Manuscript title: **Evaluating the role of alcohol consumption in breast and ovarian cancer susceptibility using population-based cohort studies and two-sample Mendelian randomization analyses**

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Novelty and Impact:

Using a combination of large scale observational data from three European population-based cohorts and Mendelian randomization analyses on two of the largest cancer consortia, our observational and genetic-derived findings combined reveal that alcohol consumption is unlikely to be a major risk factor for both breast and ovarian cancer susceptibility.

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Abbreviations:

ADH - Alcohol dehydrogenase
BCAC - Breast Cancer Association Consortium
CCHS - Copenhagen City Heart Study
CGPS - Copenhagen General Population Study
EOC - Epithelial ovarian cancer
ER - Estrogen receptor
GWAS - Genome-wide association studies
HR -Hazard ratio
IVW - Inverse variance weighted
KARMA - Karolinska Mammography Project for Risk Prediction of Breast Cancer
LD - Linkage Disequilibrium
MAF - Minor Allele Frequency
MR - Mendelian randomization
MVMR - Multivariable Mendelian randomization
OCAC - Ovarian Cancer Association Consortium
OR - Odds ratio
SNP - Single nucleotide polymorphism
UKB - UK Biobank
WCRF - World Cancer Research Fund

Abstract

Alcohol consumption is correlated positively with risk for breast cancer in observational studies, but observational studies are subject to reverse causation and confounding. The association with epithelial ovarian cancer (EOC) is unclear. We performed both observational Cox-regression and two-sample Mendelian randomization (MR) analyses using data from various European cohort studies (observational) and publicly available cancer consortia (MR). These estimates were compared with World Cancer Research Fund (WCRF) findings. In our observational analyses, the multivariable-adjusted hazard ratios (HR) for a one standard drink/day increase was 1.06 (95% confidence interval; 1.04,1.08) for breast cancer and 1.00 (0.92,1.08) for EOC, both of which were consistent with previous WCRF findings. MR ORs per genetically predicted one standard drink/day increase estimated via 34 SNPs using MR-PRESSO were 1.00 (0.93,1.08) for breast cancer and 0.95 (0.85,1.06) for EOC. Stratification by EOC subtype or estrogen receptor status in breast cancers made no meaningful difference to the results. For breast cancer, the confidence intervals for the genetically derived estimates include the point-estimate from observational studies so are not inconsistent with a small increase in risk. Our data provide additional evidence that alcohol intake is unlikely to have anything other than a very small effect on risk of EOC.

Introduction

The World Cancer Research Fund (WCRF) concluded that alcohol intake is a probable cause of breast cancer, with an estimated additional risk of 9% per 10g/day increase in consumption of ethanol, but that there is inadequate evidence to evaluate the association with epithelial ovarian cancer (EOC) [1,2]. It is, however, difficult to measure any effect of elevated alcohol consumption from conventional observational data because of the possible confounding issues: alcohol consumption is itself associated with many other lifestyle and socio-economic factors, which may themselves be a risk factor for cancers and are difficult to quantify. Estimates from observational studies may additionally be biased by other mechanisms [3], including recall bias due to differences in the completeness of the subjective indications of alcohol consumption across case-control status, selection bias [4,5] against heavy users of alcohol due to the preferential participation of reasonable healthy individuals and reverse causality. The WCRF estimates are based on data from population-based prospective cohort studies, where exposure information is collected before the event of interest occurs. Although cohort studies in general are less likely to suffer from bias, it is still impossible to rule out confounding. Also, such studies typically only measure exposure variables once or a few times, precluding detailed individual modelling of exposures over time [6]. In principle, double blinded randomized trials are the best way to evaluate causality, but such studies are usually logistically cumbersome and may be unethical.

A Mendelian randomization (MR) study, using genetic variants associated with alcohol consumption as an instrument for alcohol consumption, offers a way to test hypotheses of causality, since the genetic variants are less likely to be associated with other known or unknown confounders, and they are not influenced by (pre-)clinical stages of the diseases [7]. Conceptually, MR relies on the random assortment of genetic variants during meiosis to mimic a "natural" randomized trial [8]. However, for MR estimates to make valid inferences on causality, several assumptions have to be met [8]. Typically,

such a genetic instrument only explains a fraction of the variance of the exposure variable, and therefore MR studies need very large numbers of participants to answer questions of causality. For alcohol consumption, previous MR studies have used the rs1229984 variant (as this SNP is associated with high levels of acetaldehyde and facial flushing [9]) as a genetic instrument to evaluate the link between alcohol intake and disease outcomes [10–12]. Two-sample mendelian randomization is an extension of the traditional MR methodology that leverages greater statistical power for MR analyses by utilizing independent summary-based datasets to derive the genetic association on alcohol and cancer outcome separately without the need for individual level data. As larger GWAS have identified more risk loci and GWAS of outcomes of interest have increased in size, power has recently become adequate to support meaningful statistical inference [13].

Using several large independent population-based prospective cohorts, we first tested whether alcohol intake is associated with risk of breast cancer and EOC via observational analyses, to compare against previous WCRF findings. We then evaluated whether there is genetic evidence to support a causal relationship between the two using a two-sample Mendelian randomization study approach utilizing consortia data from both the Breast Cancer Association Consortium (BCAC) and Ovarian Cancer Association Consortium (OCAC) for breast and ovarian cancer, respectively. Both observational and genetically derived estimates were then used to infer whether there is evidence for a causal relationship between alcohol intake and these cancers.

Materials and Methods

Overview

For the large-scale population-based cohorts, we evaluated the observational association between self-reported alcohol consumption and risk of breast and ovarian cancer via Cox-regression analyses. Study-specific hazard ratio (HR) estimates were then combined via a fixed-effect meta-analysis (separately for each cancer). For the genetic causality analyses, we performed a two-sample MR to

assess whether genetically predicted alcohol consumption is associated with breast/ovarian cancer susceptibility using publicly available consortia data. Instruments for the MR analyses were obtained from an alcohol consumption GWAS performed on the UK Biobank white British participants.

Description of observational cohort studies

Data from Copenhagen General Population Study (CGPS) and Copenhagen City Heart Study (CCHS)

The CGPS[14] and the CCHS[15] are two large prospective general population studies from Denmark. For both studies, residents from Copenhagen were invited to complete a baseline questionnaire and undergo a physical examination. The questionnaire includes the number of alcoholic drinks consumed daily and this was used to derive standard drinks per week (for this study 1 standard drink ~ 12g ethanol). Blood samples were also obtained. In total, 69 420 women participated, 60 205 from the CGPS (enrolled between 2003 to 2015) and the remaining 9 215 from the CCHS (enrolled during four examinations from 1976-78, 1981-83, 1991-94, and 2001-03). A total of 2 312 incident breast cancer and 327 EOC cases were identified. Women with diagnosis of breast cancer prior to examination or who had missing information on covariates were excluded from the analysis. All participants gave written informed consent, and both CCHS and CGPS were approved by the Danish ethics committees (H-KF01-144/01 and KF100.2039/91). Full details on the observational HR analysis in the CGPS and CCHS are provided in Supplementary Methods.

Data from the Karolinska Mammography Project for Risk Prediction of Breast Cancer (KARMA).

The KARMA study is a large Swedish breast cancer prospective cohort study comprising 70 877 women who attend regular mammographic screening across four hospitals in Sweden [16]. The aim of the project is to identify risk factors for breast cancer such as mammographic density, genetic and lifestyle

factors. Information on tumour characteristics, such as ER status, was identified through registers. Self-reported alcohol intake (in grams) estimated via questionnaires was standardised into number of standard drinks/week using a nominal conversion scale of 10g/standard drink. For our HR analysis, we identified 985 incident breast cancer cases in a cohort of 60 903 women with non-missing data on confounders. We did not perform the analysis for EOC due to the limited number of cases (n=57). See Supplementary Methods for a complete description.

Data from the UK Biobank cohort

The UK Biobank (UKB) cohort consists of 502 000 middle-aged individuals recruited from across the United Kingdom [17]. The UKB study was approved by the North West Multi-Centre Research Ethics Committee (reference number 06/MRE09/65), participants at the time of recruitment gave informed consent to participate in UK Biobank and be followed up, using a signature capture device. 487 910 individuals passed initial genetic quality control protocols. We identified 215 830 women genetically similar to those of white-British ancestry through ancestral principal component techniques [18]. The UKB records extensive (n>2 000) phenotypes including anthropometric traits, disease status and lifestyle behaviours. Number of standard drinks per day (one standard drink in the UKBB is roughly equivalent to 12g/day of alcohol) was calculated as a weighted sum of daily consumption on various types of alcoholic beverages (Supplementary methods; see also Supplementary Table 1 and 2). Non-drinkers were given a score of zero standard drinks per day. Information about cancer diagnosis among the UKB participants was obtained through data-linkage between self-report, hospital records and cancer registries. Individual cancer types were defined based on ICD-10 definitions, as per previous work [19]. After excluding women with a history of cancer (excluding non-melanoma skin cancer) prior to enrolment including recurrent cancer cases, the cohort comprised 141 071 white British women with 4,068 women diagnosed with breast cancer and 425 with EOC. However, the proportion of individuals with missing data on the necessary covariates (e.g. menopausal status,

education attainment, nulliparity) were relatively high (i.e. only 1 771 breast cancer cases and 187 EOC cases had complete data). Cox regression was used to obtain hazard ratios for cancer risk per standard drinks/day increase in alcohol consumption. A complete description of the observational HR analysis for alcohol and cancer in the UKB is provided in Supplementary Methods.

Meta-analysis of observational findings

We then performed an observational meta-analysis of the association estimates combining the UKB results with those obtained from the CCHS+CGPS and KARMA study for breast cancer and EOC. All association estimates were scaled to reflect a one standard drink per day increase (an increase of ~10g/day of ethanol) to facilitate comparison with our MR findings. Estimates were combined under a fixed-effect inverse variance weighted model using the *rmeta* R library. These results were then compared against the existing WCRF findings on both cancers [1,2].

Genetic analyses

In this two-sample MR study, we derived instrumental variables for alcohol consumption from the UK Biobank cohort. We then evaluated whether these alcohol-associated instruments were associated with breast/ovarian cancer risk using GWAS summary statistics obtained from the Breast Cancer Association Consortium (BCAC) and the Ovarian Cancer Association Consortium (OCAC). A flowchart illustrating the complete MR procedure is shown in Figure 1.

(Figure 1 here)

Breast and ovarian cancer risk GWAS

The BCAC breast cancer GWAS summary statistics [20], derived from a total of 122 977 cases and 105 974 controls of European ancestries, were obtained from a publicly available repository

(http://bcac.ccge.medschl.cam.ac.uk/bcacdata/oncoarray). Among these, 69 501 of the cases were identified to have ER+ breast cancer, and 21 468 breast cancer cases were ER-. Participants in the BCAC were recruited from various case-control and cohort studies around the world. BCAC participants involved in the breast cancer GWAS were genotyped via one of these genotyping platforms: (i) custom Illumina iSelect genotyping arrays, (ii) OncoArray or (iii) the iCOGS array. Genotypes were then imputed against the 1000 Genomes Project Phase III reference panels using IMPUTE2 [21]. A full description of the genetic quality control procedures is given elsewhere [20]. The association between SNPs and cancer outcome were estimated using conventional multiple logistic regression adjusting for top ancestral principal components and age [20].

The OCAC EOC GWAS summary statistics [22], derived from a total of 22 406 cases and 40 941 controls of European ancestries, were obtained from a publicly available repository (http://ocac.ccge.medschl.cam.ac.uk/). The genotyping platforms used were broadly similar to those used in the BCAC breast cancer GWAS. Top ancestral principal components were fitted as covariates in both the breast cancer and EOC GWAS model to account for the presence of population substructure. Prior to our main analyses, we excluded SNPs that were poorly imputed (INFO<0.6) or had very low minor allele frequencies (MAF<0.01) for both GWAS datasets. Similarly, the association between SNPs and cancer outcome were estimated using conventional multiple logistic regression adjusting for top ancestral principal components and age [22].

Deriving genetic instruments for alcohol consumption (UKB data)

The complete description of how estimated standard drinks per week was derived via self-reported consumption of alcoholic beverages is provided in Supplementary Methods. In brief, we computed the participants' total alcohol standard drinks per week using both frequency and quantity of alcohol consumption, summing across the alcohol content (in std drinks) from self-reported quantities of

various types of alcoholic beverage consumed weekly. Non-drinkers are included in the GWAS, and assigned a score of zero standard drinks per week. We performed a GWAS on standard alcoholic drinks per week to calibrate genetic instruments that are predictive of self-reported alcohol consumption among white British women in the UKB. We used the software BOLT-LMM [23], a Bayesian linear mixed model GWAS framework to explicitly model the genetic relatedness within the sample. Genetic sex, age and 10 ancestral principal components were included as covariates. We performed 2 separate GWAS: using i) the estimated alcohol quantity in both sexes (n=432 178) and ii) the estimated alcohol quantity in females only (n=197 948). For each alcohol GWAS result, only SNPs that were genome-wide significant and had MAF>0.01 were retained. Clumping on the SNPs were then performed based on LD (r^2 <0.01) and maximum distance of 1 000 kb apart to ensure that selected instruments are strictly independent. We identified 72 instruments (including SNP rs1229984) from the combined-sex drinks/week GWAS (Supplementary Table 3). The combined-sex GWAS was used to robustly identify alcohol associated SNPs but in our main MR analysis we adopted SNP effect sizes estimated among females only. In order to ensure that our analyses were protected against weak instrument bias, we only used 34 out of 72 SNPs that were successfully replicated in the female-only alcohol GWAS (p<1e-5 in females).

Two-sample Mendelian randomization

GWAS summary statistics were used to obtain association estimates for genetic predictors of alcohol on cancer outcomes (breast or ovarian cancer) from the respective consortia (BCAC and OCAC). The estimated statistical power to detect MR associations at various effect sizes (ORs) is shown in Supplementary Table 4. We extracted the SNP-cancer association estimates and minor allele frequency information for each of the 34 alcohol-associated SNP instruments. We fitted a multiplicative random effect inverse variance weighted (IVW) model to obtain a combined estimate of the causal effect inferred via multiple SNPs [24]. For each test, palindromic SNPs with strands that

could not be inferred via allele frequency were excluded. The global Cochran Q statistics was first evaluated to determine MR findings with heterogeneous effect sizes. For tests with strong evidence of causal effect heterogeneity, we then repeated our IVW MR analyses by manually filtering out SNPs that showed strong evidence for having heterogenous effect estimates, defined by SNPs with a Cochran' Q statistics exceeding 3.84. The heterogeneity-adjusted MR estimate will be reported in the main results section for traits that have inflated global Cochran Q statistics (p-heterogeneity < 0.05).

Previous studies have shown that SNPs associated with alcohol intake might be pleiotropically linked with changes in adiposity, or simply markers for smoking behaviour or education attainment, some of which might confound the association with these cancers. Discarding SNPs on the basis of a pleiotropic association might result in loss of power if the association is linked through the same causal pathway (vertical pleiotropy). However, determining the modes of pleiotropy for each SNP instrument is not a trivial task. Instead, we applied a multivariable MR (MVMR) model to evaluate the direct effect between genetically predicted alcohol intake on breast/ovarian cancer by regressing out the genetic effect of these variants on BMI, BMI-adjusted waist-to-hip ratio, education attainment and cigarettes smoked per day. The marginal OR for alcohol intake (drink/day) on cancer risk(s) after adjusting for the aforementioned risk factors were reported. Curation of the phenotypes used in the MVMR framework is described in Supplementary Methods.

We scaled our MR estimate to reflect a genetically predicted one drink/day increase in alcohol consumption (by multiplying the predicted change in log(OR) of cancer for 1 standard drink/week by 7). All statistical analyses (including MR sensitivity analyses) were performed in statistical package R using the *TwoSampleMR* library implemented in the MR-Base platform [25].

Sensitivity analyses

Observational. We assessed evidence for a non-linear relationship between alcohol and breast cancer or EOC outcomes by evaluating the dose-response relationship over strata of increasing alcohol intake in the observational analyses. For the KARMA study (where ER status was available for breast cancer cases), we performed stratified analyses to evaluate whether the alcohol-breast cancer association differed by ER status. There is no information available for ER-status in both the UK Biobank and Copenhagen cohort studies. Due to the high rate of missingness in the covariate data in our UKB (multivariable adjusted) observational HR analyses, we finally performed missing data imputation to evaluate whether the associations varied with more recovered samples (see Supplementary Methods).

MR analyses For the genetically derived estimates, we first attempt to re-evaluate our MR findings using only the rs1229984 SNP as an instrument fitted through a Wald-type estimator [26]. Next we ensure that our findings were not biased by violation of the MR assumptions by repeating our analyses using the following alternative MR models: MR Egger regression [27], weighted median [28] and the penalised weighted median model[29]. Deviations of the MR Egger regression intercept from the null for each tested outcome were used to assess evidence of directional pleiotropy. The multi-SNP MR analyses were also repeated using the MR-PRESSO technique [30] which provides adjusted causal estimates after filtering out heterogeneous SNP-outliers. MR estimates derived using these models were reported alongside the main (IVW) MR results to ensure robustness of findings as different techniques relax different assumptions. Funnel plots and leave-one-out MR plots were also generated to evaluate whether the causal estimates were driven by strong outliers. We also performed a SNP-lookup on the recently published alcohol drinks/week GWAS summary data to evaluate whether our instrument-alcohol associations replicate well in the much larger GSCAN alcohol GWAS [31]. Detailed descriptions of the MR sensitivity analyses are provided in the Supplementary Methods.

For the MR analysis on breast cancer, we additionally performed stratified analyses based on estrogen receptor (ER) status, whilst for EOC, we subsequently evaluated the association of alcohol across different histotypes including the most common HGSOC histotype.

Results

Conventional observational analyses on alcohol consumption

Observational association between alcohol consumption with breast and ovarian cancer risk

Breast cancer. Alcohol consumption was associated with increased risk of breast cancer in the CCHS+CGPS cohorts with a HR of 1.09 per standard drink/day (95% C.I. 1.05, 1.13), and the Swedish KARMA study with HR 1.07 (0.97,1.19) while the HR in the UKB dataset was lower (HR 1.04 (1.01, 1.07)). Meta-analysing all these estimates yielded an HR of 1.06 (1.04,1.08) for risk of breast cancer per one standard drink/day (Figure 2 upper panel)

Ovarian cancer. In the UK Biobank, higher alcohol consumption was associated with a reduction in risk for cancers in the ovary with an age-adjusted HR of 0.92 (0.85,0.99). Using the multivariable adjusted model (N=61,267, N=187 cases), the log(HR) was unchanged, albeit with wider confidence intervals (adjusted HR 0.92 (0.83,1.03)) due to missing information on covariates. In CCHS+CGPS, the estimated HR (HR=1.07 (0.96,1.20)) was in the opposite direction, but with 95% CIs that overlapped the estimates from the UKBB. Combining both these estimates yielded an meta-analysed HR of 1.00 (0.92,1.08) for the risk of EOC per one standard drink of 10 g alcohol per day increase in alcohol consumption (Figure 2 lower panel).

(Figure 2 here)

Instrumental variable analyses - Genetically predicted alcohol consumption

Association of genetically predicted alcohol consumption with breast cancer and EOC

(Figure 3 here)

For a one unit increase in genetically predicted daily alcohol intake (using 34 variants), the odds ratio on breast cancer was 1.03(0.93,1.14) in standard IVW analysis, with a tighter confidence interval when MR-PRESSO was used to discard one heterogeneous SNPs (OR 1.00 [0.93,1.08], figure 3). For EOC, the point estimate was less than one, although with relatively wide confidence intervals (OR 0.89 (0.73,1.08)). The MR-PRESSO MR OR estimate for EOC was attenuated slightly towards the null (OR 0.95 [0.85,1.06]). Given that all but one of the SNPs (rs62055546) used in our genetic instrument appeared homogeneous in our MR-PRESSO analysis we adopted the MR-PRESSO results as our primary results. Estimates of the MR association under alternative models are shown together in Figure 3. The comparison of our genetically derived estimate against our new observational findings and the WCRF results for breast cancer and EOC risk is provided in Figure 4. The original results not manually filtered for heterogenous SNPs are shown in Supplementary Table 11.

For the multivariable analyses, the estimated marginal OR on breast cancer for one drinks/week increase is 1.03 (0.97,1.10) in the MVMR model after excluding SNPs with high heterogeneity scores (Q>3.84), showing no evidence of effect size attenuation in the univariate MR model due to negative pleiotropy. The MVMR OR estimate for ovarian cancer was 0.97 (0.87,1.09), see Supplementary Table 12.

(Figure 4 here)

Sensitivity analyses

The observational HR association between alcohol and breast cancer and EOC for different levels of alcohol consumption indicated no strong evidence for a non-linear relationship (Supplementary Table 5-6). There was limited evidence that the alcohol-breast cancer association differed by ER status in the KARMA study (Supplementary Table 7). Furthermore, the age-adjusted and fully-adjusted models

gave similar estimates suggesting minimal evidence for confounding on the factors that were controlled for (Supplementary Table 8). To address the high rate of missingness in the UK Biobank multivariable-adjusted HR analyses, multiple imputation was performed to recover missing information in the covariates. We first verified that missingness of our covariates of interests can be predicted by our set of auxiliary variables and other covariates (satisfying the MAR assumption for accurate imputation) (see Supplementary Methods 8; Supplementary Figure 5 and 6). We generated a total of five sets of imputation datasets and pooled the estimates from each individual imputed dataset. The pooled regression estimates from imputed data revealed minor attenuation of the estimate between alcohol drinks/day and both cancers (HR on breast cancer =1.02 [1.00, 1.03] ; ovarian cancer = 0.94 [0.90, 0.99]) towards the null, providing more precise estimates upon modelling the covariates that previously had high rates of missingness adequately. However, these revised estimates were not meaningfully different from the original multivariable HR estimates as shown by the overlapping confidence intervals (Supplementary Table 9).

(Figure 5 here)

The confidence interval of the estimate from our single SNP MR analyses using the rs1229984 (*ADH1B*) variant (strongest instrument, explaining 0.23% of variation in alcohol intake, p=1e-128) largely overlaps those of the multi-instrument MR results (Supplementary Figure 1). The MR scatter plots for both cancers using the original 34 alcohol SNP instruments are shown in Figure 5. The F-statistics for our instruments suggest that each of our 34 SNPs are strong instruments, with SNP-alcohol associations (female only) being replicated successfully in the subsequent GSCAN GWAS revealing limited evidence for weak instrument bias (Supplementary Table 13-14). Our Steiger Z-test (Supplementary Table 15) also indicated no evidence for instrument mis-specification (i.e. our SNP instrument r^2 on alcohol >> r^2 on outcome) in our study design. Estimates derived from alternative MR methods (before/after filtering heterogenous instruments) reveal that our findings were robust against weak violation of MR assumption, with the MR-Egger intercepts showing no evidence for

directional pleiotropy (Supplementary Table 10, 11, 15 and 16). In our pleiotropy assessment, we did not observe evidence for an association between our genetic instruments with potential confounders including age at menarche, oral contraceptive use, smoking quantity, coffee consumption and psychiatric traits, except for BMI in the UKB (See Supplementary Table 17 and 18). However, the magnitude of association between rs1229984 and BMI is so small that it is very unlikely to have substantially biased our estimates. Moreover, our MR-PRESSO findings were statistically consistent with the IVW estimates for each trait. The distribution of effect sizes around the null across multiple sensitivity MR analyses provide strong support for an overall null or a very weak positive relationship between alcohol and breast cancer or EOC.

In our exploratory MR analyses, stratification by ER status produced essentially unchanged the MR results for breast cancer (Supplementary Table 19). Similarly, for subtypes of EOC, results were indistinguishable from those for overall EOC: the high-grade serous estimate was 0.95 [0.85,1.06] (Supplementary Figure 2).

Discussion

In this study, we evaluated the association between alcohol consumption and breast and ovarian cancer using conventional observational prospective designs and MR approaches. The point estimates for breast cancer from the observational findings were slightly higher than those from MR, but with overlapping confidence intervals. Although the confidence intervals are wider for the MR estimates, the MR design is likely to be robust to some of the issues which can hamper interpretation of observational studies, such as confounding. Taken together, although our MR estimate overlaps the null, the confidence interval from our MR estimate remains consistent with a modest increase as consistently reported in observational findings. For EOC, the effect appears null in both the observational and MR analyses.

Comparison with previous literature

Earlier molecular investigations found that alcohol may be implicated in the development of breast cancer, especially ER+ breast cancer, as it modulates estrogen levels. This adverse influence of alcohol is supported by a study investigating the link between alcohol intake and percentage of breast density (PBD), postulating a potential relationship between alcohol intake and breast cancer susceptibility via increased PBD [32]. Similarly, many observational findings have found that alcohol consumption is associated with risk of breast cancers [33–36]. Results from a large meta-analysis of 27 cohort studies showed that even light drinking (<1 drink/day) is associated with increased risk of breast cancer in women [37]. In our study, we found suggestive evidence from our observational study meta-analysis that increased alcohol consumption is associated with susceptibility for breast cancers, although the magnitude of association was slightly lower than those reported by the WCRF [2]. Here we add MR analysis to provide additional evidence as to whether the association seen in the observational studies represents a true causal association. Whilst the MR estimates had confidence intervals overlapping those from the observational studies, the most likely causal effect was zero (point estimate from MR analysis), with the relatively narrow MR confidence intervals suggesting the causal effect of alcohol intake is at most very small.

The null association between alcohol and EOC was previously shown in the study by Kelemen et al. [38] pooling together data from 12 case-control studies in OCAC, , and in other pooled case-control[39] and cohort[40] studies. In contrast, Cook et al.[41] showed that self-reported wine consumption was associated with a reduction in EOC risk in a recent Canadian study. One possible explanation for such an association is that the relationship may have been driven by residual confounding with other exposures correlated with socio-economic factors such as educational attainment [38,41]. While the estimated direction of effect for alcohol and ovarian cancer differed (non-significantly) for the UKB

and Copenhagen cohorts, it is difficult to draw any definitive statement given the overlapping CIs and low number of cases. In our observational meta-analyses, we did not find strong evidence to support a protective association between alcohol and overall EOC, consistent with the WCRF findings [1]. Our MR results for EOC were concordant with the observational study results.

Strength and limitations

Our large sample size combining data from various sources allow us to assess the role of alcohol consumption on breast/ovarian cancer with reasonably good precision. The MR approach provides additional evidence to triangulate evidence for causality. Our additional MR analysis using alcohol consumption instruments calibrated only among European women helps protect against biased inferences due to weak instruments [42]. While these SNPs combined explain only a small amount (~0.92%) of variation in alcohol consumption among women (Supplementary Table 3), due to the large sample sizes from both OCAC and BCAC, the confidence intervals on our MR estimates are reasonably precise. The use of a larger set of SNP instruments also enabled better assessment of potential bias in MR findings through the use of alternative MR models which allow inference under a range of different assumptions (heterogeneity of effect sizes, horizontal pleiotropy).

This study had some limitations. While genetically derived estimates are unlikely to be affected by confounding, the magnitude of association between these genetic instruments and estimated standard drinks detected in GWAS analyses relies on the accuracy of self-reported data, which may contain self-report bias. This might also apply to the UKBB with known healthy volunteer bias[43], in which the genetic instruments were derived. In recent years, investigators have used multi-instrument MR experiments due to availability of genetic data on large cohorts. The multi-instrument approach is expected to minimise the standard errors around the causal estimates (relative to just a single SNP), although in practice we only found this to be the case when one heterogeneous SNP was discarded

using MR-PRESSO (Figure 3). Furthermore, the strength of our instruments (explaining ~1% of variance) remain insufficient to detect small ORs and make further inference on causality, evident from the large degree of overlap in CIs between the observational and MR estimates. The presence of weak instruments in the multi-instrument MR can potentially bias the overall causal estimates towards the null [42], however this is unlikely the case given that our instruments satisfy the strong instrument criteria (median F-stat 22.2; Interquartile range: 19.5 - 30.3) and most of our variants show evidence of replication in the recent combined-sex drinks/week GWAS from GSCAN [31]. Of the 98 associations reported in the GSCAN alcohol GWAS meta-analysis[31], 95 replicated in our combined-sex alcohol GWAS in UKBB alone, suggesting that the genotype-alcohol associations used in this study are fairly stable. Results from alternative MR estimators that are robust against horizontal pleiotropy were similar to the IVW findings, although the confidence intervals were wider for those techniques. In contrast, assessing the rs1229984-only estimate remains informative because rs1229984 by itself is by far the strongest and most extensively studied instrument among the SNP set with well-studied biological insights to justify its association with alcohol consumption. Apart from the ADH1B variant, the biological pathways linking the SNP instruments with alcohol consumption are not well understood; for a trait like alcohol, unmeasured pleiotropy remains a concern as variants might consequently influence alcohol intake through changes in socio-economic status, cultural factors and other social behaviours. In our assessment for bias, the MR-Egger intercept for alcohol-EOC did not show any evidence of directional pleiotropy affecting our MR findings (Supplementary Table 16).

Earlier studies have suggested a link between acetaldehyde (ADH) and cancer cell growth [44,45], but it is unclear whether these associations were mainly driven by a change in alcohol consumption. Disentangling the complex effects of the rs1229984 variant is difficult, as previous studies have shown that the variant is associated with esophageal carcinoma [46], potentially due to accumulation of acetaldehyde among minor allele carriers although our single SNP MR analyses using the rs1229984

(*ADH1B*) variant find weak evidence for an association with breast cancer risk. Our PheWAS findings on rs1229984 found no strong evidence that the *ADH1B* instrument was associated with potential risk factors linked with breast or ovarian cancer that are unlikely to be mediated through increased alcohol consumption (Supplementary Table 17). However, we cannot exclude the possibility of rs1229984 being associated with other factors related to carcinogenesis and unmeasured confounders. We are unable to assess whether our MR causal inference remain consistent when we conservatively excluded rs1229984 from the main analyses, as it resulted in wide confidence intervals on the estimate (rs1229984 being the instrument that explains the highest amount of genetic variance, Supplementary Table 3).

For our observational analyses, selection bias might be present for the study cohorts if participants are more healthy than non-participants. Our reliance of self-reported consumption data for the observational analyses is vulnerable to recall error, and the definition of standard drinks may differ slightly across regions, contributing to higher heterogeneity in our exposures. In the covariate-adjusted model, the power to detect meaningful associations were hindered by a large degree of missingness on information for the covariates, especially the UK Biobank cohort where the number of cancer events from the multivariable-adjusted analyses were essentially halved. Our multiple imputation analyses recovered valuable information on both smoking pack/years and duration of hormone-replacement therapy and in the pooled regression estimate adjusted for these covariates in the imputed dataset there was a small degree of attenuation of the effect size between alcohol and both cancers (towards the null). Whilst the regression analysis on the imputed dataset characterises confounding better, the estimates based on imputed data (with twice the number of events) were not meaningfully different to the original multivariable adjusted findings for the UKB (Supplementary Table 9) and our conclusions remain essentially unchanged.

When we compare the observational findings across different cohorts, the breast cancer estimates for one standard drink/day increase from the Copenhagen cohorts and KARMA were broadly consistent with the overwhelming evidence from previous studies. However the estimated HR from the UKB cohort is slightly lower (HR 1.04). This might be explained by the healthy-volunteer selection bias in the recruitment for the UKB cohort, resulting in under-estimation of the true effect size. Conversely, genetic estimates are conceivably less affected by these biases, but they can be vulnerable to biases in the presence of horizontal pleiotropy. We performed sensitivity analyses based on filtering out SNPs with heterogeneous causal effects to reduce the chances of horizontal pleiotropy biasing the estimate including the use of MVMR to adjust for confounding risk factors, although in practice this made no meaningful changes to our results. If participants in the genetic study under-reported their true alcohol consumption this may lead to an underestimate of the SNP-alcohol effect sizes, resulting in an inflated estimate from the MR analysis. If the SNP-alcohol effect sizes were underestimated, it would suggest more strongly that alcohol intake is not causally associated with breast cancer. Finally, our risk estimates from the observational and MR analyses were on similar but not identical scales (i.e. OR versus HR); however for low prevalence outcomes any discrepancy is likely to be small. Individuals with high alcohol consumption are less likely to participate in the studies included, and competing risks after study participation may have influenced our estimates of the effect of risk factor on outcome, but since the observational studies largely reproduced results from other observational studies, this bias is likely to have been small.

The MR estimates for alcohol and risk of breast cancer or EOC remain valid under the assumption that alcohol consumption and log(OR) of these disease outcomes have a linear relationship. This is a strong assumption, given previous speculation about a J-shaped relationship between alcohol and other disease outcomes (e.g. cardiovascular diseases) where abstainers are at higher risk similar to those drinking more than moderate amounts [47,48]. Despite our inability to perform MR-by-stratum

(evaluating effect of genetically predicted alcohol consumption on risk of disease at various consumption levels) due to insufficient sample size, our observational findings show little evidence that the relationship between alcohol intake and these cancers is non-linear. Given that the alcohol variants (such as rs1229984) might predict both drinker status and quantity consumed, modelling the MR association within drinkers-only might potentially induce collider bias [49].

Taken together, Mendelian randomization analyses are not inconsistent with findings from several cohort studies showing that moderate alcohol consumption is associated with a modest increase in risk of breast cancer (upper 95% CI of OR on breast cancer being 1.10 for a genetically predicted 1 drink per day increase). For EOC alcohol intake is unlikely to have anything other than a very small effect on risk.

Additional information:

Ethics statement:

The UK Biobank study was approved by the National Research Ethics Service Committee North West—Haydock, whereby all study procedures were performed in accordance with the World Medical Association Declaration of Helsinki ethical principles for medical research. The CCHS and CGPS studies were approved by the Herlev and Gentofte Hospital and by a Danish ethical committee (H-KF-01-144/01), and was conducted according to the Declaration of Helsinki. Finally, the KARMA study was approved by the ethical board at the Karolinska Institute.

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Authorship:

SM, EMD, PMW, GCT, SEB and PH designed the study and obtained funding. JSO performed the UKB analyses. ME provided data for the KARMA study cohort. SEB provided data for the Copenhagen City Heart Study and Copenhagen General Population Study. JSO, JA, EMD, ME, SEB and SM analysed the data. JSO, SM, SEB, PMW and GCT wrote the first draft of the paper. LEK, KM, PP, AB, DFE and LDH provided critical feedback on the manuscript. All authors contributed to the final version of the paper.

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Conflict of interest:

The authors declare no conflict of interest.

Data availability statement:

Individual level genetic and phenotypic data of UK Biobank participants are available at http://biobank.ctsu.ox.ac.uk through formal application. GWAS meta-analyses data for BCAC and OCAC were publically available and downloaded from the corresponding consortium sites. The authors declare that summary statistics data supporting the findings of this study are available within the paper and its supplementary information files.

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Figure Legends

Figure 1. Schematic diagram illustrating the Mendelian randomization (MR) framework for the main analysis.

Figure 2 Meta-analysis of the observational hazard ratio estimates for daily alcohol consumption on breast and ovarian cancer. Estimates were adjusted for BMI, oral contraceptive use, nulliparity, physical activity and education attainment. Please refer to supplementary table 7 for the estimated HR adjusted for age only. There is no strong evidence for effect heterogeneity ($p>0.1$) among estimates from each of these studies.

Figure 3. Mendelian randomization estimates for the relationship between alcohol consumption and risk of breast/ovarian cancers. The confidence interval around the estimates narrowed down after we removed SNPs via the outlier test in MR-PRESSO.

Figure 4. Comparison of observational and genetic (MR) estimates for the association between standard drink per day with breast and ovarian cancer risk. Observational HR estimates were obtained via fixed effect meta-analysis of the studies used in the main analysis. The MR-PRESSO outlier-adjusted estimates were reported here as the MR-analysis findings.

Figure 5. Scatter plot for the genetic association between alcohol drinks/week SNP instruments and risk of breast and ovarian cancers. The slope of the fitted line in the scatter plots reflect the MR causal estimates for each type of MR estimator. The scatter plot shows the association of a genetically predicted one standard drinks/week increase on log(OR) of the outcome (cancer) risk inferred via each alcohol SNP instrument. The panel (A) refers to the plot for overall EOC; (B) refers to the plot for the risk of overall breast. For both plots, the right-most point refers to the rs1229984 SNP estimate. The forest plot for the individual SNP estimates along with the leave-one-SNP-out MR forest plot is shown in Supplementary Figure 3 and 4.

Supplementary Material for manuscript "Evaluating the role of alcohol consumption in breast and ovarian cancer susceptibility using population-based cohort studies and two-sample Mendelian randomization analyses"

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Supplementary materials and methods

Method 1. Estimating phenotypic variance tagged by genetic variants

For a set of k SNP instruments and phenotype of interest Y, we used the following equation to estimate the total phenotypic variance explained by instruments:

$$r^2 = \sum_{i=1}^{k} 2p_i(1-p_i)\beta_i^2 \, / Var(Y)$$

Where p_i and β_i refers to the minor allele frequency and the magnitude of association of the i-th SNP.

Method 2. Deriving number of alcoholic standard drinks in UK Biobank

Information on *quantity* and *frequency* of alcohol consumption, was obtained through self-report questionnaires in the UKB. Frequency of consumption (AC-Frequency) was assessed in 501,718 participants (UKB field IDs: 1558) with the item "About how often do you drink alcohol?". Frequency was originally assessed at a scale ranging from 1 (daily or almost daily) to 6 (never), but was recoded so that a lower score represented less frequent drinking. For individuals who reported multiple instances (via multiple visits) of alcohol intake, only the first assessment was used.

In those who drank at least once or twice a week, information on quantity of consumption (AC-Quantity) was assessed (*n*=348,039). AC-Quantity was assessed based on the average weekly alcohol intake for five general classes: red wine (1568), champagne plus white wine (1578), spirits (1598), beer plus cider (1558), and fortified wine (1608). The following item was used: "In an average WEEK, how many servings of {class of alcohol} would you drink?". To combine the different classes of alcohol, we followed the procedures developed by Clarke et al. (2) with some minor changes, as discussed below. To calculate the total number of alcohol standard drinks, the number of reported drinks was multiplied with a conversion factor depending on the class of alcohol (Supplementary Table 1). For the less-frequent drinkers, we repeated the same procedure using equivalent assessments available for their monthly (instead of weekly) quantity. These values were converted to weekly units by dividing by 4.3 (~30 days/7). The average value for total drinks/day was then calculated for each frequency category (see Supplementary Table 2). We subsequently identified outliers as those who had a score that deviated >5 SD from the average in each female drinker category.

We then imputed the missing values with the sex-specific average of total standard drinks/week for each of the 6 frequency categories. This allowed us to utilise data from the maximum number of female participants for the GWAS analyses, further improving the statistical power to detect robust genetic instruments for alcohol intake. Finally, we selected only individuals of white-British ancestry based on clustering via ancestral principal components and performed the GWAS analyses as per description in

the main text. Unlike Clarke et al. (2), we did not include weight (in kg) as a covariate in the GWAS model.

Method 3. Observational data for the Copenhagen General Population Study and the Copenhagen City Heart Study

We performed cox regression analyses of the 69,420 women to evaluate the relationship between daily number of standard alcohol drinks of 10 g alcohol per day on breast cancer risk or ovarian cancer risk, adjusted for cohort, age, parity, use of contraceptives, hormone therapy, daily and cumulative tobacco use, height, weight, BMI, nulliparity and menopausal status. Follow up began at date of examination, and ended at next examination, date of first breast or ovarian cancer diagnosis, date of death, or end of follow up (December 31st 2016), whichever came first. Each woman from CCHS could contribute with up to 4 observations, depending on the number of examinations of that woman. Recurrent events were not considered. Women with event prior to entry were excluded from that particular analysis. Analyses were performed using STATA 13.1 SE.

Method 4. Observational data for the Karolinska Mammography Project for Risk Prediction of Breast Cancer (KARMA).

In the KARMA study, alcohol drinking behaviour of the participants was captured through diet questionnaires, along with information on other breast cancer related risk factors (Parity, age at menarche, anthropometric traits, smoking and menopausal status). Self-reported alcohol intake (in grams) estimated via questionnaires was standardised into number of standard drinks/week using a nominal conversion scale of 10g/standard drink. All individuals diagnosed with breast/ovarian cancer prior to recruitment were excluded. In total, 60,903 women (985 incident breast cancer cases) with non-missing data on confounders were used for the KARMA observational analyses. Among the 985 cases, 867 were ER+ while the remaining 118 were ER-. We derived both a crude estimate adjusting for only age, and a multivariable-adjusted estimate accounting for parity, age at menarche, BMI, height, hip circumference, smoking and menopausal status. The Software SAS v9.4 was used for the KARMA observational analyses.

Method 5. Modelling the association between alcohol and BrCa/OvCa in UK Biobank

Using our derived alcohol intake phenotype (estimated standard drinks per week), we used Cox proportional hazards models to quantify the association between alcohol consumption and breast/ovarian cancer risk. All cancers diagnosed prior to recruitment (prevalent cases) were excluded, retaining only 427 ovarian cancer and 4,081 breast cancer incident cases (cohort size, n=145,089 women) all of which are filtered to be genetically unrelated. The crude model adjusts for only top 10 ancestral principal components, with age at last follow-up as the underlying time variable. The adjusted model (complete_model) additionally included the following covariates: number of live births, cigarette pack years, smoking status (former, current, non-smoker), coffee intake, education attainment, BMI, height, physical activity, age at menarche and menopausal status. Individuals that are cryptically related

were removed by filtering individual-pairs (and retaining only one of each) with a strong degree of genetic relatedness ($\hat{\pi} > 0.2$) estimated through genetic data. The sample size for the adjusted model were lower due to missing data on some of the covariables (See Supplementary Table 7). The Cox proportional hazard models were implemented using Surv() in R.

Method 6. Sensitivity analyses for the Mendelian randomization study

Weak violation of the MR assumptions (such as SNP-confounding and invalid instruments) can severely bias MR inferences. To address these issues, we performed sensitivity analyses using alternative MR models (MR Egger, Weighted median, MR-PRESSO) that are least affected by these violations. The MR-Egger intercepts were computed to evaluate evidence of directional pleiotropy, which would be captured by a non-zero intercept estimate. To generate a reliable causal estimate, we attempted to screen for heterogeneity in MR causal estimates through the global and individual SNP heterogeneity Cochran Q test (Supplementary Table 10). We conservatively excluded any SNPs with a Q-score greater than 3.84 (3). These estimates are provided in Supplementary Table 11. In the main text, we included the MR-PRESSO estimates alongside the IVW estimate to provide an estimate of the MR association that are robust against SNP-heterogeneity. Furthermore, leave-one-out MR analyses (MR estimates leaving out one SNP at a time) were also used to detect outliers in our MR estimates. For the single instrument (rs1229984) MR analyses, the causal estimate was computed using a wald-type ratio estimator. We further performed a phenome-wide association scan (PheWAS) on the rs1229984 SNP, which is the strongest alcohol-associated genetic variant against publicly available GWAS datasets to assess potential pleiotropic association with known confounders. The Steiger-Z test was also used to perform MR directionality test to ensure SNP instruments are acting on the correct hypothesized causal pathway and avoid instrument mis-specification.

For the MR-PRESSO analysis, each trait (cancer outcomes) was performed using the mr_presso() function in the mr-presso R package (4), with default setting at Nb=5000 iterations, enabled distortion test, enabled outlier test and applied an outlier-significance threshold of 0.05. SNP alleles were harmonized prior to running these analyses via the harmonize() feature in the MR-Base R-package. All sensitivity analyses apart from the MR-PRESSO tests were performed using the MendelianRandomization and MR-Base R package (5,6).

Method 7. Multivariable Mendelian randomization analyses

When the exposure of interest is correlated with various other risk factors, MR estimates can be severely biased by horizontal pleiotropy. However, some of the SNP association on these risk factors might manifest in the same causal pathway, where manual removal of these variants would reduce statistical power for MR. The multivariable MR model can hence be used to evaluate the direct effect between our exposure of interest on the outcome while adjusting the genetic effect our SNPs exert on a

set of risk factors. This is implemented through the mv_multiple() function curated within the TwoSampleMR R package. The risk factors that we adjusted for includes BMI, BMI-adjusted waist-to-hip ratio (WHR), education attainment and cigarette smoked per day, all of which had data available in the UK Biobank cohort. We performed the GWAS for BMI and WHR using identical procedure to those described in previous work (Gharahkhani et al. 2019). We recoded education attainment as an ordinary variable, with the lowest score reflecting lowest attained academic qualification, with individuals that reported to have "Other professional qualification" excluded from the GWAS analysis. The variable is then ranked-transformed to allow effect size to be interpreted in changes in SD units. For cigarette per day, we excluded individuals reported to have smoked more than 99 cigarettes per day but retained individuals that reported zero consumption levels. For both education attainment and cigarette/day, the GWAS was performed on 438,609 white British individuals in the UK Biobank using the BOLT-LMM software, adjusting for recruitment age, sex and top 10 ancestral principal components. The effect estimate of the alcohol instruments on these risk factors obtained directly from the GWAS summary statistics were then applied into the MVMR model.

Method 8. Imputation of missing covariate data in UK Biobank observational analyses

In our primary cox-regression analyses, participants with missing information on any of the covariates are removed from the analyses. For the UK Biobank cohort, this eventually resulted in a much lower sample size (cases were essentially halved) for the multifactor-adjusted cox regression analyses. To be able to include additional samples, we imputed missing confounders via multiple imputation analyses in R. We extracted a collection of phenotypes on socio-economic status, sexual development, and female sex-specific risk factors (see list below). The pairwise correlation between these phenotypes and the covariates (for imputation) are shown in Supplementary Figure 5.

Finalised Variables considered in the multiple imputation analyses including auxiliary variables (AX):
"Ever smoked", "Age at menarche", "Duration of daily moderate physical activity", "Duration of daily vigorous physical activity", "coffee cups/day", "drink temperature (AX) ", "Townsend deprivation index", "Duration of hormone replacement therapy (HRT)", "Ever had HRT", "Menopausal status", "Smoking pack years", "Overall health rating (AX)", "Body mass index", "Frequency of visit from friends and family (AX)", "Number of live births", "Age at first sexual intercourse (AX)", "Renting or privately owns an apartment (AX)", "Academic qualifications", "Age completed full time education (AX)", "Number of medications (AX)", "Number of vehicles in the household (AX)", "Birth weight (AX)", "Height"

Before we can proceed to impute the missing values on the covariates, we first extracted a series of auxiliary variables from the UK Biobank to assess whether missingness on any of these covariate of interests can be predicted by these auxiliary variables (along with the other covariates with low missingness). This can be done via performing a logistic regression fitting all auxiliary variables against the missingness status of the covariate (i.e. we defined a 'missingness status' phenotype where we recoded missing values as 1, and non-missing values as 0 for the trait under assessment). We performed this check for each covariate we intend to impute, to help assess plausibility of the Missing-at-random

(MAR) assumption prior to multiple imputation. In our dataset, the missingness for each of our covariate of interests can be predicted by atleast one auxiliary variable (average predictors ~ 3), hence there is strong evidence to support the MAR assumption on the pattern of missingness for these covariates. We also compared these predictability estimates against those from the predictor matrix derived from the MiCE initiation process (accessible via imp$predictorMatrix upon executing "imp=mice(dat,m=1,max_iter=0,..))". The comparison of the predictability of each covariate and the specific models used for the imputation of the trait is summarized in Table M8. We did NOT impute our outcome (cancer diagnosis) and exposure (alcohol drinks/week) phenotype to prevent bias from circular associations – these variables were manually omitted from the multiple imputation process.

The missing data imputation was performed using mice() from the MiCE R package for missing data imputation (available at https://github.com/stefvanbuuren/mice). The process was set for a maximum of 5 iterations, generating 5 distinct datasets with pseudo-random seed set at seed=123. We applied different imputation models to impute covariate data of varying characteristics: the predictive mean matching model (*pmm)* for quantitative trait, logistic regression (*logreg)* for binary trait, proportional odds model (plr) for ordered categorical traits and polytomous regression (*polyreg)* for ordered/unordered categorical traits. Cox proportional hazard estimates from the 5 distinct imputed datasets were then computed using Surv() and then pooled together via the pool() function in MiCE. The pooled estimates were finally compared against the original estimate (multivariable adjusted Cox-model) for both cancers to evaluate potential difference in observational findings. Note that the multiple imputation analysis was performed on the entire set of UK Biobank female participants used in the main analysis but were evaluated separately for both cancers.

Table M8. Evaluation of missingness in covariate data in the UK Biobank breast/ovarian cancer cohort

Variable	Data structure	Proportion of missing values		Predictable from auxiliary variables	Number of predictors (based on MiCE prediction matrix)	Number of predictors (based on missingness regression)	Imputation model	Used in analyses
		Breast cancer UKB cohort	Ovarian cancer UKB cohort					
Duration of Hormone replacement therapy	Continuous	0.732	0.713	Yes	3	6	pmm	N/A
Cigarettes smoked in pack years	Continuous	0.361	0.352	Yes	12	5	pmm	N/A
Qualification	Categorical (ordered)	0.172	0.167	Yes	13	8	polr	Yes
Coffee consumption (cup/day)	Continuous	0.073	0.071	Yes	1	2	pmm	Yes
Duration of daily moderate physical activity	Categorical (ordered)	0.055	0.054	Yes	3	1	polr	Yes
Duration of daily vigorous physical activity	Categorical (ordered)	0.051	0.049	Yes	3	2	polr	Yes
Age at menarche	Continuous	0.029	0.028	Yes	1	1	pmm	Yes
Ever smoked	Binary	0.003	0.003	Yes	3	3	logreg	Yes
BMI	Continuous	0.003	0.002	Yes	11	3	pmm	Yes
Height	Continuous	0.002	0.002	Yes	7	2	pmm	Yes
Menopausal status	Categorical (unordered)	0.002	0.001	Yes	7	3	polyreg	Yes
Number of live births	Continuous	0.001	0.001	Yes	8	4	pmm	Yes
Townsend deprivation index	Continuous	0.001	0.001	Yes	0	0	pmm	Yes
Participant ID	N/A	0	0	N/A	N/A	N/A	N/A	Reference only
Sex	N/A	0	0	N/A	N/A	N/A	N/A	N/A

The model “pmm” refers to the predictive mean matching model for quantitative traits, “polr” refers to proportional odds model for ordered categorical variable(s), “polyreg” refers to polytomous logistic regression for unordered categorical

variable(s) and “logreg” refers to logistic regression for binary coded variable(s). The performance of the logistic regression on missingness is poor (since ncases [missing=1] for traits with very low missingness is very small) apart from duration of HRT and cigarette smoked in pack years; note that these estimates are indicative of plausibility for MAR assumption only, since the multiple imputation process was wholly performed via the MiCE R package.

Supplementary tables

Supplementary Table 1. Conversion table for various alcoholic beverage into units of standard drinks.

Type of alcoholic beverage	UKB-ID for weekly; monthly	Standard drinks equivalence
Red wine	1568 ; 4407	1.67
White wine	1578 ; 4418	1.67
Fortitude wine	1608 ; 4451	2.25
Spirits	1598 ; 4440	1
Pint of beer	1588 ; 4429	2.3
Other alcoholic drinks	5364 ; 4462	1.1

Supplementary Table 2. Estimated average of total standard drinks per week across each consumption category among women in the UK Biobank cohort.

Alcohol frequency category	Description	Average no. of standard drinks/week
Category 1	Daily or almost daily	12.5
Category 2	3 or 4 times a week	8.0
Category 3	Once or twice a week	5.0
Category 4	1 or 3 times a month	0.8
Category 5	Special occasion only	0.5
Category 6	Never	0
Total	-	9.4

Supplementary Table 3. Phenotypic variance (weekly alcohol intake) explained by alcohol-associated SNP instruments used in MR analysis. The variance explained by instrument (r^2) was estimated using the formula provided in Supplementary methods. BETA refer to the magnitude of association on estimated standard drinks/week per effect allele (EA) of the SNP. EAF is the frequency of the effect allele. The variance for drinks/week was estimated to be 256.67 (across both sexes) and 114.60 (in females). Only SNPs with P<1e-5 in the female-only GWAS are used as instruments for the MR analyses.

SNP	CHR	EA	NEA	Estimated across both sexes				Estimated in females only			
				EAF	BETA	P-value	r^2	EAF	BETA	P-value	r^2
rs1229984	4	T	C	0.02	-3.421	1.10E-218	0.0020	0.02	-2.471	6.80E-121	0.0023
rs1260326	2	T	C	0.39	-0.529	3.50E-58	0.0005	0.39	-0.315	4.60E-23	0.0004
rs11940694	4	A	G	0.39	-0.518	9.70E-55	0.0005	0.39	-0.342	1.40E-26	0.0005
rs13107325	4	C	T	0.93	0.706	4.50E-30	0.0003	0.93	0.300	6.70E-07	0.0001
rs1302808	4	C	A	0.80	-0.446	1.90E-27	0.0002	0.80	-0.318	6.50E-16	0.0003
rs62055546	17	A	C	0.78	0.401	1.50E-24	0.0002	0.78	0.258	1.10E-11	0.0002
rs1004787	2	G	A	0.47	-0.310	9.90E-22	0.0002	0.47	-0.235	1.10E-13	0.0002
rs11604680	11	A	G	0.68	0.305	5.50E-19	0.0002	0.68	0.167	5.80E-07	0.0001
rs56094641	16	A	G	0.60	0.251	2.30E-14	0.0001	0.60	0.072	2.20E-02	0.0000
rs61873510	10	G	T	0.67	0.263	5.70E-14	0.0001	0.67	0.181	5.80E-08	0.0001
rs9822731	3	T	C	0.78	-0.288	7.80E-14	0.0001	0.78	-0.189	2.40E-07	0.0001
rs485425	11	C	G	0.45	-0.229	5.30E-13	0.0001	0.45	-0.141	3.40E-06	0.0001
rs4630328	11	G	A	0.62	0.237	6.60E-13	0.0001	0.62	0.228	4.30E-13	0.0002
rs113443718	16	G	A	0.69	0.251	9.80E-13	0.0001	0.69	0.192	1.90E-08	0.0001
rs378421	16	G	A	0.58	0.228	1.40E-12	0.0001	0.58	0.181	2.00E-08	0.0001
rs838145	19	G	A	0.46	0.229	2.60E-12	0.0001	0.46	0.216	4.70E-12	0.0002
rs6969458	7	G	A	0.53	-0.227	5.00E-12	0.0001	0.53	-0.182	6.40E-09	0.0001
rs75199129	2	A	T	0.95	0.510	1.00E-11	0.0001	0.95	0.320	7.90E-06	0.0001

rs13413953	2	T	G	0.64	0.228	1.60E-11	0.0001	0.64	0.168	1.80E-07	0.0001
rs28929474	14	C	T	0.98	0.755	2.30E-11	0.0001	0.98	0.389	4.90E-04	0.0001
rs4480324	1	A	G	0.30	0.239	2.60E-11	0.0001	0.29	0.145	3.80E-05	0.0001
rs12124523	1	C	T	0.89	0.340	2.80E-11	0.0001	0.89	0.178	2.70E-04	0.0001
rs74424378	9	T	G	0.76	0.251	3.40E-11	0.0001	0.76	0.208	1.90E-08	0.0001
rs77294902	17	G	A	0.78	0.252	5.00E-11	0.0001	0.78	0.161	1.50E-05	0.0001
rs6452788	5	G	A	0.76	-0.249	5.20E-11	0.0001	0.76	-0.223	8.20E-10	0.0002
rs7786376	7	A	G	0.72	-0.240	6.30E-11	0.0001	0.72	-0.193	4.90E-08	0.0001
rs7132908	12	G	A	0.62	0.206	1.50E-10	0.0001	0.62	0.119	1.80E-04	0.0001
rs11860773	16	T	C	0.80	0.264	1.60E-10	0.0001	0.80	0.204	1.40E-07	0.0001
rs4815366	20	G	T	0.36	-0.213	1.60E-10	0.0001	0.36	-0.162	5.90E-07	0.0001
rs2858088	4	A	G	0.38	-0.208	3.50E-10	0.0001	0.38	-0.129	5.00E-05	0.0001
rs2959005	15	C	T	0.33	-0.216	4.10E-10	0.0001	0.33	-0.100	2.10E-03	0.0000
rs9349379	6	A	G	0.59	-0.207	4.30E-10	0.0001	0.59	-0.146	4.30E-06	0.0001
rs748919	11	T	C	0.79	0.245	4.60E-10	0.0001	0.79	0.131	4.90E-04	0.0000
rs113441031	16	C	T	0.83	0.266	6.40E-10	0.0001	0.83	0.135	1.40E-03	0.0000
rs109536	9	G	C	0.73	-0.222	6.50E-10	0.0001	0.73	-0.102	2.70E-03	0.0000
rs2717053	2	G	C	0.37	-0.205	8.20E-10	0.0001	0.37	-0.074	2.10E-02	0.0000
rs35572189	17	G	A	0.64	-0.205	8.80E-10	0.0001	0.64	-0.089	5.40E-03	0.0000
rs2274793	14	C	T	0.67	0.210	9.20E-10	0.0001	0.67	0.154	1.70E-06	0.0001
rs147711594	3	G	T	0.98	0.651	9.60E-10	0.0001	0.98	0.416	2.90E-05	0.0001
rs322764	7	G	A	0.44	-0.197	1.00E-09	0.0001	0.44	-0.131	3.70E-05	0.0001
rs11692435	2	G	A	0.92	-0.364	1.10E-09	0.0001	0.92	-0.156	4.80E-03	0.0000
rs7940127	11	T	C	0.14	-0.281	1.10E-09	0.0001	0.14	-0.144	1.30E-03	0.0000
rs11648570	16	T	C	0.89	-0.318	1.30E-09	0.0001	0.89	-0.243	1.80E-06	0.0001

rs72726477	4	G	A	0.88	0.304	1.30E-09	0.0001	0.88	0.136	4.40E-03	0.0000
rs75543135	19	T	A	0.88	-0.309	1.70E-09	0.0001	0.88	-0.081	8.90E-02	0.0000
rs56197131	7	G	A	0.80	0.239	2.70E-09	0.0001	0.80	0.170	1.20E-05	0.0001
rs17177078	16	C	T	0.94	0.421	3.10E-09	0.0001	0.94	0.297	1.20E-05	0.0001
rs324012	12	C	T	0.55	-0.194	3.20E-09	0.0001	0.55	-0.084	8.40E-03	0.0000
rs142687608	16	A	G	0.98	0.661	7.50E-09	0.0001	0.98	0.508	5.30E-06	0.0001
rs6136465	20	G	A	0.60	0.190	8.00E-09	0.0001	0.60	0.124	6.10E-05	0.0001
rs7630012	3	A	G	0.57	0.186	8.00E-09	0.0001	0.57	0.165	2.00E-07	0.0001
rs77123275	9	C	T	0.95	-0.415	8.10E-09	0.0001	0.95	-0.284	5.70E-05	0.0001
rs3809162	12	A	G	0.59	-0.185	8.30E-09	0.0001	0.59	-0.195	6.40E-10	0.0002
rs28601761	8	C	G	0.58	-0.193	8.40E-09	0.0001	0.58	-0.103	1.60E-03	0.0000
rs9639559	7	C	T	0.28	-0.213	8.40E-09	0.0001	0.27	-0.136	9.70E-05	0.0001
rs4775792	15	T	G	0.37	0.192	8.50E-09	0.0001	0.37	0.094	4.50E-03	0.0000
rs17446532	9	C	T	0.51	-0.182	8.80E-09	0.0001	0.51	-0.106	8.20E-04	0.0000
rs12899560	15	C	T	0.59	-0.195	9.60E-09	0.0001	0.59	-0.096	2.20E-03	0.0000
rs11773627	7	T	C	0.81	-0.238	9.70E-09	0.0001	0.81	-0.093	2.10E-02	0.0000
rs2117760	3	C	A	0.71	0.198	1.00E-08	0.0001	0.71	0.180	1.40E-07	0.0001
rs7673993	4	A	G	0.58	-0.187	1.10E-08	0.0001	0.58	-0.161	3.60E-07	0.0001
rs142488468	10	G	C	0.82	0.238	1.30E-08	0.0001	0.82	0.146	1.90E-04	0.0001
rs7499750	16	A	C	0.22	0.217	1.40E-08	0.0001	0.23	0.170	6.00E-06	0.0001
rs2584448	4	T	G	0.43	-0.187	1.70E-08	0.0001	0.43	-0.159	5.00E-07	0.0001
rs17884691	22	G	A	0.75	0.208	2.00E-08	0.0001	0.75	0.155	2.60E-05	0.0001
rs756747	7	T	G	0.48	-0.183	2.00E-08	0.0001	0.48	-0.111	2.30E-04	0.0001
rs11030084	11	C	T	0.81	0.228	2.20E-08	0.0001	0.81	0.113	3.30E-03	0.0000
rs1713675	11	A	G	0.51	-0.183	2.40E-08	0.0001	0.51	-0.132	4.40E-05	0.0001

rs2756185	6	G	A	0.93	0.337	2.60E-08	0.0001	0.93	0.066	2.10E-01	0.0000
rs11079849	17	C	T	0.67	-0.195	2.80E-08	0.0001	0.67	-0.111	9.20E-04	0.0000
rs118784	5	T	A	0.50	-0.178	3.20E-08	0.0001	0.50	-0.110	3.30E-04	0.0001
rs78621285	16	A	T	0.91	0.312	3.20E-08	0.0001	0.91	0.175	1.10E-03	0.0000
rs1788820	18	A	G	0.35	-0.186	3.40E-08	0.0001	0.34	-0.156	1.70E-06	0.0001
rs2068650	5	A	C	0.53	0.178	3.70E-08	0.0001	0.53	0.145	2.60E-06	0.0001
rs6690101	1	T	C	0.46	-0.178	3.90E-08	0.0001	0.46	-0.114	2.80E-04	0.0001
rs1788030	18	C	T	0.54	0.179	4.70E-08	0.0001	0.54	0.096	3.00E-03	0.0000
rs11090045	22	G	A	0.69	0.194	4.80E-08	0.0001	0.69	0.129	1.50E-04	0.0001
Combined							**0.0094**				**0.0095**

Supplementary Table 4. Power calculation for MR analysis

Outcome	Number of Controls	Number of cases	OR>1.15	OR>1.20	OR>1.30
Breast cancer	105974	122977	0.91	0.99	0.99
Ovarian cancer	40941	22406	0.4	0.62	0.91

Power for MR analysis estimated for a 1SD change in alcohol intake (~1.4 stand drinks/day) using mRnd power calculator (https://shiny.cnsgenomics.com/mRnd/). Variance explained by instrument is set to be r^2=0.95%.

Supplementary Table 5. Estimated hazard ratios for the association between daily alcohol consumption and breast and ovarian cancer in the CCHS+CGPS for different consumption levels.

Breast cancer	**Total sample size**	**HR (adjusted model)**	**P-value**
Daily number of drinks (1 drink=12g)			
0.0-0.9	44451	0.91 (0.82, 1.00)	0.05
1.0-1.9	21435	REF	-
2.0-2.9	8156	1.11 (0.97, 1.27)	0.13
3.0-3.0	2765	1.24 (1.02, 1.51)	0.03
4.0-4.9	871	1.38 (1.00, 1.90)	0.05
5.0-9.9	550	1.36 (0.91, 2.03)	0.13
>=10	38	4.3 (1.61, 11.49)	0.004
			P-trend= 0.02
Ovarian cancer			
Daily number of drinks (1 drink=12g)			
0.0-0.9	45601	1.19 (0.91, 1.55)	0.22
1.0-1.9	22242	REF	
2.0-2.9	8510	1.19 (0.81, 1.74)	0.37
3.0-3.0	2873	0.62 (0.29, 1.35)	0.23
4.0-4.9	916	2.89 (1.50, 5.58)	0.002
5.0-9.9	566	1.87 (0.69, 5.12)	0.22
>=10	no events		
			P-trend=0.32

Analyses used age as the underlying time, and were adjusted for cohort (CCHS or CGPS), birth year, and examination year. For both cancers, final consumption category (>=10) was omitted from trend test.

Supplementary Table 6. Estimated hazard ratio for the association between daily alcohol drinks with breast cancer in the KARMA study for different consumption levels.

Study	Category (daily alcohol intake, in grams)	HR (only age adjusted)	HR (full adjusted model)
KARMA	0.1-9.9g	1.13 (0.93,1.36)	1.10 (0.91,1.34)
KARMA	10-20g	1.18 (0.79,1.76)	1.14 (0.76,1.69)
KARMA	20-30g	1.30 (0.62,2.74)	1.25 (0.60,2.64)

Supplementary Table 7. Estimated hazard ratio for the association between daily alcohol drinks with breast cancer in the KARMA study stratified by ER status of breast cancer.

Study	ER Status	Events	HR (only age adjusted)	HR (full adjusted model)
KARMA	All breast cancer	985	1.09 (0.99,1.20)	1.07 (0.97,1.19)
KARMA	ER+ breast cancer	867	1.10 (0.99,1.23)	1.09 (0.98,1.21)
KARMA	ER- breast cancer	118	0.97 (0.73,1.30)	0.99 (0.74,1.34)

Supplementary Table 8. Comparison of age- and fully-adjusted hazard ratios for the association between daily alcohol intake and breast and ovarian cancer risk for each study. Age was used as the underlying time variable. Participants were censored at the last visit, death or event, whichever came first.

	Only age adjustment			Multifactorial adjustment		
Study	**Events**	**Participants**	**HR_age adjusted**	**Events**	**Participants**	**HR_fully adjusted**
Breast						
KARMA	985	59918	1.12 (1.01,1.24)	985	59918	1.07 (0.97, 1.19)
CCHS+CGPS	2312	65803	1.07 (1.04, 1.10)	2055	63560	1.09 (1.05, 1.13)
UK Biobank	4081	141008	1.08 (1.06, 1.10)	1787	64622	1.04 (1.01, 1.07)
Ovarian						
CCHS+CGPS	327	67981	1.05 (0.97, 1.14)	287	62867	1.07 (0.99, 1.16)
UK Biobank	427	137394	0.97 (0.90, 1.04)	187	61267	0.92 (0.83, 1.03)

The crude HR model is adjusted for recruitment age, top 10 ancestral principal components (UKB only) and Townsend deprivation index (UKB only). The adjusted model incorporates the following additional covariates: coffee intake, BMI, height, smoking pack years, menopausal status, number of live births, ever smoked, education, duration of moderate and vigorous physical activities and age at menarche. Given that a sizeable proportion of the UK Biobank participants are cryptically related, estimates obtained from the UK Biobank had been adjusted for cryptic relatedness (i.e. related individuals removed within and between cancer cases and healthy individuals undiagnosed with any cancer).

Supplementary Table 9. Comparison of pooled HR estimates obtained through missing covariate data imputation against original estimates in the UK Biobank breast and ovarian cancer cohort.

Outcome	Events	Participants	HR (95% CI)	P-value
Age-adjusted HR model				
Breast cancer	4081	141008	1.08 (1.06, 1.10)	3.20E-15
Ovarian cancer	427	137394	0.97 (0.90, 1.04)	0.38
Multivariable-adjusted HR model				
Breast cancer	1787	64622	1.04 (1.01, 1.07)	0.01
Ovarian cancer	187	61267	0.92 (0.83, 1.03)	0.16
Pooled multivariable-adjusted HR model using imputed covariate data				
Breast cancer	4081	141008	1.02 (1.00, 1.03)	8.5E-3
Ovarian cancer	427	137394	0.94 (0.90, 0.99)	0.01

The crude HR model is adjusted for recruitment age, top 10 ancestral principal components (UKB only) and Townsend deprivation index (UKB only). The adjusted model incorporates the following additional covariates: coffee intake, BMI, height, smoking pack years, menopausal status, number of live births, ever smoked, education, duration of moderate and vigorous physical activities and age at menarche. The pooled HR estimates were averaged across 5 imputed datasets. Note that the cancer outcome of interest, recruitment age, and diagnosis age were not imputed.

Supplementary Table 10. Global test for heterogeneity on MR causal estimates. SNP-outliers are detected using the conventional Cochran Q-statistics (with df=1), where SNPs that have a heterogeneity score > 3.84 are filtered. The causal estimate derived from the filtered set of SNPs are reported in the main analysis.

		Before filtering heterogenous SNP-effects			***After filtering heterogenous SNP-effects***		
Trait	**Methods**	**nsnps**	**cochran Q-stats**	**Q-stat pvalue**	**nsnps**	**cochran Q-stats**	**Q-stat pvalue**
All BrCa	IVW(main)	34	82.4	2.53E-06	29	26.6	0.54
	MR-Egger	34	81.9	1.80E-06	29	26.6	0.49
All EOC	IVW(main)	34	73.7	6.19E-05	33	24.3	0.83
	MR-Egger	34	73.3	4.45E-05	33	22.8	0.86

The variant(s) that was dropped after heterogeneity filtering were rs11648570, rs2117760, rs61873510 and rs62055546 for the breast cancer MR analysis. The variant that was dropped was rs62055546 for the ovarian cancer MR analysis.

Supplementary Table 11. Comparison of MR estimates across alternative MR methods before and after filtering for SNP-heterogeneity. For MR-Egger(bootstrap), 1000 bootstrap iterations were performed to obtain reliable standard errors for the causal estimates. PWM stands for the penalized weighted median model.

Outcome	**MR-model**	***Before filtering for SNP-heterogeneity***					***After filtering for SNP-heterogeneity***			
		P-value	**OR**	**L_95CI**	**U_95CI**		**P-value**	**OR**	**L_95CI**	**U_95CI**
All Breast cancers	**MR-Egger**	0.96	1.00	0.86	1.17		0.83	0.99	0.90	1.09
	PWM	0.50	0.97	0.89	1.06		0.51	0.97	0.89	1.06
	IVW (random effect)	0.53	1.03	0.93	1.14		0.78	0.99	0.93	1.06
	IVW (fixed effect)	0.31	1.03	0.97	1.10		0.78	0.99	0.93	1.06
All EOC	**MR-Egger**	0.27	0.84	0.62	1.14		0.17	0.86	0.71	1.06
	PWM	0.07	0.84	0.70	1.01		0.003	0.73	0.59	0.90
	IVW (random effect)	0.23	0.89	0.73	1.08		0.39	0.95	0.85	1.07
	IVW (fixed effect)	0.07	0.89	0.78	1.01		0.46	0.95	0.83	1.08

Supplementary Table 12. Multivariable MR analysis adjusting for the effect on adiposity, smoking behaviour and education attainment. Changes in the MVMR estimate before and after filtering out SNPs with heterogeneous effect sizes indicate that the unadjusted estimates were largely driven by outliers. Estimated OR reflect the change in risk per one SD increase in the risk factors.

		Before filtering for heterogenous variants					After filtering for heterogenous variants				
Risk factors	Outcome	nsnp	pval	OR	CI_low	CI_upper	nsnp	pval	OR	CI_low	CI_upper
Cigarette/day	Breast cancer	34	0.02	1.03	1.01	1.06	30	0.00	1.03	1.01	1.05
Education attainment	Breast cancer	34	0.08	1.67	0.95	2.96	30	0.20	1.34	0.86	2.09
estimated standard drinks/week	Breast cancer	34	0.02	1.10	1.02	1.19	30	0.31	1.03	0.97	1.10
Waist-Hip Ratio adjusted for BMI	Breast cancer	34	0.00	0.45	0.29	0.70	30	0.03	0.69	0.49	0.97
BMI	Breast cancer	34	0.92	1.00	0.93	1.07	30	0.95	1.00	0.95	1.06
Cigarette/day	Ovarian cancer	34	0.19	1.04	0.98	1.11	33	0.01	1.05	1.01	1.09
Education attainment	Ovarian cancer	34	0.04	0.26	0.07	0.94	33	0.50	0.76	0.34	1.69
estimated standard drinks/week	Ovarian cancer	34	0.03	0.82	0.95	1.00	33	0.65	0.97	0.87	1.09
Waist-Hip Ratio adjusted for BMI	Ovarian cancer	34	0.42	1.53	0.55	4.26	33	0.11	0.59	0.31	1.13
BMI	Ovarian cancer	4	0.43	0.94	0.79	1.11	33	0.98	1.00	0.91	1.11

The variant(s) that was dropped after heterogeneity filtering were rs11648570, rs2117760, rs61873510 and rs62055546 for the breast cancer MR analysis. The variant that was dropped was rs62055546 for the ovarian cancer MR analysis. PheWAS analysis reveal that the dropped variant rs62055546 in both the breast and ovarian cancer analyses was strongly associated with changes in red blood cell count (http://geneatlas.roslin.ed.ac.uk/phewas/?variant=rs62055546&representation=table).

Supplementary Table 13. Comparison of instrument strength across various sets of instrument from UK Biobank drinks/week GWAS. The median value (and the interquartile range) for the partial F-statistics for each set of instruments were reported.

Test statistics	Alcohol intake (combined sex)	Alcohol intake (combined sex)	Alcohol intake (females only)
Number of SNPs	77	34	34
Total r2	0.009	0.006	0.007
Partial F-stat	30.31[26.6 - 37.0]	37.3 [29.3 - 46.3]	22.2 [19.5 - 30.3]
total F	50	75	41.7
Description	Variants identified from combined sex alcohol GWAS	Variants identified from combined sex GWAS that replicate in female only GWAS (p<1e-5)	Variants identified from combined sex GWAS that replicate in female only GWAS (p<1e-5), using EAF and effect sizes from female only GWAS

Supplementary Table 14. Evidence of replication of UKBB estimated drinks/week (females only) SNP-association in the GSCAN drinks/week GWAS summary statistics

SNP	CHR	NEA	EA	EAF	BETA_ GSCAN	SE_GSCAN	N_GSCAN	PVALUE_ GSCAN	PVAL_UKBB
rs1260326	2	T	C	0.60	0.024	0.002	532340	3.33E-33	4.60E-23
rs75199129	2	A	T	0.04	-0.026	0.005	513023	8.44E-09	7.90E-06
rs1004787	2	G	A	0.58	0.015	0.002	526940	3.31E-15	1.10E-13
rs13413953	2	T	G	0.34	-0.012	0.002	529000	5.35E-09	1.80E-07
rs7630012	3	A	G	0.46	-0.007	0.002	535602	0.000423	2.00E-07
rs2117760	3	C	A	0.28	-0.009	0.002	524866	1.01E-05	1.40E-07
rs9822731	3	T	C	0.22	0.017	0.002	531166	5.03E-14	2.40E-07
rs11940694	4	A	G	0.60	0.028	0.002	527865	3.11E-46	1.40E-26
rs7673993	4	A	G	0.41	0.012	0.002	529073	2.65E-09	3.60E-07
rs1229984	4	T	C	0.95	0.188	0.006	514602	1.60E-203	6.80E-121
rs1302808	4	C	A	0.19	0.024	0.002	516605	1.36E-23	6.50E-16
rs2584448	4	T	G	0.54	0.010	0.002	531331	1.83E-07	5.00E-07
rs13107325	4	C	T	0.07	-0.036	0.004	528164	1.23E-20	6.70E-07
rs6452788	5	G	A	0.27	0.012	0.002	535356	3.58E-07	8.20E-10
rs2068650	5	A	C	0.47	-0.009	0.002	527780	2.38E-06	2.60E-06
rs9349379	6	A	G	0.40	0.009	0.002	526515	4.90E-06	4.30E-06
rs7786376	7	A	G	0.27	0.011	0.002	525890	1.01E-06	4.90E-08
rs6969458	7	G	A	0.46	0.013	0.002	509646	5.20E-11	6.40E-09
rs74424378	9	T	G	0.26	-0.011	0.002	530826	1.72E-06	1.90E-08
rs61873510	10	G	T	0.31	-0.011	0.002	500397	8.83E-08	5.80E-08
rs11604680	11	A	G	0.32	-0.015	0.002	526748	2.87E-12	5.80E-07
rs4630328	11	G	A	0.35	-0.014	0.002	531293	3.80E-12	4.30E-13
rs485425	11	C	G	0.54	0.010	0.002	532602	2.13E-07	3.40E-06
rs3809162	12	A	G	0.41	0.010	0.002	527315	4.61E-07	6.40E-10
rs2274793	14	C	T	0.33	-0.012	0.002	531843	3.15E-09	1.70E-06
rs7499750	16	A	C	0.78	-0.009	0.002	534136	0.000192	6.00E-06
rs378421	16	G	A	0.41	-0.013	0.002	508328	2.26E-11	2.00E-08
rs113443718	16	G	A	0.28	-0.011	0.002	510879	5.26E-08	1.90E-08
rs142687608	16	A	G	0.02	-0.021	0.007	489914	0.00226	5.30E-06

rs11648570	16	T	C	0.11	0.019	0.003	526153	3.43E-09	1.80E-06
rs11860773	16	T	C	0.18	-0.015	0.002	515422	8.35E-10	1.40E-07
rs62055546	17	A	C	0.20	-0.020	0.002	529432	3.06E-17	1.10E-11
rs1788820	18	A	G	0.65	0.007	0.002	535226	0.000374	1.70E-06
rs838145	19	G	A	0.58	-0.016	0.002	521587	3.87E-16	4.70E-12
rs4815366	20	G	T	0.67	0.009	0.002	534788	2.70E-06	5.90E-07

Supplementary Table 15. MR Steiger Z-test for directionality.

Outcome	Exposure	r^2 on exposure	r^2 on outcome	Correct causal direction	Directionality test p-value
Breast cancer	alcohol (drinks/week)	0.007	0.00024	TRUE	<1e-300
Ovarian cancer	alcohol (drinks/week)	0.007	0.00078	TRUE	<1e-300

Supplementary Table 16. Estimate of the MR-Egger intercept for the MR analysis between alcohol intake with breast and ovarian cancer risk. For both cancer outcomes, the MR Egger intercept was not significantly different from zero, presenting limited evidence against the presence of directional pleiotropy biasing the IVW results.

Outcome	Intercept	se(intercept)	Pvalue	lower 95% C.I.	upper 95% C.I.
All Breast cancer	0.002	0.003	0.483	-0.004	0.008
All EOC	-0.001	0.004	0.752	-0.009	0.006

Supplementary Table 17. Pleiotropy assessment on ADH1B SNP rs1229984 using the online Phenoscanner database. Beta refer to the magnitude of association on the traits per effect allele (T), the none effect allele for the SNP is C. Note that traits that are directly related to alcohol drinking (e.g. alcohol dependence) are excluded. Traits with N_cases being zero are quantitative traits. Only association estimates derived from Europeans were included.

Trait	Beta	se	P-value	direction	N	N_cases	N_controls
Upper aerodigestive tract cancers	0.4447	0.0476	1E-20	+	-	-	-
Self-reported gout	0.0084	0.001	3.6E-18	+	337159	4807	332352
Reason for reducing amount of alcohol drunk: health precaution	-0.051	0.0063	8E-16	-	124798	40728	84070
Treatment with allopurinol	0.0068	0.0009	6.1E-15	+	337159	3819	333340
Leg fat mass right	-0.042	0.0066	9.4E-11	-	331293	0	331293
Systolic blood pressure	-0.052	0.0083	3.3E-10	-	317754	0	317754
Leg fat mass left	-0.04	0.0065	9.5E-10	-	331275	0	331275
Leg fat percentage right	-0.03	0.0052	6.5E-09	-	331296	0	331296
Whole body fat mass	-0.045	0.008	1.4E-08	-	330762	0	330762
Leg fat percentage left	-0.028	0.0051	5E-08	-	331278	0	331278

Body mass index	-0.044	0.0081	6.5E-08	-	336107	0	336107
Vascular or heart problems diagnosed by doctor: none of the above	0.02	0.0037	8.9E-08	+	336683	236530	100153
Vascular or heart problems diagnosed by doctor: high blood pressure	-0.019	0.0036	1.1E-07	-	336683	91033	245650
Sodium in urine	0.0422	0.008	1.2E-07	+	326831	0	326831
Arm fat mass left	-0.042	0.008	1.4E-07	-	331164	0	331164
Body fat percentage	-0.033	0.0063	1.5E-07	-	331117	0	331117
Arm fat mass right	-0.041	0.008	2.5E-07	-	331226	0	331226
Trunk fat mass	-0.042	0.0083	2.9E-07	-	331093	0	331093
Pulse pressure	-0.465	0.0925	5.00E-07	-	-	-	-
Arm fat percentage left	-0.031	0.0062	7E-07	-	331198	0	331198
Waist circumference	-0.036	0.0073	8.1E-07	-	336639	0	336639
Self-reported hypertension	-0.018	0.0036	8.2E-07	-	337159	87690	249469

Medication for cholesterol, blood pressure or diabetes: cholesterol lowering medication	-0.025	0.0051	9.9E-07	-	154702	35840	118862
Weight	-0.035	0.0072	1.3E-06	-	336227	0	336227
Arm fat percentage right	-0.03	0.0062	1.8E-06	-	331249	0	331249
Trunk fat percentage	-0.035	0.0075	3.9E-06	-	331113	0	331113

Supplementary Table 18. Pleiotropy assessment on ADH1B SNP rs1229984 using database GeneATLAS. Shortlisted traits are risk factors that are potentially associated with breast/ovarian cancer. These associations are obtained from the UK Biobank only. The GeneATLAS database is available at https://geneatlas.roslin.ed.ac.uk/phewas/)

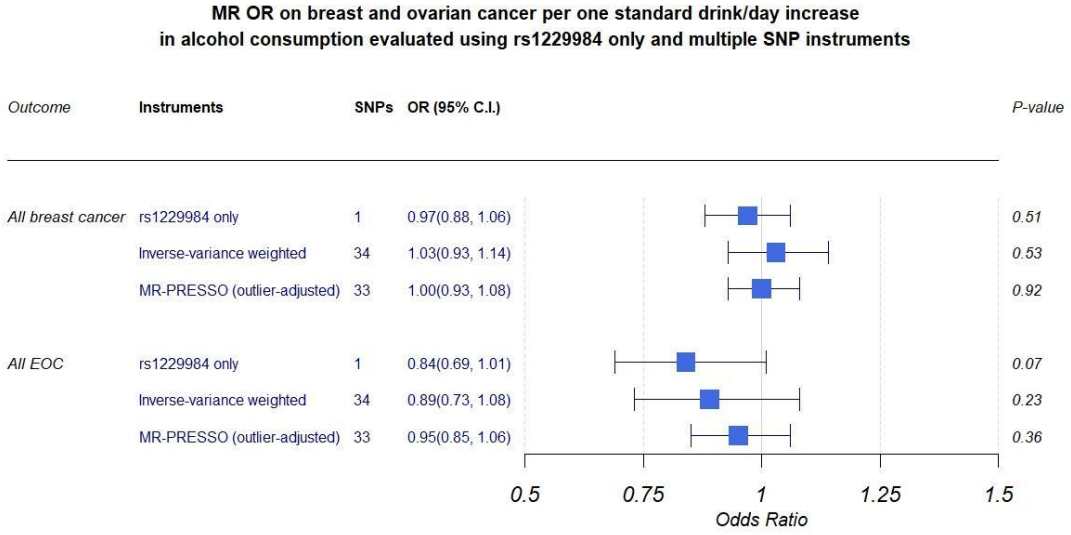
Trait	**Variant**	**Eff. allele**	**beta**	**pvalue**	**MAF**
Psychological/psychiatric problem	rs1229984	C	-0.001564	0.37201	0.0246
N95 Menopausal and other perimenopausal disorders	rs1229984	C	-0.002943	0.12659	0.0246
Smoking status	rs1229984	C	-0.003317	0.42239	0.0246
Body mass index (BMI)	rs1229984	C	0.17162	2.6542e-11	0.0246
Standing height	rs1229984	C	-0.026256	0.32622	0.0246

Supplementary Table 19. MR association between standard drink/day alcohol consumption and breast cancer risk stratified by ER status. The MR association estimates derived from alternative MR models (other than the IVW) were shown altogether. For MR-Egger(bootstrap), 1000 bootstrap iterations were performed to obtain reliable standard errors for the causal estimates. PWM stands for the penalized weighted median model. The confidence interval of the estimated OR for ER- and ER+ breast cancer were largely overlapping and included the null (OR=1), indicating minimal meaningful differences with respect to the relationship against alcohol intake.

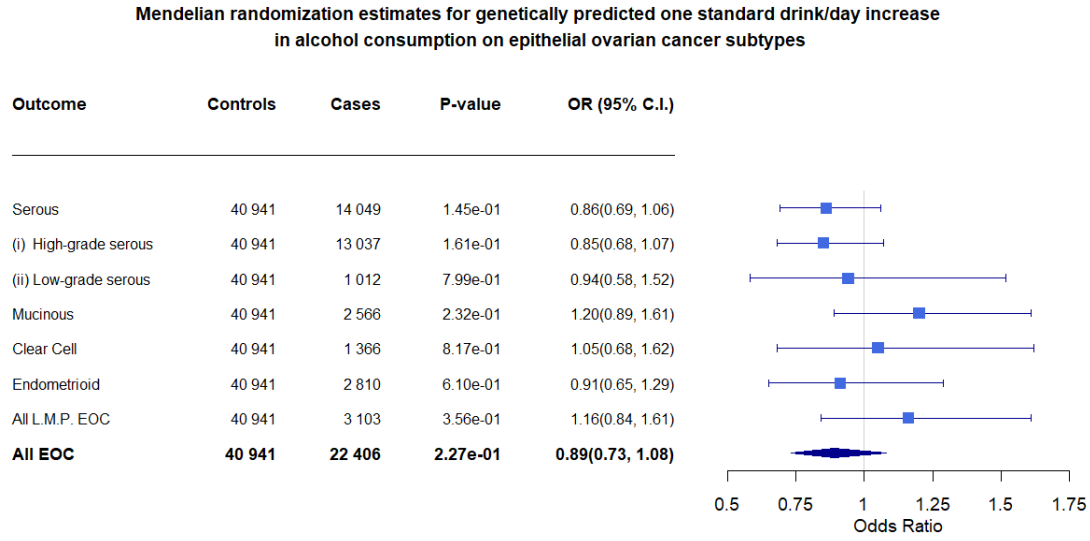
Outcome	MR-model	*Before filtering for SNP-heterogeneity*					*After filtering for SNP-heterogeneity*			
		P-value	OR	L_95CI	U_95CI		P-value	OR	L_95CI	U_95CI
ER- Breast cancers	**MR-Egger**	0.52	0.93	0.75	1.15		0.2	0.89	0.75	1.06
	PWM	0.22	0.91	0.78	1.06		0.22	0.91	0.78	1.06
	IVW (random effect)	0.77	0.98	0.85	1.13		0.71	0.98	0.89	1.08
	IVW (fixed effect)	0.71	0.98	0.88	1.09		0.74	0.98	0.88	1.09
ER+ Breast cancers	**MR-Egger**	0.64	0.96	0.82	1.12		0.37	0.95	0.85	1.06
	PWM	0.30	0.95	0.85	1.05		0.29	0.95	0.86	1.05
	IVW (random effect)	0.76	1.02	0.92	1.12		0.96	1.00	0.92	1.08
	IVW (fixed effect)	0.67	1.02	0.94	1.09		0.96	1.00	0.93	1.08

Supplementary Figures

Supplementary Figure 1. Comparison of MR association between estimated one standard drink/day and breast cancer and EOC susceptibility using single instrument and multiple instrument approaches. Estimates for the multi-instrument analyses were derived from 34 alcohol-associated SNPs as per main analysis. LMP refer to Low-malignant potential tumours.

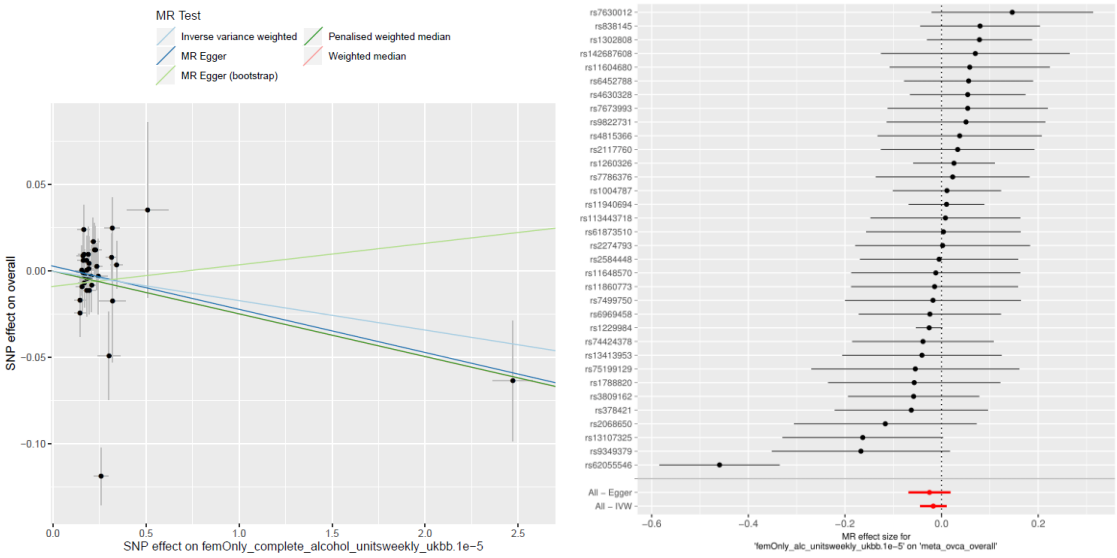


Supplementary Figure 2. MR association between estimated one standard drink/day and EOC susceptibility based on EOC subtypes. Estimates were derived from 34 alcohol-associated SNPs as per main analysis. LMP refer to Low-malignant potential tumours. The serous subtype can further be separated into high-grade serous and low-grade serous EOC.

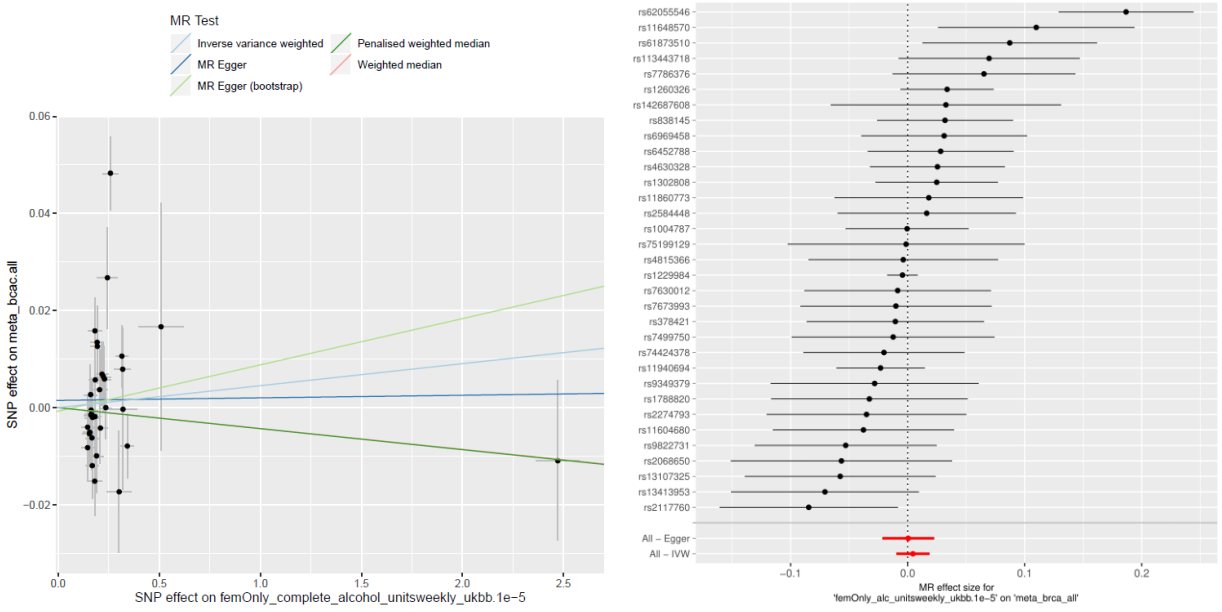


Supplementary Figure 3. Scatter plot and forest plot for the genetic association between alcohol drinks/week SNP instruments and risk of breast and ovarian cancers. The slope of the fitted line in the scatter plots reflect the MR causal estimates for each MR estimator. The forest plot shows the association of a genetically predicted one unit increase in alcohol drinks/week (need to be multiplied by 7 to obtain drink/day) on log(OR) of the outcome (cancer) risk inferred via each alcohol SNP instrument. The panel (a) refer to the plots for overall EOC and (b) refer to the plots for the risk of overall breast cancers respectively. The rs62055546 variant was consistently dropped after filtering for SNP-heterogeneity.

(a) Overall EOC MR scatter plot and forest plot

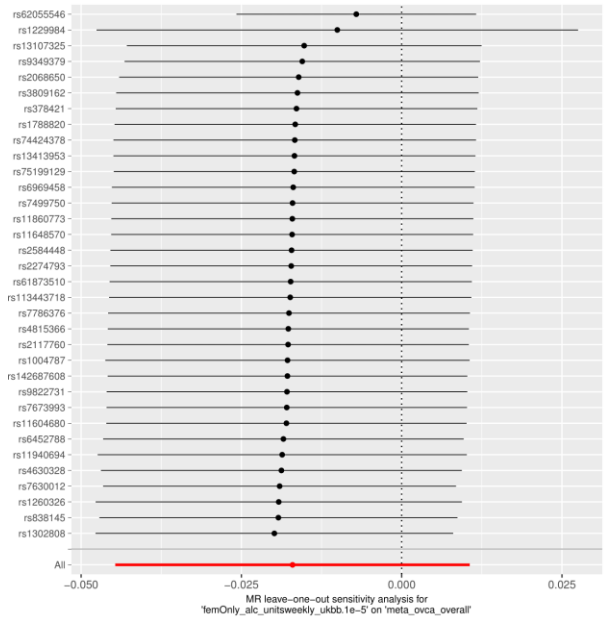


(b) All Breast cancer MR scatter plot and forest plot

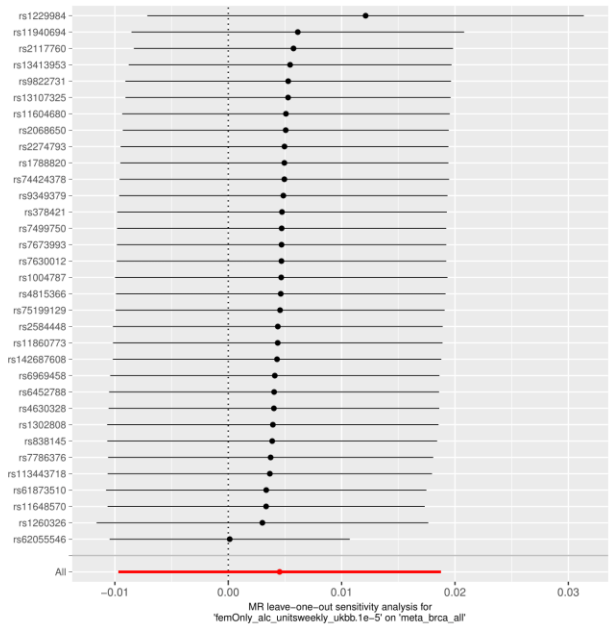


Supplementary Figure 4. MR Leave-one-out plots for the genetic association between alcohol drinks/week SNP instruments and risk of breast and ovarian cancers. The forest plot shows the IVW estimate of a genetically predicted one unit increase in alcohol drinks/week (need to be multiplied by 7 to obtain drink/day) on log(OR) of the outcome (cancer) risk inferred via excluding one alcohol SNP instrument at a time. The left and right side of panel (a) refer to the Leave-one-out plot for EOC and (b) refer to the plots for overall breast cancer.

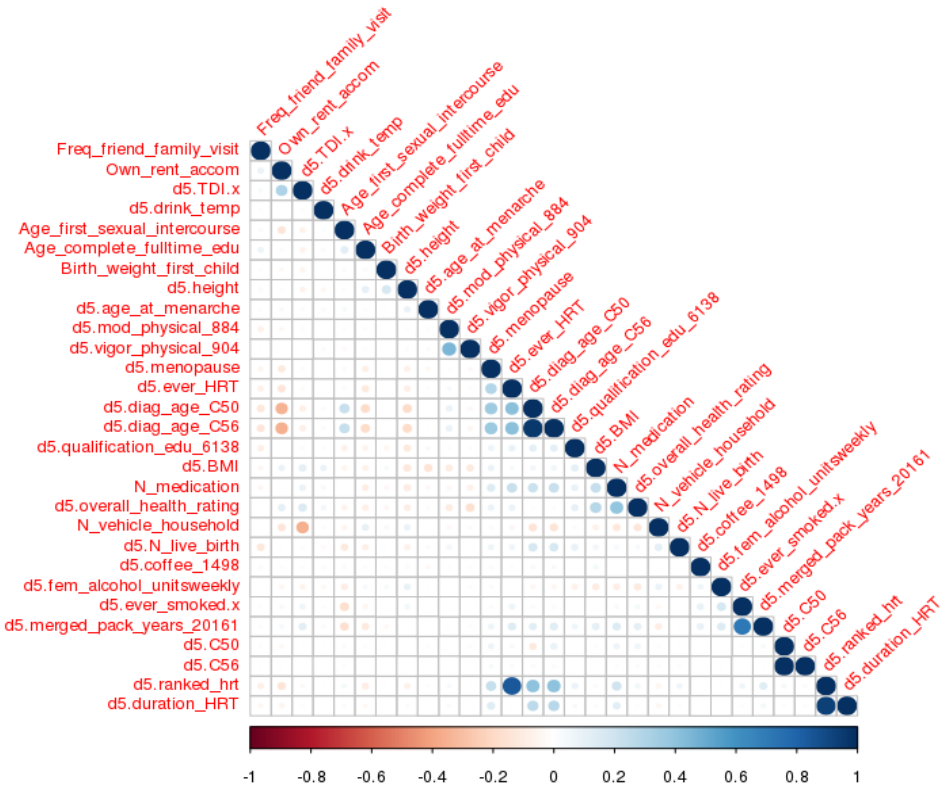
(a) Ovarian cancer



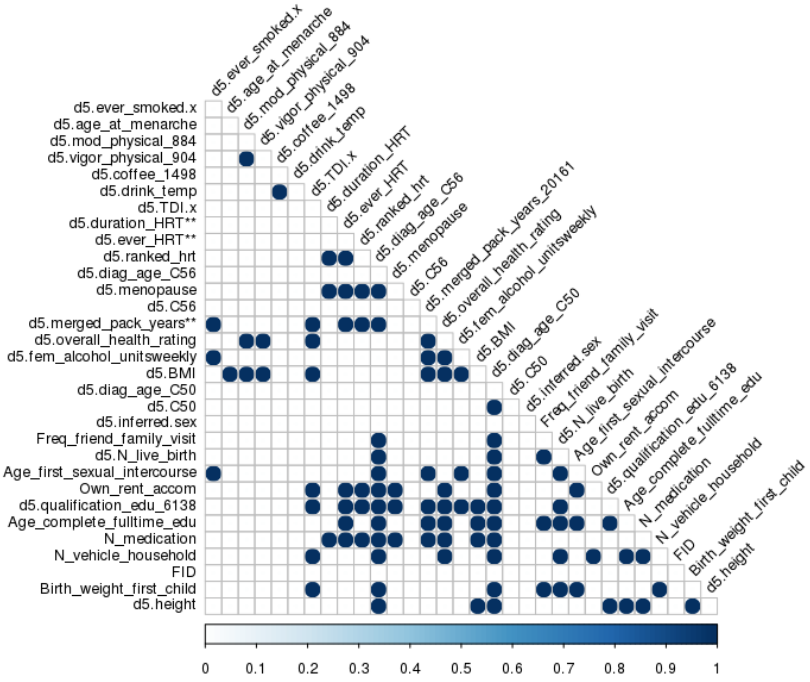
(b) MR leave-one-out breast cancer



Supplementary Figure 5. Phenotypic correlation between covariates and auxilliary variables used in the multiple imputation analysis for the UKB cohort. The figure below shows the magnitude of correlation between pairs of traits, with correlations that did not achieve nominal significance (p<0.05) left blank. The complete list of variables carried forward into the imputation process can be found in Table M8 (methods table in Supplementary material) .



Supplementary Figure 6. Indicator matrix on the predictability of covariates with missing data from other covariates and auxiliary variables estimated from MiCE. For each row, a positive indicator value (value=1) indicates that the trait of the corresponding column can be used to predict the row trait in a multiple imputation framework (vice versa, for column on row). The total number of predictors for a given trait is hence the sum of values across the row and column corresponding to that trait. We manually omit the cancer diagnosis outcome variables (diag_age_C50/C56 and C50/C56), genetic sex (inferred.sex) and the user ID (FID) phenotype from the multiple imputation analysis.



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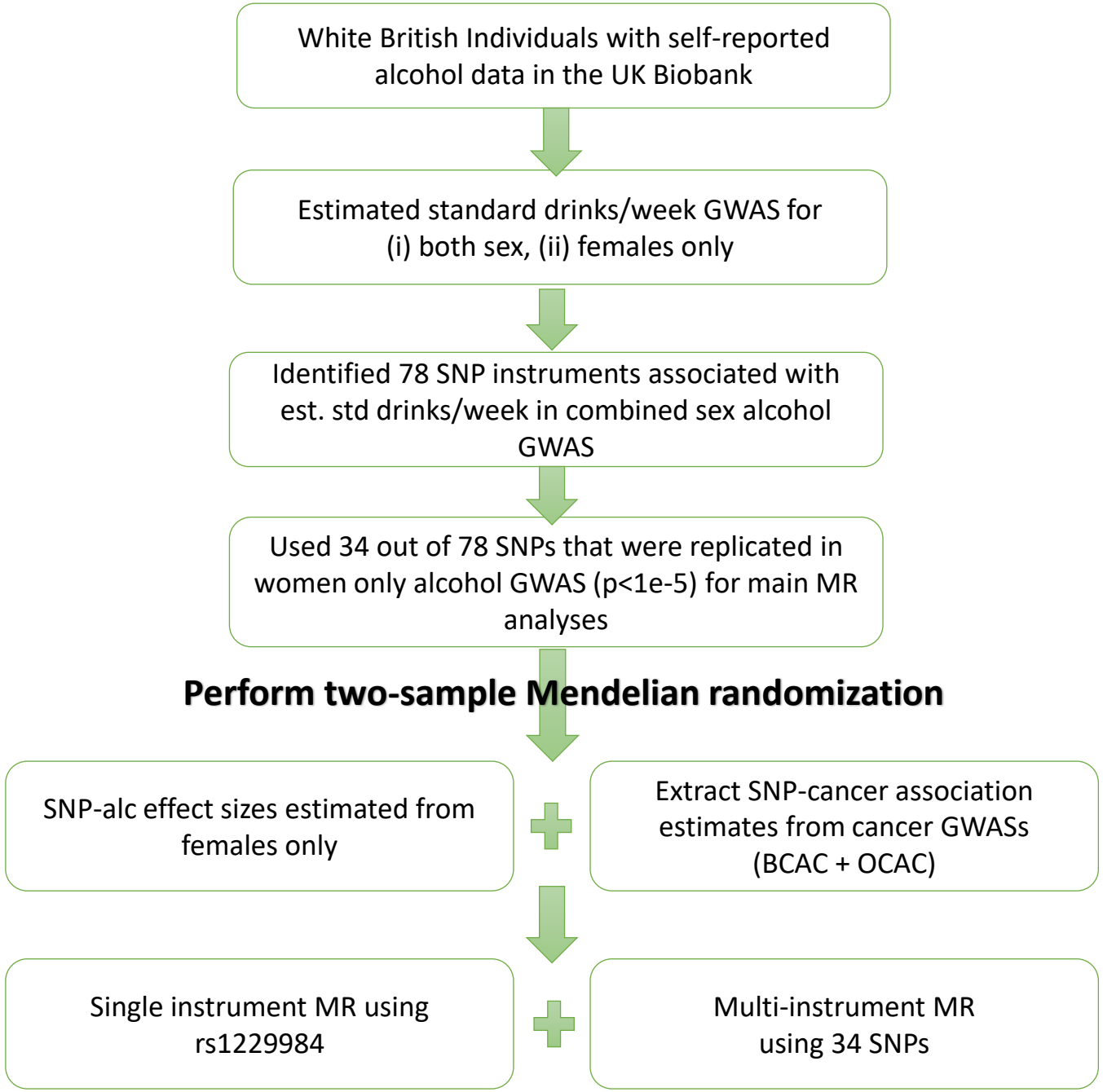
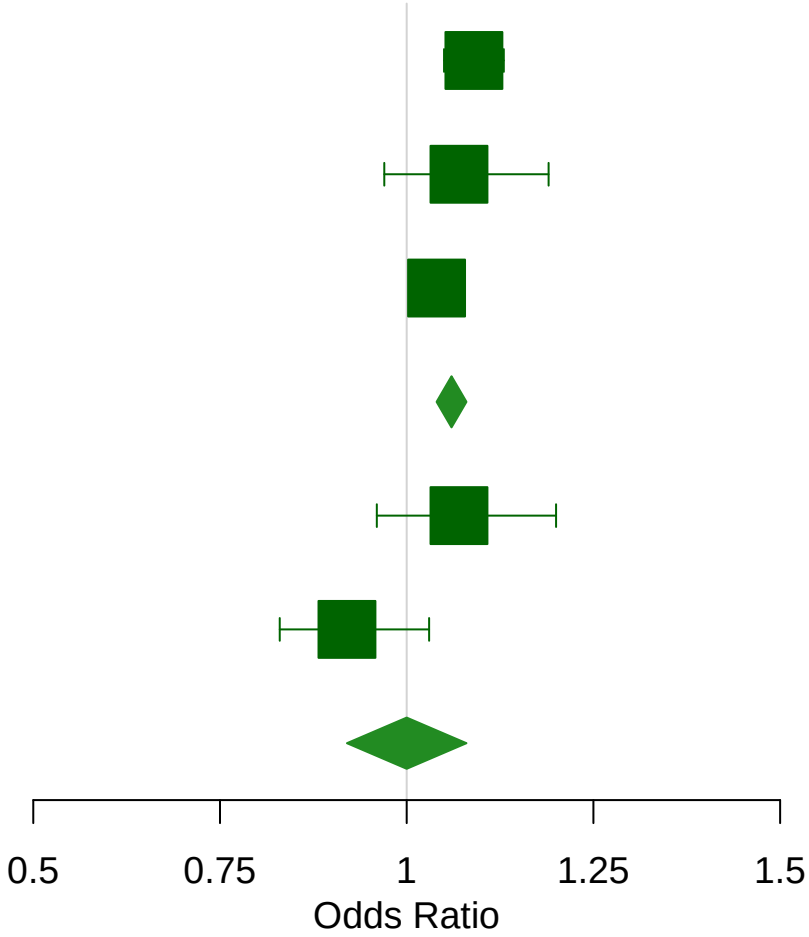

White British Individuals with self-reported alcohol data in the UK Biobank
Estimated standard drinks/week GWAS for (i) both sex, (ii) females only
Identified 78 SNP instruments associated with est. std drinks/week in combined sex alcohol GWAS
Used 34 out of 78 SNPs that were replicated in women only alcohol GWAS (p<1e-5) for main MR analyses
Perform two-sample Mendelian randomization
SNP-alc effect sizes estimated from females only
Extract SNP-cancer association estimates from cancer GWASs (BCAC + OCAC)
Single instrument MR using rs1229984
Multi-instrument MR using 34 SNPs

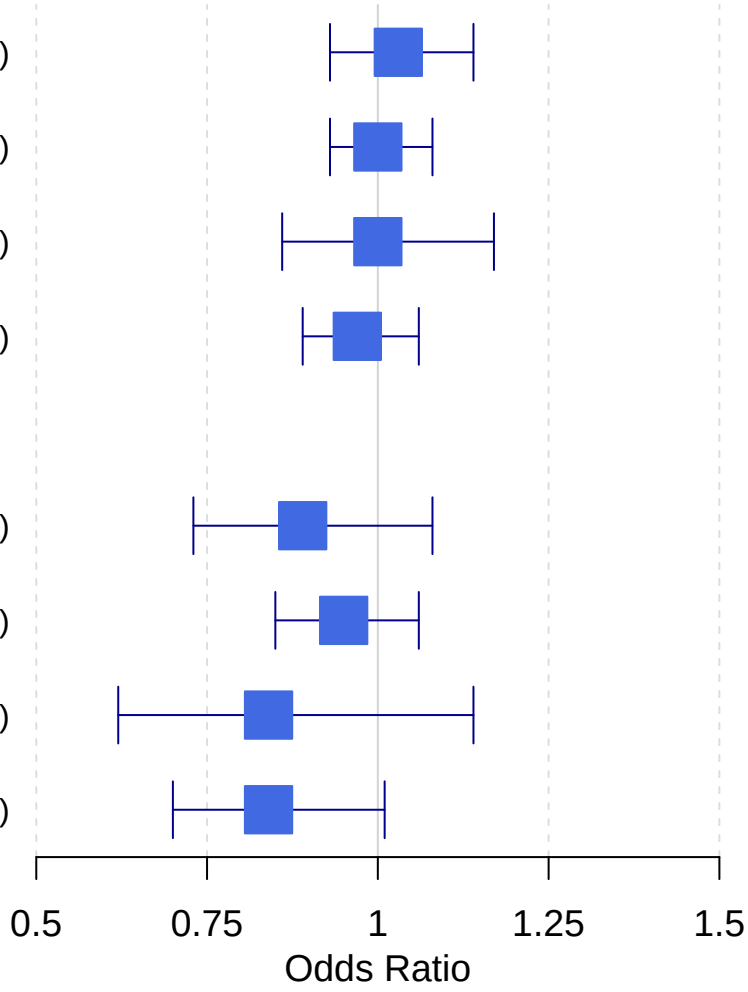
Meta−analysis of observational hazard ratio estimates for one standard drink increase on risk of breast and ovarian cancers

Studies	Cancers	Cases; Total	HR (95% C.I.)
CCHS+CGPS	Breast	2039;65126	1.09(1.05, 1.13)
KARMA	Breast	985;60903	1.07(0.97, 1.19)
UKBB	Breast	1787;62867	1.04(1.01, 1.07)
Combined (BrCa)	**Breast**	**4811;188896**	**1.06(1.04, 1.08)**
CCHS+CGPS	EOC	264;61427	1.07(0.96, 1.20)
UKBB	EOC	187;61267	0.92(0.83, 1.03)
Combined (EOC)	**EOC**	**451;122964**	**1.00(0.92, 1.08)**



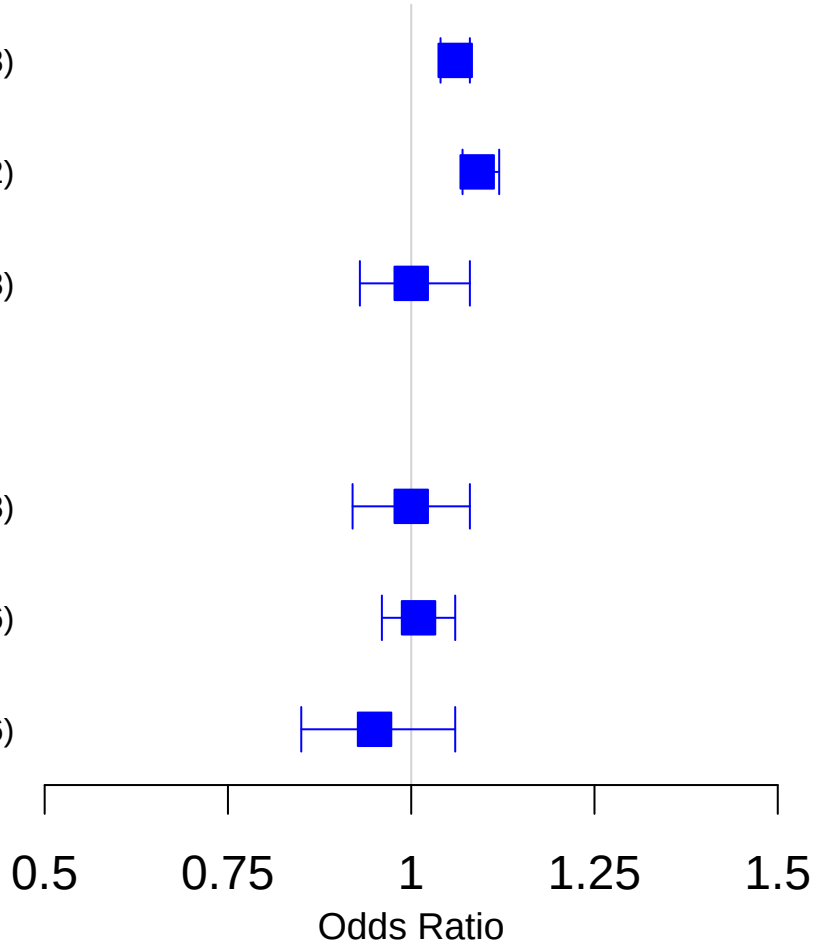
Mendelian randomization estimate for the odds ratio on breast and ovarian cancer per one standard drink/day increase in estimated alcohol consumption

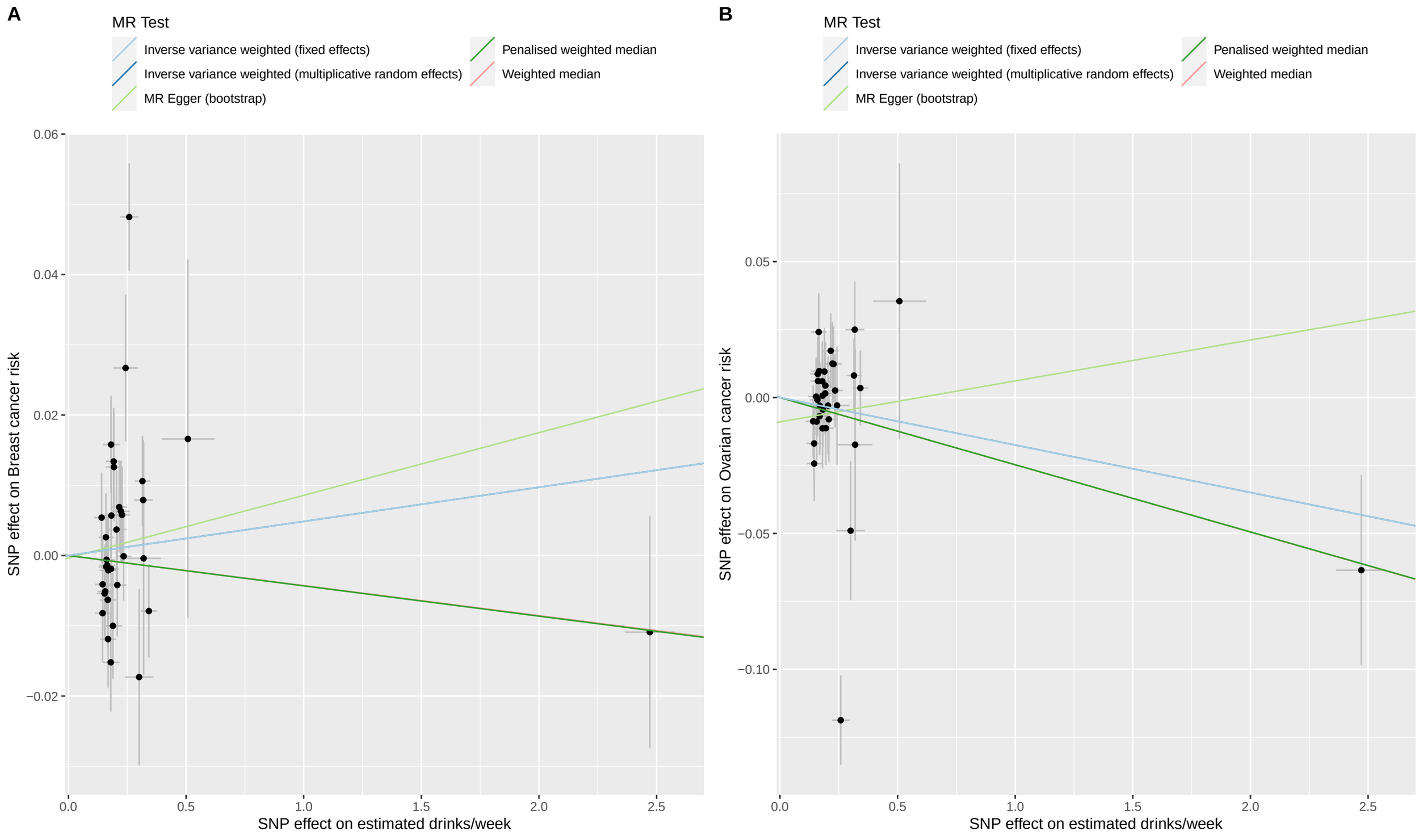
Outcome	Instruments	SNPs	OR (95% C.I.)	P−value
All breast cancer	Inverse−variance weighted	34	1.03(0.93, 1.14)	0.53
	MR−PRESSO (outlier−adjusted)	33	1.00(0.93, 1.08)	0.92
	MR−Egger	34	1.00(0.86, 1.17)	0.96
	Penalised weighted median	34	0.97(0.89, 1.06)	0.5
EOC	Inverse−variance weighted	34	0.89(0.73, 1.08)	0.23
	MR−PRESSO (outlier−adjusted)	33	0.95(0.85, 1.06)	0.36
	MR−Egger	34	0.84(0.62, 1.14)	0.27
	Penalised weighted median	34	0.84(0.70, 1.01)	0.07



Comparison of observational and genetic estimates for one standard drink/day increase in alcohol consumption on risk of breast and ovarian cancers

Studies	Cancers	Cases	Model	Measure	Estimate (95% C.I.)
Observational	Breast	4811	HR	1 std drink/day	1.06(1.04, 1.08)
WCRF CUP meta−analysis	Breast	35221	RR	10g ethanol/day	1.09(1.07, 1.12)
MR analysis (BCAC data)	Breast	122977	OR	1 std drink/day	1.00(0.93, 1.08)
Observational (HR)	EOC	451	HR	1 std drink/day	1.00(0.92, 1.08)
WCRF SLR meta−analysis	EOC	2954	RR	10g ethanol/day	1.01(0.96, 1.06)
MR analysis (OCAC data)	EOC	22406	OR	1 std drink/day	0.95(0.85, 1.06)




A
MR Test
Inverse variance weighted (fixed effects)
Inverse variance weighted (multiplicative random effects)
MR Egger (bootstrap)
Penalised weighted median
Weighted median
SNP effect on Breast cancer risk
0.06
0.04
0.02
0.00
−0.02
0.0
0.5
1.0
1.5
2.0
2.5
SNP effect on estimated drinks/week
B
MR Test
Inverse variance weighted (fixed effects)
Inverse variance weighted (multiplicative random effects)
MR Egger (bootstrap)
Penalised weighted median
Weighted median
SNP effect on Ovarian cancer risk
0.05
0.00
−0.05
−0.10
0.0
0.5
1.0
1.5
2.0
2.5
SNP effect on estimated drinks/week