meta-analysis
54 521 of Ovarian C. Global distribution pattern of histological subtypes of epithelial ovarian
55 522 cancer: a database analysis and systematic review. *Gynecol Oncol.* 2014;133(2):147-54.

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3 523 2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: a cancer journal for clinicians*. 2011;61(2):69-90.
- 4 524
- 5 525 3. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer
6 526 incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN
7 527 2012. *International journal of cancer Journal international du cancer*. 2015;136(5):E359-86.
- 8 528 4. Lu Y, Cuellar-Partida G, Painter JN, Nyholt DR, Australian Ovarian Cancer S,
9 529 International Endogene C, et al. Shared genetics underlying epidemiological association
10 530 between endometriosis and ovarian cancer. *Human molecular genetics*. 2015;24(20):5955-
11 531 64.
- 12 532 5. Salehi F, Dunfield L, Phillips KP, Krewski D, Vanderhyden BC. Risk factors for ovarian
13 533 cancer: an overview with emphasis on hormonal factors. *J Toxicol Environ Health B Crit Rev*.
14 534 2008;11(3-4):301-21.
- 15 535 6. Prescott J, Bertrand KA, Poole EM, Rosner BA, Tworoger SS. Surrogates of long-term
16 536 vitamin d exposure and ovarian cancer risk in two prospective cohort studies. *Cancers*.
17 537 2013;5(4):1577-600.
- 18 538 7. Cook LS, Neilson HK, Lorenzetti DL, Lee RC. A systematic literature review of vitamin
19 539 D and ovarian cancer. *Am J Obstet Gynecol*. 2010;203(1):70 e1-8.
- 20 540 8. Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: Circulating
21 541 vitamin D and ovarian cancer risk. *Gynecol Oncol*. 2011;121(2):369-75.
- 22 542 9. Webb PM, de Fazio A, Protani MM, Ibiebele TI, Nagle CM, Brand AH, et al. Circulating
23 543 25-hydroxyvitamin D and survival in women with ovarian cancer. *Am J Clin Nutr*.
24 544 2015;102(1):109-14.
- 25 545 10. Zheng W, Danforth KN, Tworoger SS, Goodman MT, Arslan AA, Patel AV, et al.
26 546 Circulating 25-hydroxyvitamin D and risk of epithelial ovarian cancer: Cohort Consortium
27 547 Vitamin D Pooling Project of Rarer Cancers. *Am J Epidemiol*. 2010;172(1):70-80.
- 28 548 11. Prescott J, Bertrand KA, Reid BM, Permuth-Wey J, De Vivo I, Cramer DW, et al.
29 549 Evidence of differential effects of vitamin d receptor variants on epithelial ovarian cancer
30 550 risk by predicted vitamin d status. *Frontiers in oncology*. 2014;4:286.
- 31 551 12. Bakhru A, Mallinger JB, Buckanovich RJ, Griggs JJ. Casting light on 25-hydroxyvitamin
32 552 D deficiency in ovarian cancer: a study from the NHANES. *Gynecologic oncology*.
33 553 2010;119(2):314-8.
- 34 554 13. Holick CN, Stanford JL, Kwon EM, Ostrander EA, Nejentsev S, Peters U.
35 555 Comprehensive association analysis of the vitamin D pathway genes, VDR, CYP27B1, and
36 556 CYP24A1, in prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2007;16(10):1990-9.
- 37 557 14. Wranicz J, Szostak-Wegierek D. Health outcomes of vitamin D. Part II. Role in
38 558 prevention of diseases. *Rocz Panstw Zakl Hig*. 2014;65(4):273-9.
- 39 559 15. Malloy PJ, Feldman D. Genetic disorders and defects in vitamin d action. *Endocrinol*
40 560 *Metab Clin North Am*. 2010;39(2):333-46, table of contents.
- 41 561 16. Walentowicz-Sadlecka M, Sadlecki P, Walentowicz P, Grabiec M. [The role of vitamin
42 562 D in the carcinogenesis of breast and ovarian cancer]. *Ginekol Pol*. 2013;84(4):305-8.
- 43 563 17. Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory
44 564 actions of vitamin D. *Annual review of pharmacology and toxicology*. 2011;51:311-36.
- 45 565 18. Toriola AT, Surcel HM, Agborsangaya C, Grankvist K, Tuohimaa P, Toniolo P, et al.
46 566 Serum 25-hydroxyvitamin D and the risk of ovarian cancer. *Eur J Cancer*. 2010;46(2):364-9.
- 47 567 19. Arslan AA, Clendenen TV, Koenig KL, Hultdin J, Enquist K, Agren A, et al. Circulating
48 568 vitamin d and risk of epithelial ovarian cancer. *J Oncol*. 2009;2009:672492.

- 1
2
3 569 20. Tworoger SS, Lee IM, Buring JE, Rosner B, Hollis BW, Hankinson SE. Plasma 25-
4 570 hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of incident ovarian cancer. *Cancer*
5 571 *epidemiology, biomarkers & prevention : a publication of the American Association for*
6 572 *Cancer Research, cosponsored by the American Society of Preventive Oncology.*
7 573 2007;16(4):783-8.
- 8
9 574 21. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for
10 575 Mendelian randomization. *Stat Methods Med Res.* 2015.
- 11 576 22. Sheehan NA, Didelez V, Burton PR, Tobin MD. Mendelian randomisation and causal
12 577 inference in observational epidemiology. *PLoS Med.* 2008;5(8):e177.
- 13 578 23. Pharoah PD, Tsai YY, Ramus SJ, Phelan CM, Goode EL, Lawrenson K, et al. GWAS
14 579 meta-analysis and replication identifies three new susceptibility loci for ovarian cancer. *Nat*
15 580 *Genet.* 2013;45(4):362-70, 70e1-2.
- 16 581 24. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, et al.
17 582 Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet.*
18 583 2010;19(13):2739-45.
- 19 584 25. Berry DJ, Vimalaswaran KS, Whittaker JC, Hingorani AD, Hypponen E. Evaluation of
20 585 genetic markers as instruments for Mendelian randomization studies on vitamin D. *PLoS*
21 586 *One.* 2012;7(5):e37465.
- 22 587 26. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Leong A, et al. Vitamin D and
23 588 Risk of Multiple Sclerosis: A Mendelian Randomization Study. *PLoS medicine.*
24 589 2015;12(8):e1001866.
- 25 590 27. Theodoratou E, Palmer T, Zgaga L, Farrington SM, McKeigue P, Din FV, et al.
26 591 Instrumental variable estimation of the causal effect of plasma 25-hydroxy-vitamin D on
27 592 colorectal cancer risk: a mendelian randomization analysis. *PLoS One.* 2012;7(6):e37662.
- 28 593 28. Trummer O, Pilz S, Hoffmann MM, Winkelmann BR, Boehm BO, Marz W, et al.
29 594 Vitamin D and mortality: a Mendelian randomization study. *Clin Chem.* 2013;59(5):793-7.
- 30 595 29. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common
31 596 genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet.*
32 597 2010;376(9736):180-8.
- 33 598 30. Bratke K, Wendt A, Garbe K, Kuepper M, Julius P, Lommatzsch M, et al. Vitamin D
34 599 binding protein and vitamin D in human allergen-induced endobronchial inflammation. *Clin*
35 600 *Exp Immunol.* 2014;177(1):366-72.
- 36 601 31. Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin
37 602 D concentrations and increased mortality: Mendelian randomisation analysis in three large
38 603 cohorts. *Bmj.* 2014;349:g6330.
- 39 604 32. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for
40 605 genome-wide association studies by imputation of genotypes. *Nat Genet.* 2007;39(7):906-
41 606 13.
- 42 607 33. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with
43 608 multiple genetic variants using summarized data. *Genetic epidemiology.* 2013;37(7):658-65.
- 44 609 34. Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Vitamin D
45 610 concentration, obesity, and risk of diabetes: a mendelian randomisation study. *Lancet*
46 611 *Diabetes Endocrinol.* 2014;2(4):298-306.
- 47 612 35. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, et al. Towards
48 613 a knowledge-based Human Protein Atlas. *Nature biotechnology.* 2010;28(12):1248-50.

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3 614 36. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that
4 615 the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proceedings of the National
5 616 Academy of Sciences of the United States of America. 2004;101(20):7711-5.
6 617 37. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I.
7 618 Using published data in Mendelian randomization: a blueprint for efficient identification of
8 619 causal risk factors. Eur J Epidemiol. 2015;30(7):543-52.
9 620 38. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-
10 621 binding protein and vitamin D status of black Americans and white Americans. N Engl J Med.
11 622 2013;369(21):1991-2000.
12 623 39. Taylor AE, Burgess S, Ware JJ, Gage SH, Richards JB, Davey Smith G, et al.
13 624 Investigating causality in the association between 25(OH)D and schizophrenia. Sci Rep.
14 625 2016;6:26496.
15 626 40. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid
16 627 instruments: effect estimation and bias detection through Egger regression. International
17 628 journal of epidemiology. 2015;44(2):512-25.
18 629 41. Fleet JC, DeSmet M, Johnson R, Li Y. Vitamin D and cancer: a review of molecular
19 630 mechanisms. The Biochemical journal. 2012;441(1):61-76.
20 631 42. Holick MF. Vitamin D, sunlight and cancer connection. Anticancer Agents Med Chem.
21 632 2013;13(1):70-82.
22 633 43. Ingraham BA, Bragdon B, Nohe A. Molecular basis of the potential of vitamin D to
23 634 prevent cancer. Curr Med Res Opin. 2008;24(1):139-49.
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699 **Competing Interest**

700 Mark T Goodman is a consultant for Johnson and Johnson Ltd. Usha Menon has stock
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707 **Supplementary Material**

708 See separate file.

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Table 1: Distribution of cases based on epithelial ovarian carcinoma subtypes

EOC subtypes	Number of Cases
High-grade Serous	4 121
Low-grade Serous	363
Serous of unknown grade	1 344
Mucinous	662
Clear Cell	621
Endometrioid	1 350
Others	1 604

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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/Liter)			All epithelial ovarian subtype (N=10 065 cases)				Only high grade serous epithelial ovarian subtype (N=4 121 cases)			
		β_{zx}	σ_{zx}	R^2	β_{zy}	σ_{zy}	β_{IVW}	σ_{IVW}	β_{zy}	σ_{zy}	β_{IVW}	σ_{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.

β_{zy} denotes the magnitude of association of the SNP-outcome estimate.

σ_{zx} is the standard error of the SNP-exposure estimate.

β_{zx} denotes the magnitude of association of Z, the SNP instrument on X, the modifiable exposure level (25(OH)D).

σ_{zy} is the standard error of β_{zy} .

R^2 is the proportion of variance in 25(OH)D explained by the SNP(s).

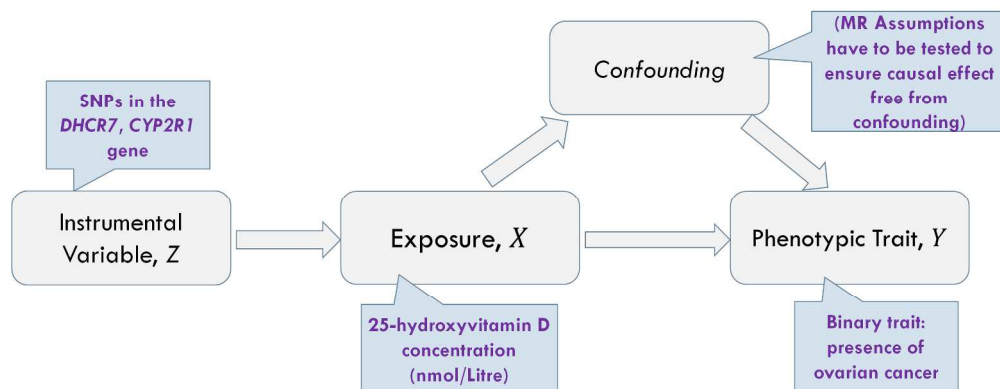
β_{IVW} is the estimate and σ_{IVW} its standard deviation. β_{zy} is presented on the log(OR) scale.

β_{IVW} is presented on the log(OR) scale for a single unit (1nmol/Liter) change in 25(OH)D – see text for OR scale changes for a 20 unit (nmol/Liter) change in 25(OH)D.

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Note: the β_{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/Litre 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 rs2822679 was computed from Mokry et al.

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Schematic representation of the Mendelian randomization framework using vitamin D SNPs as instrumental variables.

750x292mm (96 x 96 DPI)

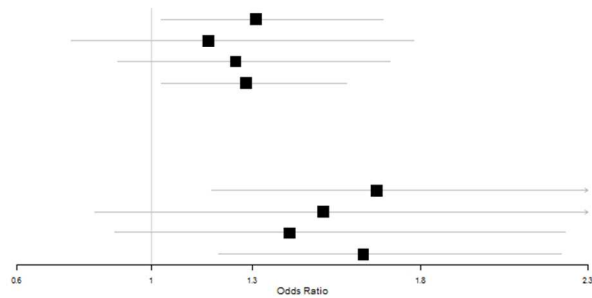
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**Causal OR for 20nmol/Liter change in 25(OH)D
on risk of all ovarian cancer and
high grade serous subtype**

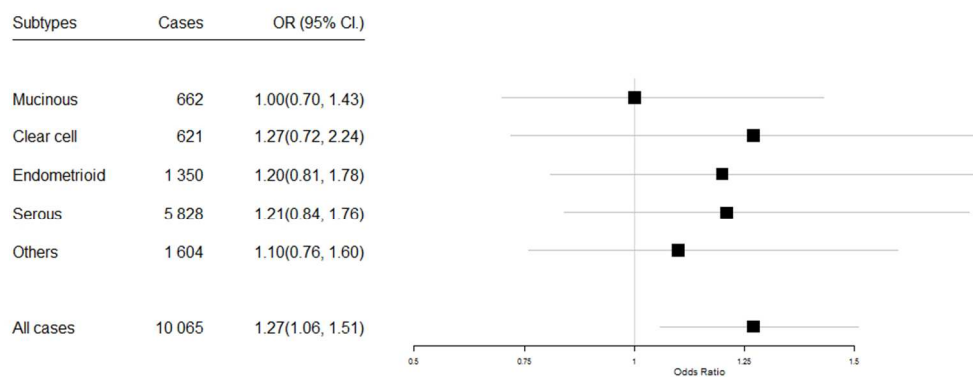
All subtypes (N=10 065)	EA/NEA	OR (95% CI)
rs12794714	A/G	1.31(1.03,1.69)
rs7944926	A/G	1.17(0.76,1.78)
rs2282679	C/A	1.25(0.90, 1.71)
Combined	-	1.28(1.03,1.58)
HG serous (N=4 121)	EA/NEA	OR (95% CI)
rs12794714	A/G	1.67(1.18,2.38)
rs7944926	A/G	1.51(0.83,2.78)
rs2282679	C/A	1.41(0.89,2.23)
Combined	-	1.63(1.20,2.22)



Causal OR of 25(OH)D on all ovarian cancer and high grade serous ovarian cancer
357x194mm (72 x 72 DPI)

Review Only

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



Causal OR of 25(OH)D on individual ovarian cancer subtypes
357x194mm (72 x 72 DPI)

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