Genetically Predicted Modifiable Lifestyle Factors in Relation to Ovarian Cancer Risk by Histologic Subtypes

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Abstract

Objective: Lifestyle factors including education, coffee intake, tea consumption, dietary fat intake, obesity, physical activities, smoking and alcohol drinking, sleep duration, and insomnia, have been linked to Ovarian Cancer (OC) in observational studies. We assessed whether the concerning factors are causally associated with the risk of OC by histological subtypes. Design: Mendelian Randomization (MR) study. Setting: Independent genetic instruments associated with thirteen lifestyle behaviors were selected from ten genome-wide association studies. Summary-level data for OC subtypes were obtained from the Ovarian Cancer Association Consortium. Population: Exposures population were including 375,833 to 1,232,091 European individuals, outcomes population were including up to 25,509 cases and 40,941 controls. Methods: Two-sample and multivariable MR study, and multiple complementary sensitivity analyses were conducted. Main Outcome Measures: Histological subtypes of OC. Results: We provided unconfounded genetic evidence of inverse associations of genetically predicted years of education and fat intake with specific OC subtypes, which are independent of BMI. Whereas consumption of coffee or tea was positively associated with endometroid OC, which may be partly mediated by BMI. Although physical activity and sleep characteristics have been reported to be the risk factors for OC, no causal associations were observed in our study. Conclusions: Our study clarified the protective and independent role of high level education and relative fat intake in particularly OC subtypes. We also showed detrimental effects of higher coffee or tea consumption on OC histotypes. Our results may provide insight into the corresponding interventions as lifestyle factors can easily be modified.

Introduction

Ovarian cancer (OC) is the most lethal gynecologic cancer, which affects around 230,000 women and with 152,000 deaths worldwide each year. One of the main factors contributing to the high death-to-incidence rate of OC is the advanced stage of the disease at the time of diagnosis. [1], [2]. OC is classified into distinct histological subtypes, which differ in their origin, pathogenesis, molecular profile, risk factors, and clinical prognosis. [3]. An understanding of the epidemiology and etiology of OC based on the heterogeneity is critical for the development of prevention strategies.

Lifestyle-related risk factors are amenable to modification and may therefore be relevant targets in the prevention of OC. While many lifestyle factors have been associated with OC and its subtypes, such as education [4], coffee or tea consumption [5]–[8], dietary fat intake [9], physical activities [10]–[13], obesity [14], cigarette smoking and alcohol drinking [15]–[17], sleep duration and insomnia [18], [19], ascertaining causality and whether their modification will reduce the risk is undetermined. For example, education and obesity are closely interrelated, but their independent association with OC subtypes is uncertain. As well, smoking and coffee or tea consumption are overlapping behaviors, so they may introduce residual confounding to observational studies. Moreover, another challenge is that OC is caused by various pathologies, which have distinct pathophysiological characteristics. Most risk factors exhibited significant heterogeneity by histology, however, much of the current epidemiological data examining risk factor modification have not studied the

relationships between modifiable risk factors and specific OC subtypes. A clear appraisal of the causality of these associations is of importance in updating the primary prevention strategy for OC and different histotypes.

Mendelian Randomization (MR) involves the use of genetic variants as instrumental variables in order to prove the causal effect of environmental exposure on a disease outcome. The MR estimates represent associations between genetically predicted levels of risk factors and outcomes, which makes MR estimates less likely to be affected by confounding factors than conventional observational epidemiology estimates [20], [21]. Additionally, since genetic codes are immune from environmental influences or preclinical disease, MR estimates are less prone to bias caused by reverse causation.

Herein, we conducted a comprehensive MR study to investigate the etiological role of multiple modifiable lifestyle factors on OC and its histologic subtypes.

Methods

Study Design and Genetic Instruments

The study design overview and the assumptions of an MR study are shown in **Fig. 1** . Genetic instruments for education [22], coffee and tea consumption [23], relative fat intake [24], BMI [25], physical activities (including three physical activity phenotypes: overall acceleration average, self-reported moderate-to-vigorous physical activity (MVPA) and vigorous physical activity (VPA) [26], smoking initiation [27], lifetime smoking index (taking into account smoking status as well as smoking duration, heaviness, and cessation in ever smokers) [28], alcohol drinking [27], sleep duration [29] and insomnia [30] were selected at genome-wide significance threshold ($P < 5\times10^{-8}$) from corresponding genome-wide association (GWAS) studies. Detailed information on used GWAS studies is presented in **Table 1** . Linkage disequilibrium between single-nucleotide polymorphisms (SNPs) was assessed based on the European population of 1000 Genomes reference panel. SNPs without linkage disequilibrium (defined by $r^2 < 0.01$ and clump distance >10,000 kb) were used as instrumental variables.

Outcome Data Sources

Summary-level data for OC were obtained from the Ovarian Cancer Association Consortium (OCAC), which involved 25,509 cases of overall OC and 40,941 controls of European descent [31]. In the subtype analyses, the data included 7 well classified OC histotypes: High grade serous (HGSOC, 13,037 cases and 53,978 controls), Low grade serous (LGSOC, 1,012 cases and 41,953 controls), Invasive mucinous (IMOC, 1,417 cases and 42,358controls), Endometrioid (EOC, 2,810cases and 43,751 controls), Clear cell (CCOC, 1,366 cases and 42,307 controls), Low malignant potential serous (LMSOC, 1,954 cases and 42,895 controls), and Low malignant potential mucinous (LMMOC, 1,149 cases and 42,090 controls). Association tests were adjusted for up to 19 genetic principal components in the data sources. Data from the consortia were extracted through the MR-Base platform [32]. Characteristics and detailed information on used data sources are displayed in **Table S1** . All studies had been approved by a relevant ethical review board and participants had given informed consent.

Statistical Analysis

We used the inverse variance-weighted (IVW) model as the main statistical method [33]. In sensitivity analyses, the weighted median method [34], MR-Egger method [35], Maximum likelihood, and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method [36] were performed to examine the consistency of associations and detect possible pleiotropy. The weighted median method provides a causal estimate when more than 50% of the weight in the analysis comes from valid instrumental variables [34]. The MR-Egger regression mode is able to detect pleiotropy by its intercept, but it compromises statistical power after pleiotropy is corrected [35]. The MR-PRESSO method, can detect and adjust for horizontal pleiotropy by outlier removal [36]. Cochran's Q statistics were calculated to test for heterogeneity produced by different genetic variants in the IVW analyses.

Multivariable MR analysis was further applied to assess whether any association of domain-specific lifestyle behaviors with OC subtypes could be affected by potential confounders, including BMI and smoking. We also conducted a 2-step multivariable MR analysis in the analysis to adjust for BMI and smoking initiation. The associations were deemed significant associations at a strict Bonferroni corrected P -value below 0.00048 (correcting for 13 exposures and 8 outcomes), and associations with P -value > 0.00048 and < 0.05 were regarded as suggestive associations. All statistical tests were 2-tailed and performed using the TwoSampleMR [32], MR-PRESSO [36], and Mendelian randomization [37] packages in the R software (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Education

Our results showed significant inverse association of genetically determined years of education with overall OC (odds ratio [OR], 0.8 [95% CI, 0.72–0.89]) (**Fig. 2A**). Suggestive associations were also observed for the protective effect of education attainment on the subtypes of LMSOC (OR, 0.64 [95% CI, 0.48–0.84]), HGSOC (OR, 0.81 [95% CI, 0.72–0.92]), IMOC (OR, 0.68 [95% CI, 0.51–0.92]), LGSOC (OR, 0.67 [95% CI, 0.46–0.98]), and EOC (OR, 0.8 [95% CI, 0.65–0.99]). The inverse associations were persistent in the weighted median and maximum likelihood mode. Although there was inconsistency in the MR-Egger method, the MR-Egger test for pleiotropy indicated that no significant pleiotropy was present. After removing outliers in the MR-PRESSO analysis, the association of education with all OC and OC subtypes persisted and the P value for the distortion test were above 0.05 (**Table S2**). Most of the associations for education and OC subtypes persisted and attenuated slightly after multivariable adjustment (**Table 2**).

Coffee or tea consumption

There were limited data supporting associations of genetically predicted coffee, or tea consumption with overall OC risk (**Fig. 2B-C**). However, higher genetically predicted coffee and tea consumption were both positively associated with the endometrioid subtype. The ORs of EOC were 1.70 [95% CI, 1.18–2.44]) for genetically predicted 50% increase in coffee consumption (**Fig. 2B**), and 1.84 [95% CI, 1.10–3.07]) for genetically predicted 50% increase in tea consumption (**Fig. 2C**). The positive association between coffee consumption and EOC was also replicated in the sensitivity analyses including the weighted median and maximum likelihood mode, although with wider CI in the MR-Egger method (**Table S3**). For tea consumption, the positive association was persistent in the weighted median method, albeit there was some inconsistency in effect estimates of other sensitivity analyses (**Table S4**). The association between coffee or tea consumption and EOC did not remain after adjusting for BMI (**Table 2**).

Relative fat intake and BMI

The results indicated that a higher relative fat intake causally increases the risk of HGSOC (OR, 1.85 [95% CI, 1.06, 3.22]), but reduces the risk of EOC (OR, 0.3 [95% CI, 0.11, 0.86]) (**Fig. 2D**). The associations remained consistent in the maximum likelihood mode, albeit with wider CI in the weighted median and MR-Egger method (**Table S5**). After adjustment for BMI, the association between fat intake and HGSOC attenuated greatly. However, the protective effect of fat intake on EOC was not affected by BMI (**Table 2**).

Genetic predisposition to higher BMI was significantly associated with an increased risk of LMSOC (OR, 1.44 [95% CI 1.18–1.77]) (**Fig. 2E**). The positive association was consistent in the further analyses including weighted median, MR-Egger, and Maximum Likelihood, suggesting little evidence for pleiotropy and potential violations of instrumental variable assumptions (**Table S6**). A suggestive association was also found between BMI and the EOC (OR, 1.27 [95% CI 1.07–1.50]). The effect estimate was persistent when performing MR-Egger and maximum likelihood and was slightly attenuated when employing weighted median mode estimators. In addition, a borderline association was observed between BMI and LMMOC in IVW (OR, 1.29 [95% CI 1.01–1.66]) and maximum likelihood mode. Although no statistically significant association was observed between BMI and all OC, sensitive analysis indicated a suggestive association (**Table S6**).

Smoking

Despite null associations between smoking initiation and risk of OC subtypes (**Fig. 2I** and **Table S10**), genetic liability to lifetime smoking index showed a suggestive association with an increased risk of overall OC (OR, 1.23 [95% CI 1.04–1.46]), HGSOC (OR, 1.25 [95% CI 1.02–1.53]) and EOC (OR, 1.51 [95% CI 1.01–2.26]) histotypes (**Fig. 2J)** . The positive associations remained consistent in the maximum likelihood mode, albeit with wider CI in the weighted median and MR-Egger method (**Table S11**).

Physical activities, alcohol drinking, sleep duration, and insomnia

Among other lifestyle factors, there were no associations to be found for physical activities (**Fig. 2F-H)** , alcohol drinking (**Fig. 2K)** , sleep duration (**Fig. 2L)** , or insomnia (**Fig. 2M)** with any type of OC in the primary analysis or the sensitivity analyses (**Table S7-9** , **S12-14**).

Discussion

In the present MR study, we systematically assessed the causal associations of a broad range of lifestyle factors with the risk of OC by histologic subtypes. We found genetically determined years of education to be inversely associated with overall OC, and subtypes of LMSOC, HGSOC, IMOC, LGSOC, and EOC. The associations of education with risk of all OC, HGSOC, IMOC, and LMSOC were independent of BMI. Genetic liability to higher coffee or tea consumption was positively associated with the risk of EOC, which may partly mediated by BMI. Our results further showed that increased dietary fat intake was positively associated with HGSOC, but inversely and independently associated with EOC. Genetic predisposition to a higher BMI was associated with an increased risk of LMSOC, LMMOC, and EOC. Although little clear evidence supports the role of smoking initiation in ovarian carcinogenesis, elevated lifetime smoking index was positively associated with overall OC, HGSOC, and EOC. In contrast, there was limited evidence for causal associations of physical activities, alcohol drinking, sleep duration, and insomnia with any type of OC.

Education level is a concerned social determinant and has been proposed as a modifiable risk factor for a number of diseases. An understanding of the causal links from education level to health outcomes may help disease prevention. A recent observational study reported that the incidence of borderline ovarian tumors decreased in women with a high educational level [4]. Our study found that genetically predicted higher education level was causally and independently associated with lower risks of many OC subtypes, further suggesting the protective effect of educational attainment on OC development. Possible pathways may link education level to OC prevention. Firstly, modifiable risk factors have been reported largely mediates the educational effects on diseases. Moreover, high level of education is more likely to drive positive health-related behaviors and thus reduce the risk of OC. [38]. The associations between education and OC subtypes were attenuated in the multivariable MR analysis with adjustment for genetically predicted BMI and lifetime smoking index liability, which may suggest that BMI and lifetime smoking index partly mediate the associations.

Studies have not demonstrated either an overall protective or detrimental effect on OC development in coffee or tea drinkers. In previous observational analyses, the RR estimates of coffee or tea consumption were significantly heterogeneous, with individual studies suggesting positive [5], [6], [39]–[41], null [8], [42]–[45], and inverse [7], [45]–[48] associations with OC risk. Inconsistencies observed in the literature may be due to the interference of confounders, lack of compatibility of categorical definitions, and differences in definitions for baseline groups. Our study is the first to use MR analyses to explore the relationship between coffee or tea consumption with OC histotypes. In the subtype analyses, we found evidence for a positive association between endometrioid OC risk and genetically predicted coffee intake levels. Similarly, a suggestive increased risk was only observed in the endometrioid subtype in the tea consumption analyses. The etiology of endometrioid OC resulting from coffee or tea consumption warrants to be established. The major endometriosis mechanism in OC is estrogen-dependent, endometriosis acts as a precursor for OC, which is easily developed in the low-progesterone and high-estrogen conditions [49]. The intake of caffeine and caffeine-containing beverages has been positively associated with estrone and sex hormone-binding globulin

concentrations and inversely with bioavailable testosterone [50], [51]. These hormonal changes may influence hormone-dependent diseases. Therefore, caffeine intake has been associated with the risk of endometriosis and has been hypothesized to exert its effects through the alteration of endogenous hormone levels [52]. Our findings of an increased risk for endometrioid carcinoma in coffee or tea drinkers are consistent with the hormonally mediated hypothesis.

The current knowledge on the association between dietary fat intake and OC was inconclusive. Pooled analyses of observational studies have reported positive [9] or null [53] association between fat intake and overall OC risk, however, there were limited data for the OC subtypes. Although we found no indication of a causal effect of relative fat intake on overall OC risk, a persistent borderline association was observed between fat intake and HGSC, which was corroborated with the findings that serous ovarian cancer was more susceptible to dietary fat consumption than other subtypes [53]. Due to the pathological specificity of endometrioid OC and the composition of dietary fat, our result of an inverse association between relative fat intake and this subtype may get support from a prospective study that higher intake of omega-3 may be protective for OC overall and endometrioid tumors in particular [54]. Further MR analyses for the effects of dietary fat components on OC subtypes are needed.

BMI has been associated with OC in observational studies [14], [55]. However, the effect of BMI increase on the etiology of histological subtypes of OC remains controversial [56] [57]. In our analyses with updated GWAS studies including 583 BMI SNPs and 25,509 OC cases, which may afford the analysis greater instrument strength and greater statistical power to detect effects, we observed positive associations of genetically predicted BMI with LMSOC, LMMOC, and EOC, which complemented the findings from previous observational studies [14]. A high BMI has been linked to benign ovarian tumors, the evidence from epidemiological, histopathological, and molecular studies suggests that borderline tumors may develop from benign tumors [58], [59]. This theory of progression for borderline tumors is supported by our finding that low malignant potential tumors were associated with BMI, but not high-grade cancers.

The association between physical activity and OC risk remains less clear. It has been hypothesized from previous meta-analysis of observational studies that physical activity may protect against the development of OC [12], [60], [61], whereas positive or null associations were found from time to time [11], [13], [62]. Across studies, the findings are inconclusive especially when considering histologic type [13], [63], indicating that this issue needs to be resolved. We identified no causal relationship for overall acceleration average, self-reported MVPA, and VPA, with overall OC by MR analyses, which supported the findings from three large prospective studies including up to 96,216 participants showing that MVPA, VPA, or overall physical activity were not independently associated with reduced risk of OC [10], [11], [62]. In this study, we further showed that physical activities do not affect the risk of OC subtypes, which contributed to the knowledge regarding the evaluation of lifestyle behaviors on OC histotypes incidence.

Studies of the association of smoking with the risk of OC have not been entirely consistent. [17], [64], [65]. One reason for the variation in these results may be the different methods employed to assess and analyze these measures of smoking exposure. Here we improve our study by using two sets of instruments for smoking [28]. Our analyses by OC histological subtypes demonstrated limited associations between smoking initiation and OC subtypes. However, we found that the lifetime smoking index is suggestively associated with overall OC, HGSOC, and EOC subtypes. Although smoking has been strongly linked to mucinous OC in previously pooled analyses, we didn't observe a consistent result. However, we still emphasize that independent GWAS and large prospective studies by OC subtypes are warranted to further validate our findings in other cohorts and ethnicities.

Results from epidemiological studies on the association between alcohol drinking and OC risk are inconsistent, reporting either a null association [66]–[69], a positive association [70], or a negative association [71], [72]. A recent study focused on overall OC and alcohol consumption, including single instrument MR using rs1229984 and multiple instruments MR using 34 SNPs, showed no causal evidence of association [15]. In our subtype analyses with 83 SNPs, we further found no causal effect of alcohol drinking on the development of OC histotypes, which complement findings of overall OC from previous studies.

Sleeping disorders are associated with the poor quality of life of women suffering from OC. However, there are limited data about the effect of sleeping disorders and lack of a proper amount of sleep on the occurrence of OC. A previous prospective study reported a null association between sleep duration and OC risk [18]. A recent observational study also shows no association between sleep duration, sleep quality, or insomnia with the risk of overall OC among postmenopausal women. Nevertheless, in subtype analyses, restful sleep quality was associated with a lower risk of invasive serous OC, and insomnia was associated with a higher one [19]. In our MR analyses by using up to 74 and 702 SNPs, we didn't observe a causal effect of sleep duration or insomnia on the development of any type of OC.

The major strengths of the present study are the MR design, the systematic assessment of multiple lifestyle factors, and the inclusion of well-classified OC subtypes. MR design strengthened the causal inference by diminishing residual confounding and other biases. In addition, consistent results from several sensitivity analyses guaranteed the robustness of our findings. Our analysis was confined to individuals of European ancestry, which largely diminished population stratification bias. However, the population confinement might limit the generalization of our findings to other populations. Limitations in our study need consideration. Although indicative pleiotropy was observed in a few analyses of BMI by the MR-Egger regression, several aspects of our results suggested a minimal probability that pleiotropy would bias our results, including a few outliers detected in the MR-PRESSO analysis and consistent results from multiple sensitivity models. In addition, we were unable to examine possible pre- and post-menopausal differences in associations of studied exposures with OC due to a lack of menopause data. However, given that most OC cases occur after menopause and that age-matched controls were used, the inclusion of some pre- or perimenopausal women in these analyses would less likely have biased results. Finally, further experimental studies to reveal the possible mechanisms, through which factors that appear to influence OC in these analyses, could help to expand the scope for prevention opportunities across the life course.

Conclusions

In conclusion, our results suggest causal associations of lower educational attainment and increased coffee or tea consumption, fat intake, BMI, and smoking with increased risk of OC histological subtypes. Specifically, our study demonstrated that genetically determined years of education was inversely associated with overall OC, and subtypes of LMSOC, HGSOC, IMOC, LGSOC, and EOC. Higher coffee or tea consumption was causally associated with EOC risk. In addition, we found increased dietary fat intake was detrimental for HGSOC, but protective for EOC subtype. Genetic predisposition to a higher BMI was positively associated with the risk of LMSOC, LMMOC, and EOC. Elevated lifetime smoking index was suggestively associated with increased risk overall OC, HGSOC and EOC. Whereas physical activity, sleep duration, insomnia, and alcohol consumption are not associated with any type of OC. These findings have major clinical and public health implications as lifestyle factors can easily be modified. However, the results should be interpreted with caution considering the difficulty in completely ruling out pleiotropy.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

Contribution

LJ and KW were responsible for the writing of the original draft; LJ, KW, FKY, YY, and PCZ for reviewing and editing the manuscript; LJ, KW, FKY, YY, and PCZ for acquisition, analysis, or interpretation of data;

LJ, KW, and FKY for the conceptualization of the study; LJ, KW and FKY for statistical analysis; and LJ for supervision. LJ, KW, and FKY accessed the database and raw data.

References

[1] N. Wentzensen *et al.* , "Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium," *J Clin Oncol* , vol. 34, no. 24, pp. 2888–2898, Aug. 2016, doi: 10.1200/JCO.2016.66.8178.

[2] S. Lheureux, C. Gourley, I. Vergote, and A. M. Oza, "Epithelial ovarian cancer," *Lancet* , vol. 393, no. 10177, pp. 1240–1253, Mar. 2019, doi: 10.1016/S0140-6736(18)32552-2.

[3] P. Gaona-Luviano, L. A. Medina-Gaona, and K. Magana-Perez, "Epidemiology of ovarian cancer," *Chin Clin Oncol* , vol. 9, no. 4, p. 47, Aug. 2020, doi: 10.21037/cco-20-34.

[4] L. Baandrup, M. T. Faber, G. L. Aalborg, and S. K. Kjaer, "Borderline ovarian tumors in Denmark 1997-2018: Time trends in incidence by histology, age and educational level," *Acta Obstet Gynecol Scand* , vol. 100, no. 3, pp. 436–443, Mar. 2021, doi: 10.1111/aogs.14013.

[5] A. C. Y. Leung *et al.* , "Tea, coffee, and caffeinated beverage consumption and risk of epithelial ovarian cancers," *Cancer Epidemiol* , vol. 45, pp. 119–125, Dec. 2016, doi: 10.1016/j.canep.2016.10.010.

[6] H. Kuper, L. Titus-Ernstoff, B. L. Harlow, and D. W. Cramer, "Population based study of coffee, alcohol and tobacco use and risk of ovarian cancer," *Int J Cancer* , vol. 88, no. 2, pp. 313–318, Oct. 2000, doi: 10.1002/1097-0215(20001015)88:2<313::aid-ijc26>3.0.co;2-5.

[7] C. F. Gosvig, S. K. Kjaer, J. Blaakaer, E. Hogdall, C. Hogdall, and A. Jensen, "Coffee, tea, and caffeine consumption and risk of epithelial ovarian cancer and borderline ovarian tumors: Results from a Danish case-control study," *Acta Oncol* , vol. 54, no. 8, pp. 1144–1151, 2015, doi: 10.3109/0284186X.2014.1001035.

[8] N. A. Lueth, K. E. Anderson, L. J. Harnack, J. A. Fulkerson, and K. Robien, "Coffee and caffeine intake and the risk of ovarian cancer: the Iowa Women's Health Study," *Cancer Causes Control* , vol. 19, no. 10, pp. 1365–1372, Dec. 2008, doi: 10.1007/s10552-008-9208-8.

[9] A. Khodavandi, F. Alizadeh, and A. F. A. Razis, "Association between dietary intake and risk of ovarian cancer: a systematic review and meta-analysis," *Eur J Nutr* , vol. 60, no. 4, pp. 1707–1736, Jun. 2021, doi: 10.1007/s00394-020-02332-y.

[10] R. G. Biesma, L. J. Schouten, M. J. M. Dirx, R. A. Goldbohm, and P. A. van den Brandt, "Physical activity and risk of ovarian cancer: results from the Netherlands Cohort Study (The Netherlands)," *Cancer Causes Control* , vol. 17, no. 1, pp. 109–115, Feb. 2006, doi: 10.1007/s10552-005-0422-3.

[11] M. F. Leitzmann *et al.* , "Prospective study of physical activity and the risk of ovarian cancer," *Cancer Causes Control* , vol. 20, no. 5, pp. 765–773, Jul. 2009, doi: 10.1007/s10552-008-9291-x.

[12] J. Lee, "Physical activity, sitting time, and the risk of ovarian cancer: A brief research report employing a meta-analysis of existing," *Health Care Women Int* , vol. 40, no. 4, pp. 433–458, Apr. 2019, doi: 10.1080/07399332.2018.1505892.

[13] A. Grundy *et al.* , "Lifetime recreational moderate-to-vigorous physical activity and ovarian cancer risk: A case-control study," *Int J Cancer* , vol. 146, no. 7, pp. 1800–1809, Apr. 2020, doi: 10.1002/ijc.32513.

[14] C. M. Olsen *et al.* , "Obesity and risk of ovarian cancer subtypes: evidence from the Ovarian Cancer Association Consortium," *Endocr Relat Cancer* , vol. 20, no. 2, pp. 251–262, Apr. 2013, doi: 10.1530/ERC-12-0395.

[15] J.-S. Ong *et al.* , "Evaluating the role of alcohol consumption in breast and ovarian cancer susceptibility using population-based cohort studies and two-sample Mendelian randomization analyses," *Int J Cancer* , vol. 148, no. 6, pp. 1338–1350, Mar. 2021, doi: 10.1002/ijc.33308.

[16] M. T. Goodman and K. H. Tung, "Alcohol consumption and the risk of borderline and invasive ovarian cancer," *Obstet Gynecol* , vol. 101, no. 6, pp. 1221–1228, Jun. 2003, doi: 10.1016/s0029-7844(03)00050-4.

[17] I. Licaj, B. K. Jacobsen, R. M. Selmer, G. Maskarinec, E. Weiderpass, and I. T. Gram, "Smoking and risk of ovarian cancer by histological subtypes: an analysis among 300 000 Norwegian women," *Br J Cancer* , vol. 116, no. 2, pp. 270–276, Jan. 2017, doi: 10.1038/bjc.2016.418.

[18] S. Hurley, D. Goldberg, L. Bernstein, and P. Reynolds, "Sleep duration and cancer risk in women," *Cancer Causes Control* , vol. 26, no. 7, pp. 1037–1045, Jul. 2015, doi: 10.1007/s10552-015-0579-3.

[19] X. Liang *et al.* , "Sleep Characteristics and Risk of Ovarian Cancer Among Postmenopausal Women," *Cancer Prev Res (Phila)* , vol. 14, no. 1, pp. 55–64, Jan. 2021, doi: 10.1158/1940-6207.CAPR-20-0174.

[20] G. Davey Smith and G. Hemani, "Mendelian randomization: genetic anchors for causal inference in epidemiological studies," *Hum Mol Genet* , vol. 23, no. R1, pp. R89-98, Sep. 2014, doi: 10.1093/hmg/ddu328.

[21] N. M. Davies, M. V. Holmes, and G. Davey Smith, "Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians," *BMJ* , vol. 362, p. k601, Jul. 2018, doi: 10.1136/bmj.k601.

[22] J. J. Lee *et al.* , "Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals," *Nat Genet* , vol. 50, no. 8, pp. 1112–1121, Jul. 2018, doi: 10.1038/s41588-018-0147-3.

[23] V. W. Zhong *et al.* , "A genome-wide association study of bitter and sweet beverage consumption," *Hum Mol Genet* , vol. 28, no. 14, pp. 2449–2457, Jul. 2019, doi: 10.1093/hmg/ddz061.

[24] S. F. W. Meddens *et al.* , "Genomic analysis of diet composition finds novel loci and associations with health and lifestyle," *Mol Psychiatry* , vol. 26, no. 6, pp. 2056–2069, Jun. 2021, doi: 10.1038/s41380-020-0697-5.

[25] S. L. Pulit *et al.* , "Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry," *Hum Mol Genet* , vol. 28, no. 1, pp. 166–174, Jan. 2019, doi: 10.1093/hmg/ddy327.

[26] Y. C. Klimentidis *et al.* , "Genome-wide association study of habitual physical activity in over 377,000 UK Biobank participants identifies multiple variants including CADM2 and APOE," *Int J Obes (Lond)* , vol. 42, no. 6, pp. 1161–1176, Jun. 2018, doi: 10.1038/s41366-018-0120-3.

[27] M. Liu *et al.* , "Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use," *Nat Genet* , vol. 51, no. 2, pp. 237–244, Feb. 2019, doi: 10.1038/s41588-018-0307-5.

[28] R. E. Wootton *et al.* , "Evidence for causal effects of lifetime smoking on risk for depression and schizophrenia: a Mendelian randomisation study," *Psychol Med* , vol. 50, no. 14, pp. 2435–2443, Oct. 2020, doi: 10.1017/S0033291719002678.

[29] H. S. Dashti *et al.* , "Genome-wide association study identifies genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates," *Nat Commun* , vol. 10, no. 1, p. 1100, Mar. 2019, doi: 10.1038/s41467-019-08917-4.

[30] P. R. Jansen *et al.* , "Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways," *Nat Genet* , vol. 51, no. 3, pp. 394–403, Mar. 2019, doi: 10.1038/s41588-018-0333-3.

[31] C. M. Phelan *et al.* , "Identification of 12 new susceptibility loci for different histotypes of epithelial ovarian cancer," *Nat Genet* , vol. 49, no. 5, pp. 680–691, May 2017, doi: 10.1038/ng.3826.

[32] G. Hemani *et al.* , "The MR-Base platform supports systematic causal inference across the human phenome," *Elife* , vol. 7, p. e34408, May 2018, doi: 10.7554/eLife.34408.

[33] S. Burgess, A. Butterworth, and S. G. Thompson, "Mendelian randomization analysis with multiple genetic variants using summarized data," *Genet Epidemiol* , vol. 37, no. 7, pp. 658–665, Nov. 2013, doi: 10.1002/gepi.21758.

[34] J. Bowden, G. Davey Smith, P. C. Haycock, and S. Burgess, "Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator," *Genet Epidemiol* , vol. 40, no. 4, pp. 304–314, May 2016, doi: 10.1002/gepi.21965.

[35] J. Bowden, G. Davey Smith, and S. Burgess, "Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression," *Int J Epidemiol* , vol. 44, no. 2, pp. 512–525, Apr. 2015, doi: 10.1093/ije/dyv080.

[36] M. Verbanck, C.-Y. Chen, B. Neale, and R. Do, "Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases," *Nat Genet* , vol. 50, no. 5, pp. 693–698, May 2018, doi: 10.1038/s41588-018-0099-7.

[37] O. O. Yavorska and S. Burgess, "MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data," *Int J Epidemiol* , vol. 46, no. 6, pp. 1734–1739, Dec. 2017, doi: 10.1093/ije/dyx034.

[38] S. Yuan, Y. Xiong, M. Michaelsson, K. Michaelsson, and S. C. Larsson, "Genetically predicted education attainment in relation to somatic and mental health," *Sci Rep* , vol. 11, no. 1, p. 4296, Feb. 2021, doi: 10.1038/s41598-021-83801-0.

[39] M. T. Goodman, K.-H. Tung, K. McDuffie, L. R. Wilkens, and T. A. Donlon, "Association of caffeine intake and CYP1A2 genotype with ovarian cancer," *Nutr Cancer* , vol. 46, no. 1, pp. 23–29, 2003, doi: 10.1207/S15327914NC4601_03.

[40] "Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer - PubMed." https://pubmed.ncbi.nlm.nih.gov/18213613/ (accessed Apr. 10, 2022).

[41] J. Kotsopoulos *et al.* , "Coffee intake, variants in genes involved in caffeine metabolism, and the risk of epithelial ovarian cancer," *Cancer Causes Control* , vol. 20, no. 3, pp. 335–344, Apr. 2009, doi: 10.1007/s10552-008-9247-1.

[42] Y. J. Song, A. R. Kristal, K. G. Wicklund, K. L. Cushing-Haugen, and M. A. Rossing, "Coffee, tea, colas, and risk of epithelial ovarian cancer," *Cancer Epidemiol Biomarkers Prev* , vol. 17, no. 3, pp. 712–716, Mar. 2008, doi: 10.1158/1055-9965.EPI-07-2511.

[43] R. Arthur, V. A. Kirsh, and T. E. Rohan, "Associations of coffee, tea and caffeine intake with risk of breast, endometrial and ovarian cancer among Canadian women," *Cancer Epidemiol* , vol. 56, pp. 75–82, Oct. 2018, doi: 10.1016/j.canep.2018.07.013.

[44] M. Hashibe *et al.* , "Coffee, tea, caffeine intake, and the risk of cancer in the PLCO cohort," *Br J Cancer* , vol. 113, no. 5, pp. 809–816, Sep. 2015, doi: 10.1038/bjc.2015.276.

[45] S. J. Jordan, D. M. Purdie, A. C. Green, and P. M. Webb, "Coffee, tea and caffeine and risk of epithelial ovarian cancer," *Cancer Causes Control* , vol. 15, no. 4, pp. 359–365, May 2004, doi: 10.1023/B:CACO.0000027482.00077.8b.

[46] S.-Y. Park, N. D. Freedman, C. A. Haiman, L. Le Marchand, L. R. Wilkens, and V. W. Setiawan, "Prospective Study of Coffee Consumption and Cancer Incidence in Non-White Populations," *Cancer Epidemiol Biomarkers Prev* , vol. 27, no. 8, pp. 928–935, Aug. 2018, doi: 10.1158/1055-9965.EPI-18-0093.

[47] K. Hirose, Y. Niwa, K. Wakai, K. Matsuo, T. Nakanishi, and K. Tajima, "Coffee consumption and the risk of endometrial cancer: Evidence from a case-control study of female hormone-related cancers in Japan," *Cancer Sci* , vol. 98, no. 3, pp. 411–415, Mar. 2007, doi: 10.1111/j.1349-7006.2007.00391.x.

[48] C. M. Nagle, C. M. Olsen, C. J. Bain, D. C. Whiteman, A. C. Green, and P. M. Webb, "Tea consumption and risk of ovarian cancer," *Cancer Causes Control* , vol. 21, no. 9, pp. 1485–1491, Sep. 2010, doi: 10.1007/s10552-010-9577-7.

[49] R. B. Ness, "Endometriosis and ovarian cancer: thoughts on shared pathophysiology," *Am J Obstet Gynecol* , vol. 189, no. 1, pp. 280–294, Jul. 2003, doi: 10.1067/mob.2003.408.

[50] R. L. Ferrini and E. Barrett-Connor, "Caffeine intake and endogenous sex steroid levels in postmenopausal women. The Rancho Bernardo Study," *Am J Epidemiol* , vol. 144, no. 7, pp. 642–644, Oct. 1996, doi: 10.1093/oxfordjournals.aje.a008975.

[51] C. Nagata, M. Kabuto, and H. Shimizu, "Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women," *Nutr Cancer* , vol. 30, no. 1, pp. 21–24, 1998, doi: 10.1080/01635589809514635.

[52] F. Parazzini, P. Vigano, M. Candiani, and L. Fedele, "Diet and endometriosis risk: a literature review," *Reprod Biomed Online* , vol. 26, no. 4, pp. 323–336, Apr. 2013, doi: 10.1016/j.rbmo.2012.12.011.

[53] W. Qiu, H. Lu, Y. Qi, and X. Wang, "Dietary fat intake and ovarian cancer risk: a meta-analysis of epidemiological studies," *Oncotarget* , vol. 7, no. 24, pp. 37390–37406, Jun. 2016, doi: 10.18632/oncotarget.8940.

[54] M. A. Merritt, D. W. Cramer, S. A. Missmer, A. F. Vitonis, L. J. Titus, and K. L. Terry, "Dietary fat intake and risk of epithelial ovarian cancer by tumour histology," *Br J Cancer* , vol. 110, no. 5, pp. 1392–1401, Mar. 2014, doi: 10.1038/bjc.2014.16.

[55] Collaborative Group on Epidemiological Studies of Ovarian Cancer, "Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies," *PLoS Med* , vol. 9, no. 4, p. e1001200, 2012, doi: 10.1371/journal.pmed.1001200.

[56] J. Yarmolinsky *et al.* , "Appraising the role of previously reported risk factors in epithelial ovarian cancer risk: A Mendelian randomization analysis," *PLoS Med* , vol. 16, no. 8, p. e1002893, Aug. 2019, doi: 10.1371/journal.pmed.1002893.

[57] S. C. Dixon *et al.* , "Adult body mass index and risk of ovarian cancer by subtype: a Mendelian randomization study," *Int J Epidemiol* , vol. 45, no. 3, pp. 884–895, Jun. 2016, doi: 10.1093/ije/dyw158.

[58] S. Jordan, A. Green, and P. Webb, "Benign epithelial ovarian tumours-cancer precursors or markers for ovarian cancer risk?," *Cancer Causes Control* , vol. 17, no. 5, pp. 623–632, Jun. 2006, doi: 10.1007/s10552-005-0370-y.

[59] S. J. Jordan, A. C. Green, D. C. Whiteman, and P. M. Webb, "Risk factors for benign serous and mucinous epithelial ovarian tumors," *Obstet Gynecol* , vol. 109, no. 3, pp. 647–654, Mar. 2007, doi: 10.1097/01.AOG.0000254159.75977.fa.

[60] C. M. Olsen *et al.* , "Recreational physical activity and epithelial ovarian cancer: a case-control study, systematic review, and meta-analysis," *Cancer Epidemiol Biomarkers Prev* , vol. 16, no. 11, pp. 2321–2330, Nov. 2007, doi: 10.1158/1055-9965.EPI-07-0566.

[61] L.-M. Zhou, "Recreational physical activity and risk of ovarian cancer: a meta-analysis," *Asian Pac J Cancer Prev* , vol. 15, no. 13, pp. 5161–5166, 2014, doi: 10.7314/apjcp.2014.15.13.5161.

[62] J. S. Hildebrand, S. M. Gapstur, M. M. Gaudet, P. T. Campbell, and A. V. Patel, "Moderate-to-vigorous physical activity and leisure-time sitting in relation to ovarian cancer risk in a large prospective US cohort," *Cancer Causes Control* , vol. 26, no. 11, pp. 1691–1697, Nov. 2015, doi: 10.1007/s10552-015-0656-7.

[63] M. A. Rossing, K. L. Cushing-Haugen, K. G. Wicklund, J. A. Doherty, and N. S. Weiss, "Recreational physical activity and risk of epithelial ovarian cancer," *Cancer Causes Control* , vol. 21, no. 4, pp. 485–491, Apr. 2010, doi: 10.1007/s10552-009-9479-8.

[64] M. T. Faber *et al.* , "Cigarette smoking and risk of ovarian cancer: a pooled analysis of 21 case-control studies," *Cancer Causes Control* , vol. 24, no. 5, pp. 989–1004, May 2013, doi: 10.1007/s10552-013-0174-4.

[65] Collaborative Group on Epidemiological Studies of Ovarian Cancer *et al.* , "Ovarian cancer and smoking: individual participant meta-analysis including 28,114 women with ovarian cancer from 51 epidemiological studies," *Lancet Oncol* , vol. 13, no. 9, pp. 946–956, Sep. 2012, doi: 10.1016/S1470-2045(12)70322-4.

[66] "Wine and other alcohol consumption and risk of ovarian cancer in the California Teachers Study cohort - PubMed." https://pubmed.ncbi.nlm.nih.gov/17186425/ (accessed Apr. 11, 2022).

[67] N. B. Peterson *et al.* , "Alcohol consumption and ovarian cancer risk in a population-based case-control study," *Int J Cancer* , vol. 119, no. 10, pp. 2423–2427, Nov. 2006, doi: 10.1002/ijc.22137.

[68] S. S. Tworoger, D. M. Gertig, M. A. Gates, J. L. Hecht, and S. E. Hankinson, "Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer," *Cancer* , vol. 112, no. 5, pp. 1169–1177, Mar. 2008, doi: 10.1002/cncr.23275.

[69] L. J. Schouten, M. P. A. Zeegers, R. A. Goldbohm, and P. A. van den Brandt, "Alcohol and ovarian cancer risk: results from the Netherlands Cohort Study," *Cancer Causes Control* , vol. 15, no. 2, pp. 201–209, Mar. 2004, doi: 10.1023/B:CACO.0000019512.71560.2b.

[70] C. La Vecchia, E. Negri, S. Franceschi, F. Parazzini, A. Gentile, and M. Fasoli, "Alcohol and epithelial ovarian cancer," *J Clin Epidemiol* , vol. 45, no. 9, pp. 1025–1030, Sep. 1992, doi: 10.1016/0895-4356(92)90119-8.

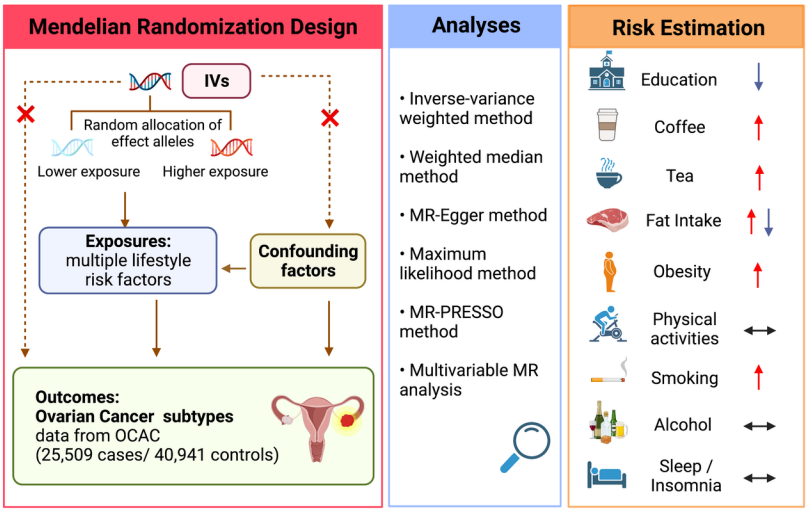
[71] L. E. Kelemen, T. A. Sellers, R. A. Vierkant, L. Harnack, and J. R. Cerhan, "Association of folate and alcohol with risk of ovarian cancer in a prospective study of postmenopausal women," *Cancer Causes Control* , vol. 15, no. 10, pp. 1085–1093, Dec. 2004, doi: 10.1007/s10552-004-1546-6.

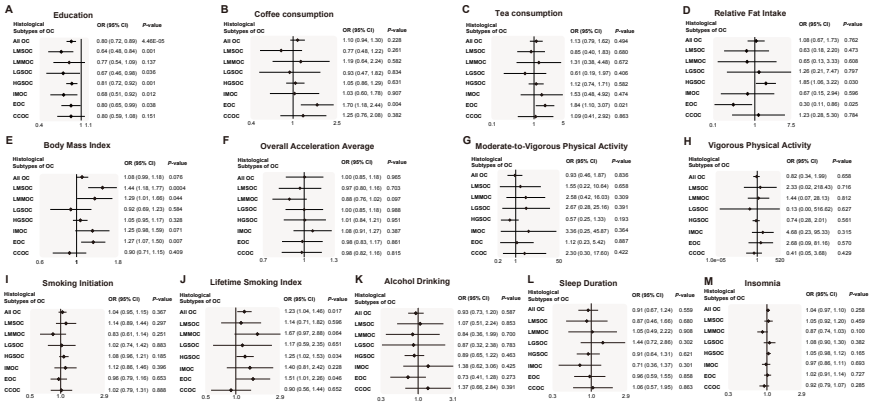
[72] P. M. Webb, D. M. Purdie, C. J. Bain, and A. C. Green, "Alcohol, wine, and risk of epithelial ovarian cancer," *Cancer Epidemiol Biomarkers Prev* , vol. 13, no. 4, pp. 592–599, Apr. 2004.

Figure legends

Figure 1 . Graphical overview of the MR study design. The Mendelian Randomization (MR) design indicates that the genetic variants proposed as instrumental variables (IVs) should be robustly associated with the risk factor of interest; the selected IVs should not be associated with potential confounders and affect the risk of the outcome merely through the risk factors, not via alternative pathways. The genetic variants, selected as IVs for studying the effect of modifying the exposure, are randomly allocated at conception and are therefore less vulnerable to confounding from environmental factors and reverse causation. Multiple analyses are conducted to assess the causal relationship between lifestyle factors and the risk of ovarian cancer subtypes.

Figure 2 . Associations of genetically predicted lifestyle factors and ovarian cancer subtypes. The OR (95% CI) of ovarian cancer subtypes were estimated using an inverse-variance weighted meta-analysis.





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