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Mendelian Randomization

Association between genetically predicted polycystic ovary syndrome and ovarian cancer: a Mendelian randomization study

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a complex endocrine disorder with an estimated prevalence of 4–21% in reproductive aged women. Recently, the Ovarian Cancer Association Consortium (OCAC) reported a decreased risk of invasive ovarian cancer among women with self-reported PCOS. However, given the limitations of self-reported PCOS, the validity of these observed associations remains uncertain. Therefore, we sought to use Mendelian randomization with genetic markers as a proxy for PCOS, to examine the association between PCOS and ovarian cancer.**Methods:** Utilizing 14 single nucleotide polymorphisms (SNPs) previously associated with PCOS we assessed the association between genetically predicted PCOS and ovarian cancer risk, overall and by histotype, using summary statistics from a previously conducted genome-wide association study (GWAS) of ovarian cancer among European ancestry women within the OCAC (22 406 with invasive disease, 3103 with borderline disease and 40 941 controls).**Results:** An inverse association was observed between genetically predicted PCOS and invasive ovarian cancer risk: odds ratio (OR)=0.92 [95% confidence interval (CI)=0.85–0.99; $P = 0.03$]. When results were examined by histotype, the strongest inverse association was observed between genetically predicted PCOS and endometrioid tumors (OR = 0.77; 95% CI = 0.65–0.92; $P = 0.003$). Adjustment for individual-level body mass index, oral contraceptive use and parity did not materially change the associations.**Conclusion:** Our study provides evidence for a relationship between PCOS and reduced ovarian cancer risk, overall and among specific histotypes of invasive ovarian cancer. These results lend support to our previous observational study results. Future studies are needed to understand mechanisms underlying this association.

Key words: Polycystic ovary syndrome, ovarian cancer, histotype, Mendelian randomization

Key Messages

- Previous observational studies of PCOS and ovarian cancer risk have reported conflicting results.
- We used Mendelian randomization, an analytical method that capitalizes on the presumed random assortment of genes from parents to offspring, to examine the association between PCOS and ovarian cancer risk.
- An inverse association was observed between genetically predicted PCOS and invasive ovarian cancer risk, with the most robust association observed for the endometrioid histotype.

Introduction

Polycystic ovary syndrome (PCOS) is a complex, heterogeneous endocrine disorder with a prevalence of 4–21% in women of reproductive age.[1] It has been estimated to affect approximately 5 million women in the USA,[2] and is characterized by oligomenorrhoea (i.e. infrequent or irregular periods), infrequent ovulation and abnormal hormone levels including hyperandrogenism, hyperinsulinaemia, and gonadotropin imbalance that can influence ovarian cancer risk. Previous observational studies have produced inconsistent results in the examination of PCOS and ovarian cancer risk, with most demonstrating null or suggestive increases in risk.[3,4] Yet recently, in the largest study to date, the Ovarian Cancer Association Consortium (OCAC), an international collaboration of ovarian cancer studies, reported a suggestion of a decreased risk of invasive ovarian cancer among women with self-reported PCOS.[5] However, given the current limitations in the accuracy of self-reported PCOS and the potential influence of other risk factors that are common among women with PCOS (e.g. oral contraceptive use) the validity of these observed associations remains uncertain.

While PCOS was first described in 1935, standard diagnostic criteria were not established until 1990 by the National Institutes of Health (NIH),[6] with the two other commonly used criteria, Rotterdam[7] and Androgen Excess (AD)-PCOS Society,[8] established in 2003 and 2006, respectively. Owing to the historically poor understanding of this condition, under-diagnosis of PCOS has been common. This under-diagnosis is evident in existing case-control studies of ovarian cancer where, on average across studies, only 0.4–2.3% of control women reported a clinical diagnoses of PCOS,[5] well below the expected population prevalence. This may explain the lack of clear results observed in the recent OCAC study examining PCOS and ovarian cancer risk.[5]

To address these limitations, we used Mendelian randomization (MR), an analytical method that capitalizes on the presumed random assortment of genes from parents to offspring, to examine the association between PCOS and ovarian cancer risk. This method is largely independent of the biases inherent in standard observational studies (e.g. residual or unmeasured confounding) when specific assumptions are met.[9] Twin studies indicate that PCOS has a large heritable component.[10] This suggests that MR may provide a means to examine the PCOS–ovarian cancer relation. Thus, we sought to employ information from recent genome-wide association studies (GWAS) of PCOS and ovarian cancer to examine this association.

Methods

Identification of SNPs associated with PCOS

We conducted a literature search to identify and extract information for single nucleotide polymorphisms (SNPs) that were associated with PCOS at the genome-wide significance level ($P = 5 \times 10^{-8}$). We identified 16 SNPs associated with PCOS from the largest and most recent GWAS publication.[11] Of these SNPs, 14 reached genome-wide significance in European ancestry populations and none was in linkage disequilibrium, thus all 14 were included in our instrument (rs2178575, rs10739076, rs7864171, rs9696009, rs11031005, rs1784692, rs2271194, rs1795379, rs8043701, rs853854, rs11225154, rs13164856, rs7563201 and rs804279) (Supplementary Table 1, available as Supplementary data at *IJE* online). We obtained information about effect alleles, trait-specific effect estimates and their standard errors from Day *et al.*[11]

GWAS of ovarian cancer

To assess whether genetically predicted PCOS is associated with risk of ovarian cancer, we used data from a GWAS of epithelial ovarian cancer (EOC) using DNA samples from participants in studies from the OCAC, an international collaboration. Details of this GWAS have been described previously.[12] Briefly, 22 406 women with invasive disease (1012 low-grade serous, 13 037 high-grade serous, 2810 endometrioid, 1366 clear cell, 1417 mucinous and other 2764 EOC), 3103 with borderline disease (non-invasive) (1954 serous borderline and 1149 mucinous borderline) and 40 941 controls of European ancestry from seven genotyping projects were included. Genotypes for OCAC samples were preferentially selected from the different projects in the following order: OncoArray, Mayo GWAS, Collaborative Oncological Gene-environment Study (COGS) project and other EOC GWAS. SNP quality control (QC) was carried out according to standard QC guidelines[13] and imputation was performed using the 1000 Genomes reference panel phase 3 version 5. Associations between genotype and risk of ovarian cancer were examined using logistic regression models. We extracted overall and histotype-specific ovarian cancer specific effect estimates and standard errors from the OCAC GWAS summary statistics for each of the identified PCOS SNPs.

Sensitivity analyses and covariate dataset

One of the assumptions of MR is that the genetic variants included in the instrument are not associated with any other factors that are associated with both PCOS

and ovarian cancer risk. Body mass index (BMI) is associated with risk of specific ovarian cancer histotypes[14] and may increase risk of PCOS, and is thus a potential confounder of the association between PCOS and ovarian cancer.[15] If the PCOS-associated genetic variants included in our instrument were also associated with BMI, our MR analysis would not be able to provide an accurate estimate of the causal effect of PCOS on ovarian cancer. In addition, we further sought to address whether the association between PCOS and ovarian cancer was independent of other ovarian cancer risk factors that are common among women with PCOS. Specifically, it is well-established that oral contraceptives reduce ovarian cancer risk[16] and are a first-line treatment for women with PCOS to manage menstrual irregularities, hyperandrogenism and acne.[17] Further, women with PCOS often have reduced fertility, and increasing parity is known to have a protective effect on ovarian cancer risk.[18] Consequently, as BMI could be considered a confounder of the PCOS–ovarian cancer association and oral contraceptive use and parity could be considered mediators, we evaluated the influence of BMI, oral contraceptive use and parity in a subset of six studies with individual-level data (DOV,[19] HOP,[20] MAL,[21] NCO,[22] NEC,[23] TOR[24]). All studies had ethics approval and all study participants provided informed consent. Information on known and suspected risk factors for ovarian cancer was collected in each study as well as individual-level genetic data. More details on the covariates and studies included are provided elsewhere.[5] Analyses using covariate data included 2860 women with invasive disease, 601 with borderline disease and 4945 controls.

Statistical analysis

We conducted 2-sample MR analyses using an inverse variance weighted (IVW) average to estimate the association between PCOS and ovarian cancer using summary genetic association statistics[25] calculated as:

$$\widehat{\beta}_{IVW} = \frac{\sum_k X_k Y_k \sigma_{Y_k}^{-2}}{\sum_k X_k^2 \ \sigma_{Y_k}^{-2}}$$

$$\sigma_{IVW} = \sqrt{\frac{1}{\sum_k X_k^2 \sigma_{Y_k}^{-2}}}$$

$\widehat{\beta}_{IVW}$ is the ratio estimate of the effect of PCOS (X) on ovarian cancer (Y) using genetic variants $k = 1, \ldots, K$ (where $K = 7$). X_k is the per-allele effect of SNP k on

PCOS, Y_k is the per-allele change in the log odds ratio (OR) for ovarian cancer for SNP k, and σ_{Y_k} is the standard error for Y_k. We only included the 14 SNPs associated with PCOS at genome-wide significance levels in European ancestry populations. We estimated ORs [95% confidence intervals (CI)] for all invasive ovarian cancers, borderline disease and by histotype (serous borderline, mucinous borderline, low-grade serous, high-grade serous, mucinous invasive, clear cell and endometrioid). Sensitivity analyses were conducted using MR Egger regression to assess bias from directional pleiotropy,[26] and using a weighted median estimator that can provide a consistent estimate of the effect when ≤50% of the information comes from invalid instrumental variables.[27]

In secondary analyses we assessed the influence of BMI, oral contraceptive use and parity in two ways. First, among six studies with individual-level data (described above), we examined the association between a PCOS-weighted allele score[28,29] created with the 14 instrument SNPs and ovarian cancer risk with and without adjustment for each of these covariates. Finally, in a separate analysis, we examined the association between each instrument SNP and BMI, oral contraceptive use (ever/never) and number of live births using publicly available GWAS data for over 180 749 women in the UK Biobank (UKBB).[30] Analyses were conducted using STATA version 15 (StataCorp LP, College Station, TX, USA) and R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) using the MendelianRandomization package.

Results

The overall association between genetically predicted PCOS and invasive ovarian cancer risk was 0.92 (95% CI = 0.85–0.99; $P = 0.03$). When results were examined by histotype, an inverse association was observed between PCOS and the high-grade serous (OR = 0.91; 95% CI = 0.82–1.00; $P = 0.046$) and endometrioid (OR = 0.77; 0.65–0.92; $P = 0.003$) histotypes (Table 1). We did not observe evidence of directional pleiotropy in our MR Egger regression, with an intercept that was not significantly different from zero (intercept = −0.01; 95% CI = −0.06 to 0.03; $P = 0.55$). In sensitivity analyses using a weighted median estimator the results were not materially different than the IVW method (Table 2). Effect estimates from the MR Egger regression were not entirely consistent with the IVW or weighted median estimator results, with the exception of the endometrioid histotype which was consistently inversely associated with genetically predicted PCOS across all methods (Table 2).

We then evaluated whether the association between genetically predicted PCOS and ovarian cancer was influenced by BMI (potential confounder), oral contraceptive

Table 1. Associations between genetically predicted PCOS and ovarian cancer overall and by histotype using 14 SNPs associated with PCOS in European ancestry populations

	IVW method		Cochran Q statistic	
	Odds ratio (95% CI)	*P*-value	Test statistic	*P*-value
Borderline	1.08 (0.94–1.25)	0.27	7.66	0.86
Serous	1.06 (0.89–1.27)	0.52	12.32	0.50
Mucinous	1.13 (0.90–1.41)	0.29	10.92	0.62
Invasive	0.92 (0.85–0.99)	0.03	17.31	0.19
Low-grade serous	1.09 (0.83–1.43)	0.53	16.26	0.24
High-grade serous	0.91 (0.82–0.998)	0.046	18.71	0.13
Mucinous	1.13 (0.92–1.38)	0.25	12.08	0.52
Endometrioid	0.77 (0.65–0.92)	0.003	18.69	0.13
Clear cell	0.90 (0.73–1.10)	0.30	12.00	0.53

Table 2. MR Egger and weighted median approaches to assess the association between genetically predicted PCOS and ovarian cancer risk, overall and by histotype

	MR Egger		Weighted median	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Borderline	0.96 (0.50–1.88)	0.91	1.05 (0.87–1.27)	0.63
Serous	0.89 (0.39–2.03)	0.79	1.07 (0.85–1.37)	0.56
Mucinous	1.28 (0.45–3.64)	0.65	1.19 (0.88–1.60)	0.26
Invasive	1.02 (0.71–1.48)	0.91	0.92 (0.84–1.01)	0.09
Low-grade serous	1.89 (0.52–6.86)	0.33	1.28 (0.90–1.83)	0.17
High-grade serous	1.23 (0.80–1.89)	0.36	0.91 (0.81–1.01)	0.09
Mucinous	0.81 (0.32–2.10)	0.67	1.20 (0.91–1.58)	0.20
Endometrioid	0.43 (0.20–0.93)	0.03	0.70 (0.56–0.87)	0.001
Clear cell	1.15 (0.44–2.96)	0.78	0.85 (0.64–1.12)	0.24

use (potential mediator) or parity (potential mediator). In our individual level data, the point estimate between the PCOS-weighted allele score and ovarian cancer risk was not materially altered with adjustment for each of these covariates (Table 3). Results were not materially different when only invasive ovarian cases were included (results not shown). In addition, none of our instrument SNPs were associated with BMI, ever use of oral contraceptives or parity at a genome-wide significance level in the UKBB (Supplementary Table 2, available as Supplementary data at *IJE* online).

Discussion

In this study, involving data from over 63 000 women in studies from the OCAC, we used a MR approach to assess the relationship between PCOS and ovarian cancer risk. We observed an inverse association between genetically predicted PCOS and risk of invasive ovarian cancer, with the strongest inverse association observed for the endometrioid histotype.

The associations we observed between genetically predicted PCOS and ovarian cancer risk by histotype, lend

Table 3. Associations between PCOS-weighted allele score and ovarian cancer risk with and without adjustment for covariates among six case-control studies in the Ovarian Cancer Association Consortium (3461 cases[a] and 4945 controls)

	Odds ratio (95% CI)
Model without covariate adjustment	0.88 (0.66–1.16)
Model with adjustment for BMI	0.88 (0.66–1.17)
Model with adjustment for oral contraceptive use	0.89 (0.67–1.19)
Model with adjustment for parity	0.88 (0.67–1.17)

[a]Cases included 2860 with invasive disease and 601 with borderline disease.

support to our previous observational study results. Among 14 OCAC case-control studies with individual-level epidemiologic data (16 594 women with invasive ovarian cancer, 2875 with borderline disease and 17 718 controls), a decreased risk of ovarian cancer was reported among women who self-reported PCOS (OR = 0.87; 95% CI = 0.65–1.15). Lack of statistical significance in this previous study may be due in part to misclassification (specifically

under-diagnosis) of PCOS, as most of the participating women were of reproductive age prior to the 1990 establishment of the PCOS diagnostic criteria established by the NIH/ National Institute of Child Health and Disease (NIH/ NICHD).[5] To address this issue, infrequent and irregular periods were examined as a proxy for PCOS, as these menstrual cycle irregularities occur in 75–85% of women with PCOS and are easier to assess via self-reported questionnaire.[31–33] Similar to the results observed in this MR analysis, a decreased risk of invasive ovarian cancer was observed among women who reported irregular menstrual cycles (OR = 0.83; 95% CI = 0.76–0.89). Further, when examined by histotype, these inverse associations were observed for high-grade serous (OR = 0.86; 95% CI = 0.78–0.95), endometrioid (OR = 0.84; 95% CI = 0.72–0.98) and clear cell (OR = 0.68; 95% CI = 0.55–0.84) ovarian cancer.[5]

Whereas our results are consistent with the largest observational study of PCOS and ovarian cancer risk to date, they are inconsistent with several previous observational studies of PCOS and ovarian cancer risk that generally demonstrated null or a suggestive increase in risk of ovarian cancer among women with PCOS.[3,4] Of eight previous studies examining PCOS and ovarian cancer risk (two of which were included in the OCAC analysis),[34–41] five did not adjust for BMI[34,35,39–41] and four of these reported effect estimates indicating an increased risk of ovarian cancer among women with PCOS.[34,35,39–41] In contrast, among the three studies adjusting for BMI,[36–38] only one reported a positive association with invasive disease,[38] with the others reporting null or the suggestion of a decreased risk. While not part of the diagnostic criteria for PCOS, a higher BMI is common among women with PCOS[42–44] and has recently been shown to potentially increase risk of PCOS.[15] Notably, of the studies mentioned above that did not adjust for BMI, the single study that observed a null association between PCOS and ovarian cancer risk was conducted among women in Taiwan,[40] which may reflect the fact that obesity is more common among women with PCOS in Caucasian women than Asian women.[43] Given the positive association between BMI and some ovarian cancer histotypes,[14,45] it is possible that some of the increased ovarian cancer risk observed with PCOS in prior studies is partially attributable to confounding due to higher BMI among women with PCOS. In this regard, MR provides a tool to examine the association between PCOS and risk of ovarian cancer, unconfounded by BMI, when the required assumptions are met. Further, previous GWAS of PCOS have concluded that genes associated with overweight and obesity are not strongly influential with regard to the genetics of PCOS.[46]

A limitation of our study is that we were not able to examine the association between different PCOS phenotypes and ovarian cancer risk. PCOS is a heterogeneous disorder,

and currently three differing diagnostic criteria are used to define PCOS, set by the NIH/NICHD, the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM, called the Rotterdam criteria) and the Androgen Excess and PCOS Society.[6–8] Although features of these clinical definitions overlap, they are not entirely consistent, and a consensus on the most appropriate definition has not been reached. The Rotterdam criteria define four phenotypes of PCOS: (i) oligoanovulation (OA) with polycystic ovaries (PO), (ii) PO with hyperandrogenism (HA), (iii) OA with HA, and (iv) OA, PO and HA,[7] and hormonal and metabolic differences have been demonstrated between these groups.[47] Limited research has been conducted on the genetic susceptibility to specific phenotypes of PCOS.[48] However, the most recent PCOS GWAS conducted by Day *et al.*, included PCOS cases defined by the NIH/NICHD criteria, the Rotterdam criteria and self-report, and for all SNPs the same direction of effect was observed across the three diagnosis types.[11] A further limitation is our use of a binary risk factor (PCOS). PCOS is a heterogeneous disorder and it is possible that some of the genetic variants associated with PCOS are not associated with all criteria underlying a PCOS diagnosis, making the estimation of the causal effect, considered as one disorder, not valid. In particular, the effect estimate of PCOS (yes/no) on ovarian cancer represents the average effect among individuals for whom the presence or absence of the included genetic effects determines their PCOS status. Thus, if our included genetic variants do not represent the risk of all subtypes of PCOS, our obtained effect estimate is difficult to interpret. We further assume that the effect of PCOS on ovarian cancer is constant for all individuals, which may not be the case. However, it is important to note that the MR test for an association between PCOS and ovarian cancer is still valid.[49] For an extended discussion about the use of binary exposures in MR studies, see Burgess and Labrecque.[49]

OA is generally common among women with PCOS, resulting in fewer ovulatory cycles and more cycles that are anovulatory.[50] One of the theories of initiating events in ovarian cancer involves factors associated with greater lifetime numbers of ovulations: the 'incessant ovulation hypothesis,' which posits that each ovulation involves damage and repair that could promote ovarian carcinogenesis.[51] Under this hypothesis, a decreased risk of ovarian cancer among women with PCOS, adjusted for histories of childbearing and oral contraceptive use, would provide evidence in support of this hypothesis, and is consistent with results from both our current MR results and our previous observational study.[5] However, we cannot rule out the possibility that other characteristics of PCOS that cause altered hormone levels (e.g. HA) could play a role in the association with ovarian cancer.

For our MR analyses to be valid, key assumptions must be met. First, valid associations must exist between the exposure of interest (i.e. PCOS) and the genetic variants. This was met, as in the main analyses we only used SNPs associated with PCOS at a genome-wide significance level. Second, genetic variants must not be associated with any other risk factors for both ovarian cancer and PCOS (e.g. BMI). We confirmed through literature review and genetic database search that none of our included SNPs was associated with BMI, oral contraceptive use or parity at a genome-wide significance level. In addition, using individual-level data, we examined the association between a PCOS-weighted allele score and ovarian cancer risk adjusting for BMI, oral contraceptive use and parity and did not observe any material change in the effect estimates. This is a strength of our study as we were able to leverage individual-level risk factor data to complement our use of summary level statistics. Further, the outcome (i.e. ovarian cancer) must be independent of genetic variants except through the exposure of interest. To evaluate this assumption, we conducted a pleiotropy assessment using MR Egger regression and found no evidence of directional pleiotropy. However, MR Egger regression relies on the instrument strength independent of direct effect (InSIDE) assumption, that is, that the strength of the association with the risk factor is independent of the pleiotropic effect. If this assumption is not valid other methods will be more appropriate for calculating effect estimates. Thus, we conducted additional sensitivity analyses using the weighted median method that does not depend on the InSIDE assumption and observed similar results to the main IVW analyses.

In conclusion, these findings provide evidence for a relationship between PCOS and reduced ovarian cancer risk, with the most robust association observed for the endometrioid histotype. These results are consistent with our previous analysis of a large pooled epidemiologic study. Future studies are needed to understand the mechanisms underlying this association.

Supplementary data

Supplementary data are available at *IJE* online.

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Commentary: Mendelian randomization and women's health

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Women and men are not biologically identical; differences in body shapes and compositions, hormone levels, enzymes, lifestyles and other factors lead to alterations in the presentation, diagnosis and natural history of disease, as well as drug efficacy and safety.[1–3] Yet, such differences have historically been disregarded and women's health conditions continue to be under-researched, under-diagnosed and under-treated.

Estimates suggest that up to 10% of women between 18 and 45 years are affected by polycystic ovarian syndrome (PCOS), making it the most common endocrinopathy among women of reproductive age.[4,5] Despite this, most PCOS studies have had small sample sizes,[6–10] and survey data suggest that over a third of PCOS patients have to wait more than 2 years for diagnosis.[11] At the same time, comorbidities are under-diagnosed and under-treated,[7–9] despite substantial effects on patient health and quality of life.[10,12]

Women are disproportionately affected by common diseases such as Alzheimer's and osteoarthritis, as compared with men, and experience more disease-related disability.[13] In addition to such disease that affects both women and men, it is estimated that 5% of disability-adjusted life years (DALYs) in women arises from diseases specific to women, with the corresponding value being almost 10-fold lower in men at 0.7% of DALYS.[13] Despite this, therapeutic options for women's health conditions remain limited. In the 5-year period between 2014 and 2018, the US Food and Drug Administration (FDA) approved 213 novel drugs.[14] Of these, only seven (3.3%) drugs were for female-specific indications, with a further five for breast cancer-related indications.[14] The corresponding value for male-specific indications was two (both related to prostate cancer), a value that is iniquitous to the proportion of DALYs arising from sex-specific disease.[14] Historically, the FDA also excluded women of childbearing potential