

Application of Mendel Randomization in Etiology Study of Polycystic Ovary Syndrome

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Abstract: PCOS (polycystic ovary syndrome, PCOS) is a complex disease involving endocrine and metabolism, but its specific etiology and risk factors are not clear. Mendel randomization is to use instrumental variables to verify the causal relationship between risk factors and outcomes. This method can effectively reduce the interference of confounding factors and reverse causality. In recent years, Mendel's randomization research method has been widely used in the etiology research of various diseases. This paper reviews this method and its application progress in PCOS research. The current research results support the causal relationship between PCOS and breast cancer, low-grade serous ovarian cancer, intestinal flora, circulating metabolites, lifestyle and so on. Although many studies have certain limitations, they provide new ideas for the causal relationship research of PCOS, and also play a potential guiding role in the treatment and intervention of the disease.

1. Introduction

PCOS is a heterogeneous disease ^[1] characterized by androgen excess and ovarian dysfunction. The development of this disease is often related to many factors such as heredity, chronic inflammation and insulin resistance ^[2]. PCOS will lead to infertility, obesity, hirsutism, acne and many other problems ^[3]. Although PCOS has a great influence on women's health, its etiology is still unclear. Many studies show that in recent years, with the development of Mendel randomization research method and its unique advantages of avoiding confounding factors and reducing the influence of reverse causality, it has been increasingly applied to the etiology research of various diseases. This paper mainly introduces the application progress of Mendel randomization in PCOS research, hoping to provide new ideas for the causality research of PCOS.

Mendelian randomization is a statistical analysis method used to evaluate the causal relationship between observed changeable exposure or risk factors and clinically relevant results^[4]. Common Mendelian randomization includes independent sample, two-sample Mendelian randomization, multivariate Mendelian randomization, bidirectional Mendelian randomization, etc.^[5]. In Mendel randomization, the choice of instrumental variables should generally meet three assumptions and

apply to each genetic variation ^[6]: (1) Correlation hypothesis: instrumental variables are reliably related to risk factors; (2) Independence hypothesis: there is no correlation between instrumental variables and confounding factors; (3) Exclusivity hypothesis: instrumental variables can only affect the outcome through exposure factors. If the hypothesis of relevance is not satisfied, it will lead to the problem of weak instrumental variables, which will affect the results to be biased.

Through Mendel's randomized research method, the potential causes of diseases can be identified more accurately. For example, studies have shown that there is a positive correlation between depression and the increased risk of breast cancer ^[7], but there is no direct causal relationship between vitamin D level and the risk of non-melanoma skin cancer ^[8]. These findings help scholars to better understand the pathogenesis of diseases, thus providing new ideas for the prevention and treatment of diseases. At the same time, Mendel randomization research can also help identify new drug targets. For example, some studies have identified biomarkers such as coagulation factor VII and apolipoprotein α as potential drug targets for ischemic stroke ^[9], which is of great significance for the development of new drugs. In the future, with the continuous accumulation of genetic data and the progress of technology, Mendel randomized research method is expected to play a greater role in revealing the nature of diseases and guiding clinical practice.

2. The application of Mendel randomization in PCOS research

At present, Mendel randomization has been applied in the field of PCOS research to a certain extent, including the causal relationship between PCOS and malignant tumor, thyroid function, intestinal flora, circulating metabolites, lifestyle, menarche age, etc., and each aspect is described below.

2.1 Malignant tumor and PCOS

Xu Yaxiong ^[10] and others used female breast cancer, cervical cancer, vulvar cancer, endometrial cancer and ovarian cancer as the outcome variables, and PCOS as the exposure factor for Mendel randomized analysis of two samples. By selecting 10 (SNP) as tool variables. It was found that the overall risk of breast cancer (OR = 1.065, 95% CI: 1.014 ~ 1.120, P = 0.013) had a causal relationship with PCOS, but not with cervical cancer, vulvar cancer, endometrial cancer and ovarian cancer. When the subgroup analysis based on tissue type found that estrogen receptor positive breast cancer (OR = 1.081, 95% CI: 1.020 ~ 1.145, P = 0.008) and low-grade serous ovarian cancer (OR = 1.349, 95% CI: 1.014 ~ 1.796, P = 0.040).

2.2 Thyroid function and PCOS

Han Xinyu et al. ^[11] used two-sample bidirectional Mendelian randomization to explore the causal relationship between hypothyroidism and PCOS. It is found that there is a causal relationship between hypothyroidism and PCOS risk, but there is no causal relationship in reverse Mendel analysis. Zhao ^[12] and others did not find a causal relationship between hypothyroidism and PCOS (OR = 1.06, 95% CI: 0.87-1.28, P = 0.559) through Mendel randomized study. PCOS was found to be associated with the increased risk of hyperthyroidism (OR = 1.08, 95% CI = 1.02-1.13, P = 0.004). At the same time, it is found that there is no positive or negative causal relationship between free thyroxine and thyrotropin and PCOS, so the specific mechanism needs further study. Due to the limited sample size of this research database, it is necessary to further expand the sample size in the future to prove the causal relationship between thyroid function and PCOS.

2.3 Intestinal microorganisms and PCOS

Intestinal microflora is indispensable to human body and plays an important role in endocrine and metabolism. It is found that serum steroids and specific fecal flora can be used as characteristic markers for clinical diagnosis of hyperandrogenism -PCOS [13]. Li [14] and others used the genome-wide association studies (GWAS) data of intestinal microbiota and PCOS to conduct a two-sample Mendelian randomized study. A total of 45 SNPs were screened for Mendel randomized analysis. The results showed that the bacteria belonged to the genus *Cholephilic* (OR = 0.42, 95% CI: 0.23–0.77, $P = 4.62 \times 10^{-3}$), *Blaut* (OR = 0.16, 95% CI: 0.03–0.79, $P = 0.02$) and *Haldeman* (OR) $P = 3.55 \times 10^{-3}$) has a protective causal relationship with PCOS, while *Lachnospiraceae* (OR = 1.86, 95% CI: 1.04–3.35, $P = 0.04$) is related to the harmful effects of PCOS. Wang [15] and others used intestinal microorganisms and PCOS to conduct a two-way Mendelian randomized analysis with two samples. It is found that the increase of *Mollicutes*-like SD, *Anaerofilum*, *Coproccus* 2, *Rumicroclostridium* 5, *Enterorhabdus*, *Streptococcus*, etc. have a causal relationship with PCOS in the forward direction, but there is no causal relationship in the reverse direction. However, the positive causal relationship disappeared after Bonferroni correction. Subsequently, the researchers conducted multivariate Mendelian randomized analysis on BMI, alcohol intake frequency, SHBG, total testosterone and fasting insulin, and found that the causal effect was all reduced. Therefore, the bias of the results may be that the mixed factors have not been corrected, which affects the analysis results. Liang [16] and others found *Streptococcus* (OR = 1.52, 95% CI: 1.13–2.06, $P = 0.006$) and *Ruminococcus* UCG 005 (OR = 1.39, 95% CI: 1.04–1.86, $P = 0.028$) by using Mendel randomized study. 95%CI: 0.58–0.83, $P=0.0001$) and UCG 011 (OR = 0.76, 95% CI: 0.60–0.95, $P = 0.017$) of *Rumen Coccidae* are related to the lower risk of PCOS. Because of the complexity of PCOS, the causal relationship between PCOS and intestinal flora still needs more and more accurate evidence.

2.4 Circulating metabolites and PCOS

At present, there are many studies showing that vitamin D deficiency is common in PCOS patients [17]. Shan [18] and others used cross-sectional study to explore the relationship between vitamin D and hyperandrogenism in PCOS women in China. By measuring 25(OH)D, sex steroid hormones, glucose, blood lipid profile and other related anthropometric and biochemical parameters in 625 PCOS patients and 217 control groups. It was found that the serum 25(OH)D level of PCOS women was lower than that of the control group, and the serum 25(OH)D level of women with high testosterone was even lower. Shen [19] and others used Mendel randomization to study the effects of vitamins on PCOS and its development on insulin resistance, obesity and hyperlipidemia. It is found that there is a causal relationship between the increase of vitamin A, D, E, K and B12 and the decrease of PCOS risk. When higher vitamin E and insulin resistance; Higher vitamin A, D, E and hyperlipidemia; Higher levels of vitamin B12, vitamin K and obesity have a causal relationship that reduces their respective risks. However, it needs to be further verified in different populations to clarify the role of vitamins in the pathogenesis of PCOS and its key ways of development.

Testosterone is an androgen secreted by ovary and adrenal gland. Although its content is much lower than that of men, it plays an important role in women's psychological and physiological functions [20]. Zhao [21] and others selected SNPs of four risk factors: total testosterone (TT), bioavailable testosterone (BioT), dehydroepiandrosterone sulfate (DHEAS) and sex hormone binding globulin (SHBG) to test the causal relationship between them and female reproductive system diseases, and found high levels of TT (OR=1.606, $P=0.019$), Biot ($P=0.017$) and DHEAS (OR= 1.970, $P=0.014$) all increase the risk of PCOS, while high level of SHBG (OR=0.715, $P=0.031$) can reduce the risk of PCOS. Thus the causal relationship between testosterone and PCOS was determined.

2.5 Lifestyle factors and PCOS

Observational studies show that many lifestyle factors are related to the risk of PCOS, but it is not clear whether there is a clear causal relationship between these lifestyles and PCOS. Obesity is considered as one of the high risk factors of PCOS. In order to prevent the influence of high BMI, Brower ^[22] and others selected 750 PCOS patients and 1567 BMI-matched control groups for two-way Mendelian randomized analysis. The study showed that higher BMI would increase the risk of PCOS, but PCOS would not affect BMI. Zeng Haijing ^[23] and others used the GWAS summary data of body mass index (BMI), waist circumference (WC), hip circumference (HC), neck circumference (NC) and PCOS of European population to conduct two-sample bidirectional Mendelian randomized analysis. A total of 367, 241, 312 and 3 SNPs related to BMI, WC, HC and NC, and 4 SNPs related to PCOS were screened out for Mendel randomized analysis. It is found that BMI and WC have causal relationship with PCOS, HC and NC have no causal relationship with PCOS, and there is no evidence that PCOS is related to obesity. This shows that BMI and WC are potential factors for PCOS, which is of great significance for the prevention and control of PCOS.

Smoking, as a risk factor that can be changed in life style, will affect people's reproductive health. Tao ^[24] and others found that smoking initiation (OR=1.38, 95%CI=1.12-1.69) was positively correlated with PCOS risk. Similarly, Zhao ^[25] and others also found that there is a causal relationship between PCOS and the number of cigarettes smoked every day and the start of smoking through Mendel randomized analysis.

2.6 Mental illness and PCOS

According to the statistics of the World Health Organization ^[26], it is estimated that there are 322 million people suffering from depression in the world, and depression is only one of the mental diseases that affect PCOS women. In order to explore the causal relationship between depression and different types of female reproductive disorders, Ling ^[27] and others found that depression or dysthymia was associated with PCOS (OR=1.43, 95%CI=1.28-1.59; $P = 6.66 \times 10^{-11}$), there is a significant positive correlation causality, and there is no causality in the opposite direction. Therefore, it is very important for PCOS women to pay attention to and manage their emotional state to improve their overall health. However, Jiang ^[28] and others did not find that PCOS has a common genetic basis or causal relationship with mental diseases such as depression, anxiety, schizophrenia and bipolar disorder through a two-way Mendelian randomized study, which may be due to limited data availability or little real influence. In the future, more perfect GWAS data are needed to further verify the relationship between mental illness and PCOS.

2.7 Menarche age and PCOS

Ge Hang ^[29] and others divided the age of menarche into four quartiles, including 163 cases in group A (≤ 12 years old), 290 cases in group B (13 years old), 248 cases in group C (14 years old) and 226 cases in group D (≥ 15 years old), and made clinical observation and research respectively. The results show that there are differences in metabolism, hormones and anthropometric indexes in PCOS patients with different menarche ages. It shows that menarche age is related to PCOS. Ma et al. ^[30] used two-sample Mendelian randomization to verify the causal relationship between menarche age and the incidence of polycystic ovary syndrome. Studies have shown that the incidence of PCOS will be significantly reduced whenever the age of menarche increases by a standard deviation (OR: 0.86 95% CL=0.75-0.98). It shows that the later menarche age (15-18 years old) can reduce the risk of PCOS, but a more comprehensive investigation is needed in the future

environment.

3. Prospect

To sum up, with the rapid development of medical technology, the research on the causal relationship of PCOS has gradually deepened. There may be causal relationship between PCOS and malignant tumor, intestinal flora, thyroid function, mental illness and lifestyle, but the research results of the same factor are different, which may be related to the problems of "weak instrumental variables" and "pleiotropic effects". In order to further improve the reliability of inferring the causal relationship between PCOS and various factors, various statistical methods can be considered for interactive verification in Mendel randomization research. With the deepening of various studies, we gradually realize the diversity of this complex disease, which is not only closely related to physiological factors, but also influenced by environment, lifestyle and psychological factors. Therefore, future research needs to comprehensively consider these factors in order to reveal the pathogenesis and pathological process of PCOS more comprehensively and provide more powerful support for the diagnosis and treatment of PCOS.

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