Clinical Epidemiology

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ORIGINAL RESEARCH

Genetically Predicted Cigarette Smoking in Relation to Risk of Polycystic Ovary Syndrome

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Background: Evidence from observational studies has suggested a link between cigarette smoking and the risk of polycystic ovary syndrome (PCOS). However, it remains uncertain whether the observed relationship is causal or due to biases inherent in observational studies. Therefore, we adopted two-sample Mendelian randomization (MR) design to assess the potential causal association between smoking and the risk of PCOS.

Methods: Summary level data of PCOS was obtained from a genome-wide association study (GWAS) meta-analysis including 4,138 cases and 20,129 controls of European ancestry. Single-nucleotide polymorphisms (SNPs) associated with smoking initiation (n=360) were selected and used as genetic instrumental variables (IVs). MR analysis was performed using inverse-variance weighted (IVW) method, supplemented with the likelihood-based method, weighted median method, MR pleiotropy residual sum and outlier (MR-PRESSO) test, and MR-Egger regression.

Results: Genetically predicted smoking initiation was associated with an increased risk of PCOS in the primary analysis (odds ratio (OR) =1.38, 95% confidence interval (CI) =1.12–1.69). MR-Egger regression did not detect the horizontal pleiotropy. Sensitivity analyses using alternative MR methods and restricted IVs produced similar results.

Conclusion: Our study provided evidence to support a potential causal association between smoking initiation and an increased risk of PCOS, providing a better understanding of the etiology and prevention of PCOS. Further studies are warranted to clarify the underlying biological mechanisms of smoking in the development of PCOS.

Keywords: causal inference, cigarette smoking, Mendelian randomization, polycystic ovary syndrome, single-nucleotide polymorphism

Introduction

Polycystic ovary syndrome (PCOS) is a hormonal and metabolic disorder with a global prevalence among women of reproductive age.[1–3] It is recognized that genetic and epigenetic aspects and lifestyle changes could contribute to the development of PCOS.[4–6] Nevertheless, its etiology and underlying biological mechanisms remain unclear. A better understanding of the disease risk factors, particularly modification of adverse lifestyle factors, is critical in the prevention and management of PCOS.[7]

Smoking, an important modifiable risk factor, has been uncovered to play a role in reproductive disorders.[8] The complex mixture of chemical substances contained in cigarette smoke can exert composite effects on different targets, such as the ovary, oviduct, and uterus.[9–11] Recently, the adverse effects of smoking on the risk of PCOS has caused much research interest, although most of the evidence is gathered from

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Received: 19 March 2021
Accepted: 11 June 2021
Published: 2 July 2021

observational studies.[11,12] For instance, Legro et al revealed that nearly 40% of PCOS patients (n=626) reported a current or past smoking history.[13] Another retrospective study demonstrated that smoking exerted an adverse effect on both PCOS patients with anovulation (odds ratio [OR] = 3.69, 95% confidence interval [CI]: 2.47–5.52) and those with menses (OR=2.39, 95% CI: 1.47–3.89), compared with the normal women.[14] Although this evidence provided a suggestive link between smoking and the risk of PCOS, residual confounding and reverse causation are difficult to eliminate in observational studies.

Mendelian randomization (MR) is a genetic epidemiological approach in which genetic variants, such as single-nucleotide polymorphisms (SNPs) are used as instrumental variables (IVs) to assess the potential causality of a risk factor of interest upon an outcome.[12] Since these analyses rely on the natural random assortment of genetic variants during meiosis, the results of the MR study are less susceptible to confounding and reverse causality bias. Several studies have implemented such approaches to identify the potential casual risk factors for PCOS, such as obesity.[15,16] However, no MR reports are assessing the potential causal relationship between smoking and PCOS to date. Therefore, in the present study, we applied a two-sample MR design to examine whether genetically predicted smoking was associated with the risk of PCOS, which can provide a better understanding of the etiology and prevention of PCOS.

Methods

Study Design and Data Sources

Two-sample MR design was implemented in this study (Figure 1). There are three key assumptions for an MR approach. These include 1) the genetic variants selected as IVs should be strongly associated with the exposure; 2) the IVs should not be associated with confounders; 3) the IVs should affect the risk of the outcome only through the risk factor, not via alternative pathways.[17,18] In the present study, the SNPs which achieved genome-wide significance for smoking initiation were used as IVs in the MR analysis. Summary-level data for the genetic associations

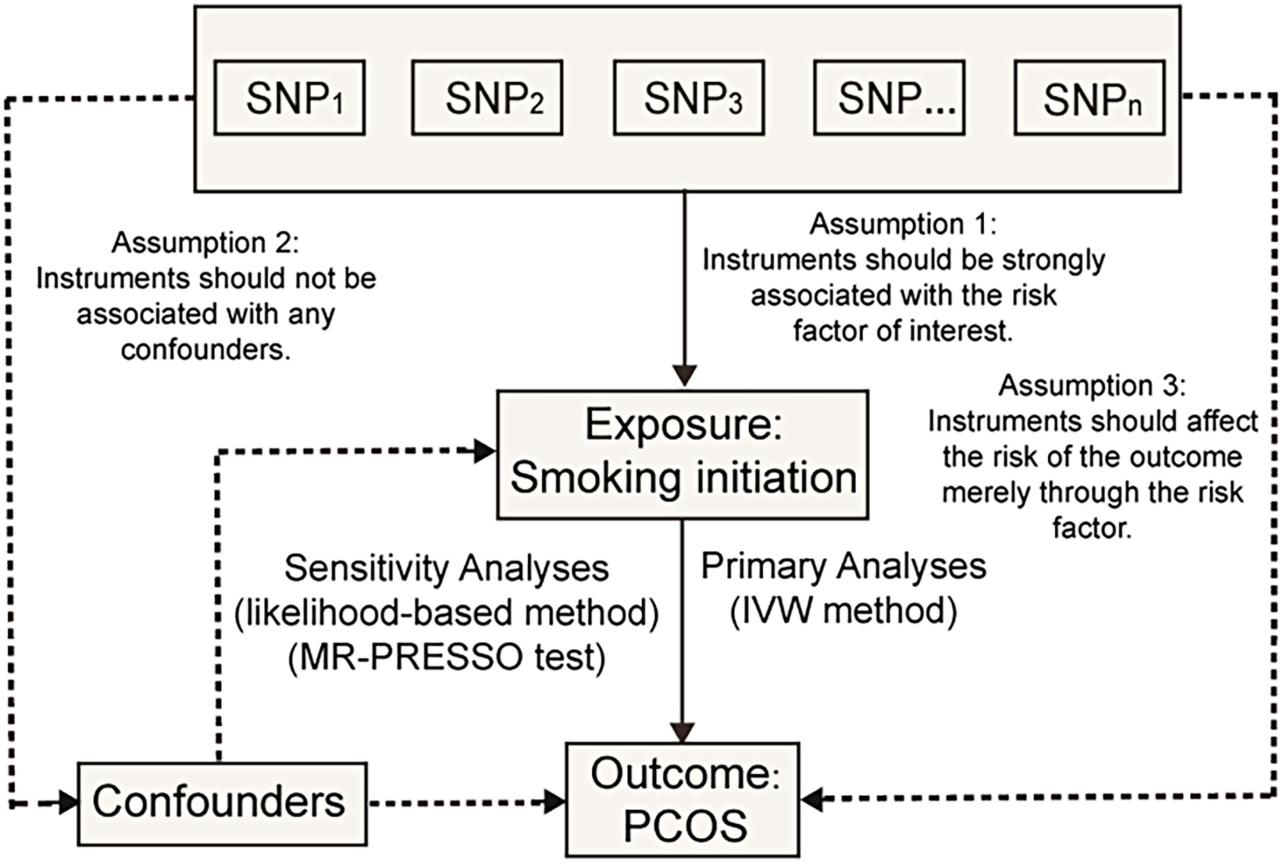

Figure 1 An overall design of the present study.
Abbreviations: IVW, inverse-variance weighted; MR, Mendelian randomization; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier; PCOS, polycystic ovary syndrome; SNP, single-nucleotide polymorphism.

between SNPs with PCOS risk was obtained from a GWAS meta-analysis published in 2018 by Day et al, which consisted of 24,357 individuals (4,138 cases and 20,129 controls) of European ancestry. Briefly, cases were diagnosed with PCOS based on criteria of the National Institute of Health (NIH) or Rotterdam Criteria.[19] Detailed information regarding the GWAS genetic dataset employed in the present study is shown in Supplementary Table 1. Since the present study was based on publicly available summary-level data of genome-wide association studies (GWAS), no additional ethical approval was required.

Genetic Instrumental Variables Selection

The genetic instruments for smoking initiation were obtained from a GWAS meta-analysis, including data from up to 1,200,000 individuals of European ancestry.[20] In total, 378 conditionally independent SNPs associated with smoking initiation (ever being a regular smoker vs never being a regular smoker) achieving the genome-wide significance threshold were identified. There were 14 SNPs excluded due to high linkage disequilibrium (LD) (r^2>0.1). Besides, four SNPs associated with smoking initiation was not available in the PCOS summary statistics. Finally, a total of 360 SNPs associated with smoking initiation were used as genetic instruments in the subsequent MR analysis. Detailed information on the included SNPs is listed in Supplementary Table 2.

Statistical Analysis

The strength of the IVs was evaluated by *F*-statistics.[21] It was defined as the ratio of the square of the gene-exposure association to the square of the corresponding standard error (SE).[22] The R^2 statistic was the proportion of variance in the smoking-related phenotypes by the SNP. It was approximately equal to $(\beta \times \sqrt{2 \times \mathrm{MAF}(1-\mathrm{MAF})})^2$, where β is the effect size estimate of the SNP in association with smoking initiation and MAF is the minimum allele frequency.[23]

The primary MR analysis was conducted using the inverse-variance weighted (IVW) method based. The estimate obtained from the IVW method is the inverse variance weighted mean of Wald ratio estimates from all genetic instruments.[24] We also used Cochran's Q test to evaluate the heterogeneity. In cases Cochran's Q statistic in the MR-IVW fixed effects analyses was significant, the MR-IVW random-effects model was adopted. Besides, the likelihood-based method and MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test were used as sensitivity analyses. In the likelihood-based method, the IVs associated with smoking initiation and with PCOS for each SNP are modeled directly by a bivariate normal distribution.[25] The MR-PRESSO test was used to detect and correct for directional pleiotropic outliers via outlier removal in multi-instrument summary-level MR testing.[26] To test potential directional pleiotropy, we also performed MR-Egger regression. In the MR-Egger method, the smoking-PCOS association was estimated by weighted linear regression of SNP-PCOS against SNP-smoking effect estimates. It can produce an intercept term indicative for directional pleiotropy.[21] Moreover, we scanned the SNPs used as genetic instruments for their potential secondary phenotypes using the GWAS Catalog (http://www.ebi.ac.uk/gwas, accessed on November 20, 2020), and further performed sensitivity analyses excluding the potentially pleiotropic SNPs.

Estimates of the association between smoking initiation and PCOS are presented as ORs with 95% CIs. All statistical analysis was performed using R software version 3.6.0 (https://www.r-project.org/). The packages used for MR analyses were "MendelianRandomization" and "MR-PRESSO".[27]

Results

For smoking initiation, all *F*-statistics were above 10, ranging from 24.9 to 73.4, suggesting less possibility of weak instrument bias. The IVs for smoking initiation can account for approximately 2.1% of the variance.

The association estimates between smoking initiation and risk of PCOS are displayed in Figure 2. Genetically predicted smoking initiation was positively correlated with an increased risk of PCOS (OR=1.38, 95% CI=1.12–1.69). The results were consistent in sensitivity analyses using the likelihood-based method (OR =1.39, 95% CI=1.12–1.71). The weighted median method produced a directionally similar estimate (OR=1.28, 95% CI: 0.93–1.71). MR-Egger regression did not indicate a potential pleiotropic bias for the IVs used for smoking initiation. By using the MR-PRESSO test, one outlier SNP (rs4759229) was identified. After correcting for the possible outlier, the effect estimate of the association between genetically predicted smoking initiation and risk of PCOS did not change markedly (OR = 1.40, 95% CI = 1.15–1.72).

In addition, we used the GWAS Catalog to scan the SNPs used as IVs for their potential-associated secondary phenotypes. A total of 69 SNPs were found to be

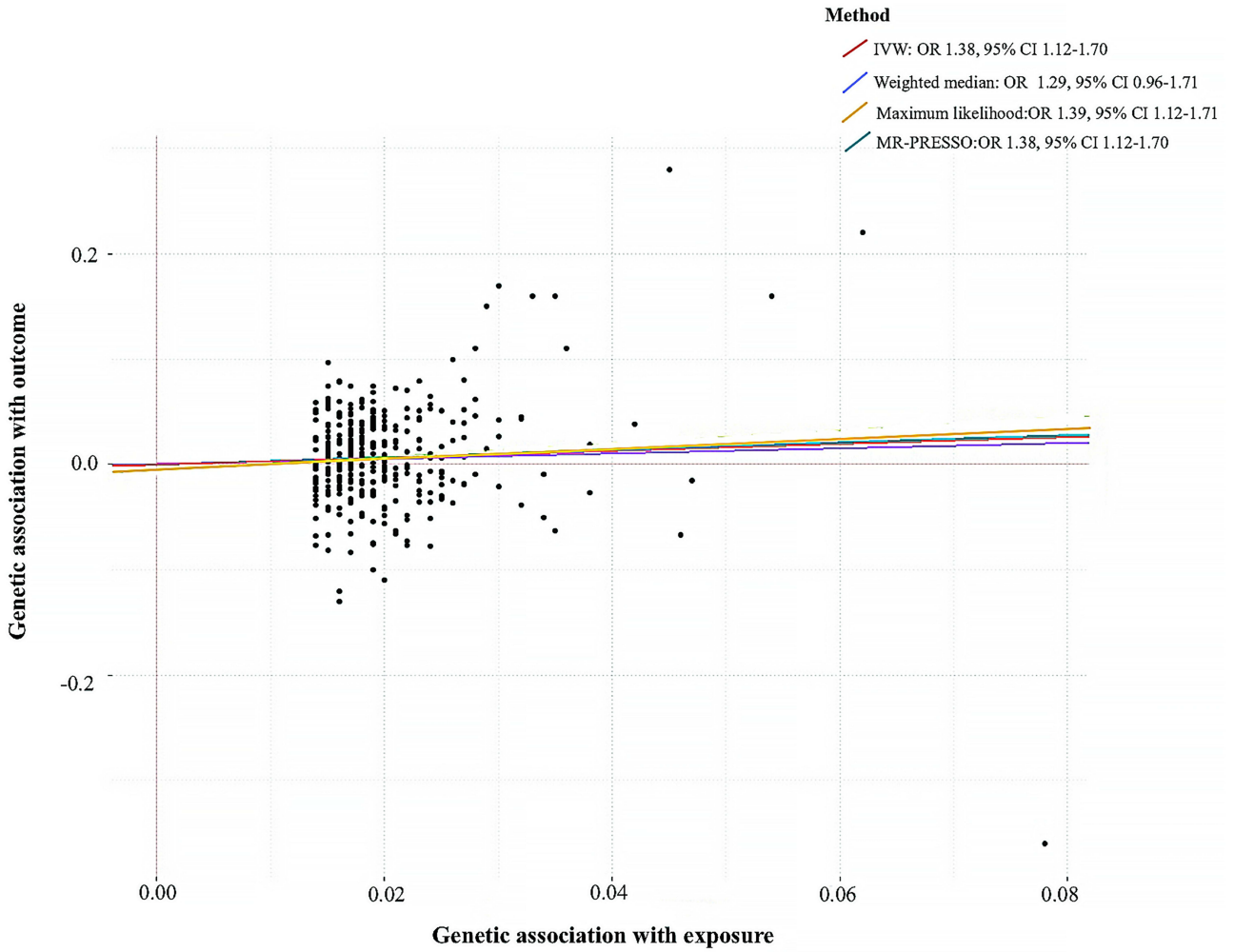

Figure 2 Association of genetically predicted smoking initiation with risk of polycystic ovary syndrome.
Abbreviations: CI, confidence interval; MR, Mendelian randomization; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier; OR, odds ratio; SNP, single-nucleotide polymorphism.

accompanied by other traits (Supplementary Table 3). After excluding these potential pleiotropic SNPs, we found the association between genetically predicted smoking initiation and risk of PCOS remained consistently in both direction and magnitude (OR = 1.32, 95% CI = 1.06–1.64 by IVW) (Supplementary Table 4).

Discussion

To the best of our knowledge, this is the first MR study to examine whether smoking is causally associated with the risk of PCOS. Our study indicated that genetically predicted smoking initiation was associated with an increased risk of PCOS. Compared with never smokers, genetically predicted smokers had a 38% increased risk of developing PCOS.

Previous observational studies have uncovered the potential association between smoking and PCOS. Legro et al found that nearly 40% of PCOS patients (n=626) in the Pregnancy in Polycystic Ovary Syndrome Study (PPCOS I) reported a current or past smoking history.[13] Similarly, a study of 346 women with PCOS from Germany revealed that 28% of the study participants were current smokers, and another study from Denmark (n=650) showed that nearly 40% of the patients with PCOS were smokers.[28,29] Moreover, the aforementioned case–control study among 2,217 PCOS women and 279 controls demonstrated a positive association between smoking and risk of PCOS after adjusting age and BMI.[14] Consistent with these prior observational studies, our data supported the hypothesis that smoking may be associated with an increased risk of PCOS.

Although the biological role of smoking in the development of PCOS is still unclear, several studies have provided plausible explanations for this. One possible interpretation may be the adverse effect of smoking on the increase of vascular stiffness, resistance and endothelial injury of ovarian tissue, which may play a role in the development of PCOS.[30] Secondly, the positive correlation between smoking and androgen metabolism was reported.[31] For example, some chemical substances contained in cigarette smoke could cause changes in luteal steroidogenesis, affecting progesterone and estradiol production, as well as suppression of the oocyte-cumulus complex expansion.[32] Another influence of smoking could be its effect on insulin. It was reported that PCOS cases who were smoking showed increased free testosterone and risking insulin resistance.[28] Although these biologically theoretical explanations are plausible, further studies are warranted to understand the underlying mechanisms of smoking in the development of PCOS.

The main novelty of this study is the adoption of the MR approach, which has been widely used to investigate the causal nature of the association between lifestyle-related factors and the risk of PCOS. The SNPs used in this study are confirmedly associated with smoking initiation at genome-wide significance threshold, thereby reducing possible violation of the first assumption of MR. Moreover, the *F*-statistics for the IVs were all satisfying the threshold of *F*-statistics >10, indicating that the analysis was unlikely to be affected by weak instrument bias.[33] The MR-Egger regression was performed to analyze the data and did not manifest the presence of directional pleiotropy.[21] In addition, we manually scanned each of the SNPs used as IVs for potential secondary phenotypes in the GWAS Catalog, and MR analyses excluding these SNPs produced similar results. These consistent results suggested the robustness of our findings.

There several limitations of the present study. First, since our analysis was restricted to participants of European ancestry, the conclusions may not necessarily apply to other populations. However, this fact also greatly reduced the potential impacts of population stratification bias. Second, although the MR approach could provide an unbiased result due to diminishing the confounding factors, the gene–environment and gene–gene interactions may affect the development of PCOS unavoidably. Third, we were unable to use sex-specific genetic estimates for smoking initiation due to lack of data, and further study were still warranted by using sex-adjusted genetic estimates. Finally, the MR analysis of PCOS were based on summary statistics with relatively small sample sizes, and the potential adverse effect of smoking on the risk of PCOS should be explored further in larger samples.

Conclusion

In summary, our study provided evidence to support a potential causal association between smoking initiation and an increased risk of PCOS among participants of European ancestry. Lowering smoking rates should be considered when constructing the prevention strategies for PCOS. However, the exact role and its underlying biological processes of smoking in the development of PCOS warrant further investigation.

Acknowledgment

We thank all the participants and researchers involved in the relevant studies. Data on smoking initiation have been contributed by Liu et al (2019). Data on polycystic ovary syndrome have been contributed by Day et al (2018) and were downloaded from https://doi.org/10.17863/CAM.36024.

Funding

This work was supported by grants from the National Natural Science Foundation of China (81602917), the Natural Science Foundation of Zhejiang Province (LQ20H260008, LQ21H260001), the Talent Project of Zhejiang Association for Science and Technology (2018YCGC003), the Foundation of Zhejiang Chinese Medical University (KC201905, 2020ZR09, 2020ZG01, 2020ZG06), and the Medical Health Science and Technology Project of Health Commission of Zhejiang Province (2019RC134, 2020KY195).

Disclosure

The authors have declared no conflicts of interest.

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