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# Micronutrients and polycystic ovary syndrome in the IEU OpenGWAS project: a two-Sample unidirectional Mendelian randomization analysis

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**Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine and metabolic disorder. Research suggests a potential link between certain micronutrients and PCOS development, but the exact cause-and-effect relationship is unclear. This research utilizes data from extensive genome-wide association studies (GWAS) to investigate the possible association between micronutrients and PCOS via Mendelian randomization (MR). The findings aim to inform future research and guide clinical practice. We performed a two-sample unidirectional MR analysis using genetic data from European populations. The MR analysis revealed no statistically significant association between the 11 micronutrient-related genes and PCOS (all *P*-values > 0.05). Phosphate, magnesium, folate, vitamin B12, D, iron, selenium, and copper odds ratios (ORs) less than 1, calcium, vitamin C, and zinc ORs greater than 1. Sensitivity analyses revealed no indications of heterogeneity, pleiotropy, or outliers, hence affirming the robustness of these findings. High serum phosphate, magnesium, folate, vitamin B12, D, iron, selenium and copper levels may be potentially protective against PCOS. High serum calcium, vitamin C and zinc levels may be potential risk factors for PCOS. These insights are important for understanding PCOS's pathophysiology and improving clinical management.**

**Keywords** Micronutrients, Polycystic ovary syndrome, Mendelian randomization, Causal relationship, Genome-Wide association study

Polycystic Ovary Syndrome (PCOS) is a multifaceted endocrine condition impacting numerous women of reproductive age globally[1]. It is frequently associated with increased testosterone levels, insulin resistance, and ovarian hypertrophy and dysfunction[2]. Research indicates that PCOS is affected by multiple factors, including environmental, genetic, and epigenetic elements[3]. An imbalance in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), along with high levels of gonadotropin-releasing hormone (GnRH), is known to be a major cause of PCOS[4]. However, its exact causes and mechanisms are still not fully understood[4,5]. PCOS increases the likelihood of various severe problems, including cardiovascular disease, type 2 diabetes[5,6], metabolic syndrome[6], and mental health issues like depression and anxiety[7]. These complications not only impact women's overall health and quality of life but can be life-threatening. Consequently, there is an imperative necessity to investigate the etiology and processes of PCOS in order to enhance therapeutic approaches.

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Micronutrients are indispensable elements in the human body, fulfilling critical roles in numerous physiological functions. Research on the influence of micronutrients on women's health is currently expanding, particularly regarding their impacts on PCOS. After giving vitamin C to Wistar rats with PCOS, Olaniyan et al.[8] examined the changes in their ovaries. They found that vitamin C plays a key role in controlling the menstrual cycle and maintaining ovarian function; levels change throughout the cycle. Additionally, studies have found a close link between micronutrient levels and ovarian health in women[9–11]. Afshar Ebrahimi et al.[12] indicated that magnesium and zinc enhanced the metabolic conditions of PCOS patients by diminishing oxidative stress and inflammation and consequently easing clinical symptoms. Other micronutrients, including phosphate, calcium, folate, vitamin B12, vitamin D, iron, selenium, and copper, have also been associated with PCOS in various studies[13–17]. However, most of these studies are observational, and few have directly explored the causal relationship between micronutrients and PCOS. There is an urgent need for research examining these potential causal links to better understand PCOS pathogenesis and improve its diagnosis and treatment.

The authors employed Mendelian Randomization (MR) to examine the causal association between micronutrients and PCOS. MR is a genetic epidemiology method that employs naturally occurring genetic variants to replicate randomized trials, facilitating a more precise evaluation of the impact of exposures on outcomes[18,19]. This approach facilitates the examination of causal interactions by employing genetic variants as instrumental variables linked to the exposures of interest[20,21]. To check if there is a link between instrumental variables and outcomes, MR uses instrumental variables that are linked to exposure components that support the three basic assumptions as exposure proxy variables[22]. Randomly assigning single nucleotide polymorphisms (SNPs) is like running a trial within the population without taking into account environmental or other unknown confounding factors. This reduces the chance of confounding and reverse causality[18]. This study employed a two-sample unidirectional MR technique to investigate the causative associations between various micronutrients—including phosphate, calcium, magnesium, folate, vitamins B12, C, and D, iron, selenium, copper, and zinc—and PCOS. Micronutrients were used as exposure factors and PCOS as an outcome factor, and the selection of each exposure and outcome factor was based on relevant references, with corresponding definitions aligning consistently with descriptions sourced from Genome-Wide Association Studies (GWAS) databases pertinent to the respective diseases. This genetic approach sheds light on the mechanisms driving the onset and progression of PCOS and provides valuable insights to inform preventive and therapeutic strategies.

## Results

### Instrumental variables

In our examination of the causal link between micronutrients and PCOS, we initially excluded SNPs with allele frequencies under 0.01. This led to the removal of three SNPs associated with magnesium (rs140205161, rs150479966, rs146963873), two associated with folate (rs57185514, rs118164003), and one associated with vitamin B12 (rs193228340). We found no allele frequencies for vitamin D, selenium, copper, or zinc.

Following the criteria for instrumental variable selection, we extracted data on strongly correlated SNPs for phosphate, calcium, magnesium, folate, vitamin B12, vitamin C, vitamin D, iron, selenium, copper, and zinc in relation to PCOS. This process resulted in the loss of SNPs as follows: 0, 0, 1, 2, 2, 3, 0, 0, 0, 1, and 0, respectively, after excluding palindromic sequences. The elimination of the palindrome sequence finally yields the 14, 25, 26, 17, 20, 20, 12, 13, 11, 9, and 7 instrumental variables for each micronutrient. The MR analysis with the leave-one-out method revealed that three SNPs (rs7792882, rs16903307, rs12490397) were significantly correlated with phosphate, thereby influencing the entire data. After their removal, 11 SNPs remained for the final MR analysis of phosphate and PCOS. For calcium, two SNPs (rs4535437, rs1714800) were influential, resulting in 23 SNPs for the final analysis. In the case of vitamin C, three influential SNPs (rs74978963, rs11650824, rs79757038) led to 17 SNPs in the final analysis. Lastly, three significant SNPs for zinc (rs4333127, rs1532423, rs11763353) resulted in four SNPs for the final analysis. All identified SNPs (see Supplementary Materials, Table S1-11) ultimately met the criteria for instrumental variable selection.

### Effects of micronutrients on PCOS

The inverse variance weighted analysis shows that there are no statistically significant links between these eleven micronutrients and PCOS based on genetic information. The odds ratios (*ORs*) and corresponding 95% confidence intervals (*CI*) for each nutrient were as follows: phosphate ($OR = 0.302$, 95% *CI*: 0.016–5.783, $P = 0.426$), calcium ($OR = 1.326$, 95% *CI*: 0.512–3.434, $P = 0.562$), magnesium ($OR = 0.690$, 95% *CI*: 0.303–1.569, $P = 0.376$), folate ($OR = 0.527$, 95% *CI*: 0.152–1.830, $P = 0.313$), vitamin B12 ($OR = 0.644$, 95% *CI*: 0.235–1.764, $P = 0.392$), vitamin C ($OR = 1.900$, 95% *CI*: 0.666–5.416, $P = 0.230$), vitamin D ($OR = 0.505$, 95% *CI*: 0.119–2.133, $P = 0.352$), iron ($OR = 0.913$, 95% *CI*: 0.575–1.449, $P = 0.698$), selenium ($OR = 0.869$, 95% *CI*: 0.713–1.059, $P = 0.163$), copper ($OR = 1.036$, 95% *CI*: 0.885–1.213, $P = 0.658$), and zinc ($OR = 1.384$, 95% *CI*: 0.841–2.279, $P = 0.201$). The data indicate a lack of causal link between these micronutrients and PCOS (see Figs. 1 and 2).

### Sensitivity analysis

Heterogeneity tests indicated no significant variation among the micronutrients ($P > 0.05$). Additionally, horizontal pleiotropy tests provided no evidence of pleiotropic effects (see Supplementary Materials, Table S12). The MR-Egger analysis also found no evidence of directional pleiotropy among the instrumental variables ($P > 0.05$), thereby strengthening the robustness of the causal relationship. The leave-one-out analysis indicated that no single SNP had a substantial impact on the total results (see Supplementary Materials, Figure S1). Furthermore, the funnel plot displayed good symmetry, indicating that the findings are stable and reliable (see Supplementary Materials, Figure S2).

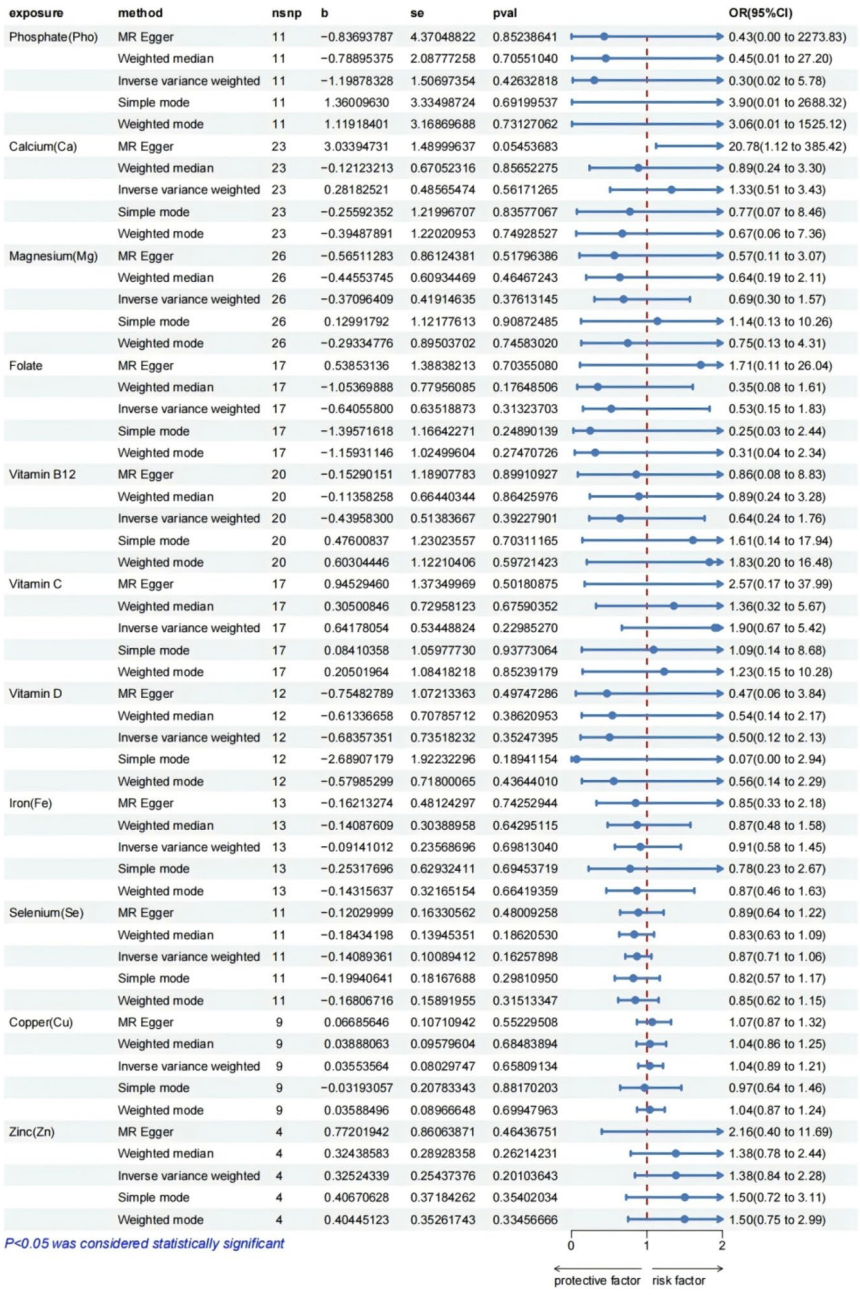


**Fig. 1**. Forest plot of the MR analysis results of micronutrients on PCOS. The forest plot, arranged vertically, displays the outcomes of five MR analysis methods on the association between (1) phosphate and PCOS, (2) calcium and PCOS, (3) magnesium and PCOS, (4) folate and PCOS, (5) vitamin B12 and PCOS, (6) vitamin C and PCOS, (7) vitamin D and PCOS, (8) iron and PCOS, (9) selenium and PCOS, (10) copper and PCOS, (11) zinc and PCOS.

## Discussion

### The MR findings on micronutrients and PCOS are reliable

This MR study sought to examine the relationship between micronutrient concentrations and the incidence of PCOS. The results indicate that the concentrations of the 11 selected micronutrients (phosphate, calcium, magnesium, folate, vitamin B12, vitamin C, vitamin D, iron, selenium, copper, and zinc) do not show a causal relationship with PCOS. Throughout the study, *Cochran's Q* test and leave-one-out analysis demonstrated that no SNPs significantly influenced the outcomes. Furthermore, both MR-Egger and MR-PRESSO analyses revealed no indication of horizontal pleiotropy. Consequently, the sensitivity analyses and corrections for multiple comparisons substantiate the validity of these findings.

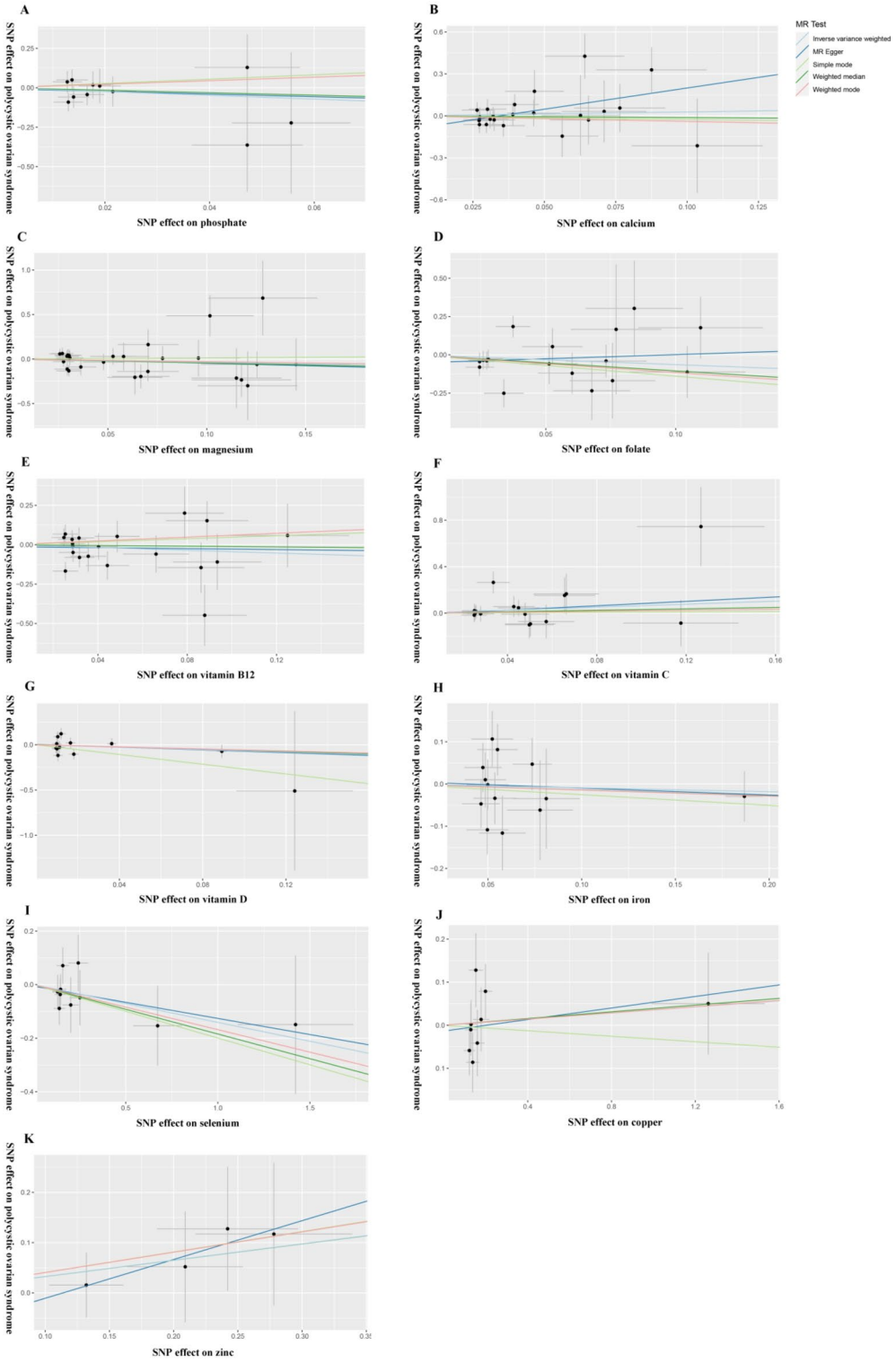


**Fig. 2**. The MR analysis results about micronutrients and PCOS are depicted in a scatter plot. The results of the MR analysis are shown in Figures A–K, which are scatter plots. They show the link between (**A**) phosphate and PCOS, (**B**) calcium and PCOS, (**C**) magnesium and PCOS, (**D**) folate and PCOS, (**E**) vitamin B12 and PCOS, (**F**) vitamin C and PCOS, (**G**) vitamin D and PCOS, (**H**) iron and PCOS, (**I**) selenium and PCOS, (**J**) copper and PCOS, (**K**) zinc and PCOS. A cross surrounding each SNP indicates the 95% *CI*. The slopes of each line indicate the causal relationship for each method. MR, Mendelian randomization; SNP, single-nucleotide polymorphism.

### The current international research status and theoretical analysis of micronutrients and PCOS

The association between micronutrients and PCOS remains inconsistent across international epidemiological studies. The authors speculate that this inconsistency may stem from variations in geographic environments, population characteristics, and confounding factors. Additionally, we noted a lack of direct studies examining micronutrients in relation to PCOS, with no research specifically focusing on the relationship between phosphate and PCOS. We hypothesize that this disparity may be due to the limited sample sizes in current case studies. These eleven micronutrients are essential for women's health. According to Szczuko et al.[23], women with PCOS typically have low levels of calcium. Furthermore, many studies have failed to achieve a consensus about the association between calcium and insulin resistance in metabolic disorders, including PCOS[24–26]. Researchers deem magnesium important for its role in regulating blood glucose and brain activity, which could potentially affect disorders associated with insulin resistance and depression, such as PCOS, cardiovascular diseases, and diabetes[27,28]. However, evidence supporting this is still limited. Recent studies, such as those by Bahmani et al.[29], suggest that folic acid administration may enhance glucose levels, inflammation, and oxidative stress markers in women with PCOS and obesity. Kaya et al.[30] discovered that diminished serum vitamin B12 levels were associated with insulin resistance, obesity, and elevated homocysteine concentrations in patients with PCOS. Additionally, meta-analyses suggest that vitamin D supplementation can positively affect glucose and lipid metabolism in individuals with PCOS, particularly in those with vitamin D deficiency, although it does not significantly impact androgen levels or inflammatory status[31]. There is also evidence that plasma selenium levels may be reduced in PCOS patients, potentially leading to increased free radicals and hyperandrogenism[32,33]. Dhar et al.[34] performed a cohort research contrasting PCOS patients to healthy women aged 18 to 35, revealing lower serum zinc levels in PCOS patients. This suggests that zinc might serve as a protective factor against PCOS, contrasting with our MR study findings. In summary, most research has focused on how micronutrients on aspects potentially contributing to PCOS, including insulin resistance, obesity, and hyperandrogenism. One study articles indicated that lower vitamin D levels correlate with obesity and insulin resistance, but not directly with PCOS itself[35]. Sharma et al.[36] reported that selenium deficiency is associated with the risk of developing PCOS, and certain genetic variations related to selenium intake are also significantly correlated with the risk of PCOS. Apart from these two studies, we found no direct studies clarifying their relationship between the other nine micronutrients and PCOS. We believe further clinical research is essential to validate the findings of our MR analysis.

### The innovation of MR studies on micronutrients and PCOS

To the best of our knowledge, this is the first study to use a GWAS dataset for MR analysis to look into the possible link between micronutrients and PCOS. It provides bioinformatic evidence of environmental risk factors for the condition. By examining the effects of micronutrients on PCOS at the genetic level, we minimized the effects of reverse causality and confounding variables[37], thereby reinforcing the conclusions of previous epidemiological research. MR overcomes the limitations of traditional observational epidemiology by enabling causal inference. It offers cross-validation for findings from cross-sectional studies and addresses the shortcomings of conventional epidemiological designs. Although our results were not statistically significant, the observed ORs greater or less than 1 suggest that these genetic variants may have risk or protective effects on PCOS, offering new insights into the condition. This may indicate a need to reassess micronutrients supplementation therapies in routine clinical practice for patients with PCOS.

### The limitations of MR studies on micronutrients and PCOS

This research possesses multiple limitations. First, the selection of instrumental variables was predicated on a *P*-value criterion of $< 1 \times 10^{-5}$, which reflects the constraints of the GWAS data on micronutrients and PCOS. This limitation may introduce unavoidable confounding factors, although it also ensures an adequate number of SNPs for analysis. Additionally, the majority of participants in the GWAS summary data were of European descent, which introduced bias in the estimations due to population stratification. Additional clinical research is required to determine the applicability of these findings to other ethnic groups. Finally, comprehensive clinical trials are necessary to draw definitive clinical conclusions. This highlights the necessity for more comprehensive GWAS databases and further analytical techniques or experimental validation to elucidate the link and processes between micronutrients and PCOS.

### Future research directions on micronutrients and PCOS

This study did not achieve statistical significance, it provides meaningful insights suggesting that micronutrients may have both protective and risk-related effects on PCOS. Future research should explore several key areas: (I) Sample Size and Diversity: Augmenting the sample size and including participants from varied ethnic and geographic backgrounds will improve the representativeness and generalizability of the findings, moving beyond a focus solely on European ancestry; (II) Selection of Genetic Variants: Optimizing the selection of genetic variants can improve the study's accuracy and reliability; (III) Mechanistic Exploration: clinical trials to investigate the impact of these genetic variations on the onset and course of PCOS, elucidating the significant connections and mechanisms involved; (IV) Expanding the micronutrient range, etc.

## Data and methods

The design of the two-sample MR study used in this research is outlined in the figure (see Fig. 3).

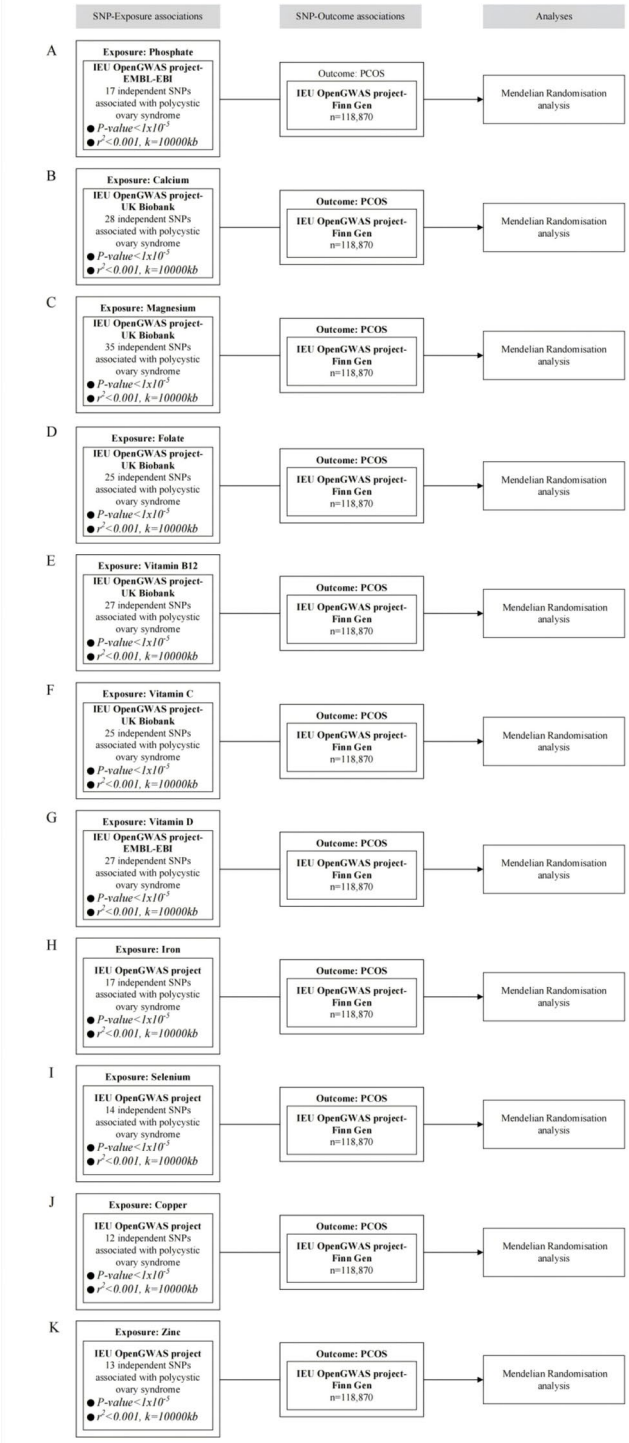


**Fig. 3**. Figure illustrating the study design of a two-sample MR analysis, which aims to examine the unidirectional associations between micronutrients and PCOS. GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; PCOS, Polycystic Ovary Syndrome.

## Design

This study follows the STROBE-MR guidelines[38] and uses a unidirectional two-sample MR approach to assess the causal relationship between micronutrients (phosphate, calcium, magnesium, folate, vitamins B12, C, and D, iron, selenium, copper, and zinc) as exposures and PCOS as the outcome. The methodology is based on three key assumptions: (I) the instrumental variables must be strongly associated with the exposure; (II) the instrumental

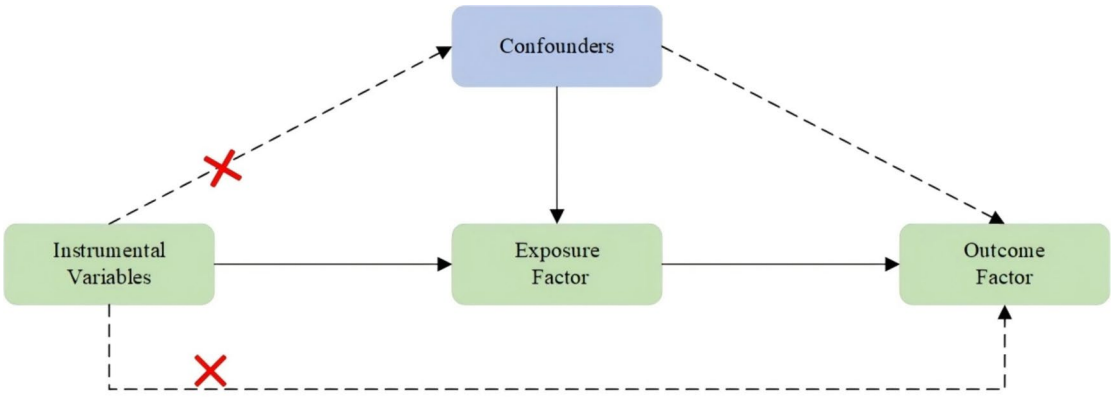


**Fig. 4**. Three Assumptions of Mendelian Randomization. Assumption 1: The instrumental variable is strongly associated with the exposure component; Assumption 2: The instrumental variable is unaffected by both known and unknown confounding variables; Assumption 3: The instrumental variable influences the outcome variable solely via the exposure variable.

| Exposure or Outcome | Data Source | GWAS Data ID | Sample Ethnicity | Number of SNPs | Sample Size | Year of Publication |
|---|---|---|---|---|---|---|
| PCOS | IEU OPEN GWAS | finn-b-E4_POCS | European | 16,379,676 | 118,870 | 2021 |
| Phosphate(Pho) | IEU OPEN GWAS | ebi-a-GCST90026038 | European | 6,815,797 | 291 | 2021 |
| Calcium(Ca) | IEU OPEN GWAS | ukb-b-8951 | European | 9,851,867 | 64,979 | 2018 |
| Magnesium(Mg) | IEU OPEN GWAS | ukb-b-7372 | European | 9,851,867 | 64,979 | 2018 |
| Folate | IEU OPEN GWAS | ukb-b-11,349 | European | 9,851,867 | 64,979 | 2018 |
| Vitamin B12 | IEU OPEN GWAS | ukb-b-19,524 | European | 9,851,867 | 64,979 | 2018 |
| Vitamin C | IEU OPEN GWAS | ukb-b-19,390 | European | 9,851,867 | 64,979 | 2018 |
| Vitamin D | IEU OPEN GWAS | ebi-a-GCST005367 | European | 2,538,249 | 79,366 | 2018 |
| Iron(Fe) | IEU OPEN GWAS | ieu-a-1049 | European | 2,096,457 | 23,986 | 2014 |
| Selenium(Se) | IEU OPEN GWAS | ieu-a-1077 | European | 2,543,617 | 2,603 | 2013 |
| Copper(Cu) | IEU OPEN GWAS | ieu-a-1073 | European | 2,543,646 | 2,603 | 2013 |
| Zinc(Zn) | IEU OPEN GWAS | ieu-a-1079 | European | 2,543,610 | 2,603 | 2013 |

**Table 1**. Summary information on data used in MR studies. Note: The GWAS data ID for Polycystic Ovary Syndrome is listed as "finn-b-E4_POCS," where "POCS" is used as the abbreviation for Polycystic Ovary Syndrome. However, according to common usage in the literature, "PCOS" is the standard abbreviation. The authors suggest that the website's ID may contain a typographical error.

variables should not be affected by confounding factors; and (III) the instrumental variables influence the outcome only through the exposure[39]. Since publicly available data were used, ethical approval and informed consent were not required. The study design is illustrated in Fig. 4.

## Date and location

The study was completed in July-August 2024 at Tianjin Medical University.

## Data

Published studies and publicly accessible summary data from GWAS provided the data for this analysis.

The datasets utilized in this research are from European populations. There is no individual overlap between micronutrients and PCOS.

## Acquisition of micronutrients data

A search of PubMed (https://www.ncbi.nlm.nih.gov/pubmed, accessed July 31, 2024) was conducted to identify observational studies and meta-analyses on micronutrients and PCOS in women. Following an evaluation of parameters including sample size, publication year, quantity of SNPs, and the ethnic composition of the samples, 11 GWAS datasets related to micronutrients (including phosphate, calcium, magnesium, folate, vitamins B12, C, and D, iron, selenium, copper, and zinc) were selected from the IEU Open GWAS Project (https://gwas.mrcieu.ac.uk/, accessed July 31, 2024) for inclusion in the study (Table 1).

### Acquisition of PCOS data

Upon assessing variables like sample size, publication year, SNP count, and sample ethnicity, we used PCOS data from the IEU Open GWAS database (https://gwas.mrcieu.ac.uk/, accessed July 31, 2024). Our analysis focuses on data from the Finnish cohort within this database, which comprises 642 cases and 118,228 controls, for a total of 118,870 European participants (Table 1).

## Methods

### Instrumental variables

We employed a sequence of quality control methodologies to ascertain genuine instrumental variables that satisfy the three fundamental assumptions of MR depicted in Fig. 4[39]. First, a genome-wide significance threshold of $P<5\times10^{-8}$ was used to select SNPs that were closely linked to the exposure factors[40]. However, this threshold led to the selection of a limited number of SNPs. In order to find additional SNPs for investigating putative causal links between exposure and outcomes, we therefore set a less strict threshold of $P<1\times10^{[-5}$ [39,41,42]. Second, we eliminated SNPs with $r^2>0.001$ that were associated with both confounding factors and outcomes to prevent linkage disequilibrium within a 10,000 kB range. Third, $R^2$ and $F$-values represent statistical power: In MR analysis, the $F$-statisti serves as a tool to measure the strength of association between the instrumental variable and the exposure[43]. The $F$-statistic is computed using the formula $F=[(N-k-1)/k)\times R^2/(1-R^2)]$, where $R^2$ is the variance elucidated by the genetic instrument, k signifies the number of genetic variations, and N indicates the sample size. The $R^2$ is computed using the formula: $2\times\beta^2\times EAF\times(1-EAF)/2\times\beta^2\times EAF\times(1-EAF)+SE^2\times2\times N\times EAF(1-EAF)$, where EAF denotes the effect allele frequency. An $F$-statistic of 10 is deemed adequate to mitigate bias, but variables with $F$-statistics below 10 were omitted from the study due to weak instrument bias[44,45]. We ultimately obtained outcome data from Finnish research within the IEU Open GWAS database. We extracted non-proxy SNPs that satisfied the assumptions from the outcome data. We subsequently integrated the exposure and result datasets, establishing connections between the selected instrumental variables and both the exposure and outcome. We eliminated palindromic SNPs and utilized the remaining SNPs as the definitive instrumental variables for the analysis.

### MR analysis

This unidirectional two-sample MR study included five MR analysis methods: inverse-variance weighted (IVW), weighted median, simple mode, weighted mode, and MR-Egger regression. These methodologies were utilized to evaluate the causal link between micronutrients and PCOS, with IVW as the principal approach[46]. IVW provides a causal estimate by integrating summary data from various genetic instruments, referred to as the Toby Johnson technique, which employs IVW meta-analysis. This approach computes the *Wald* estimates for each genetic variant utilizing the first-order variance of the delta method. It is crucial to acknowledge that IVW presumes all instrumental variables adhere to the MR assumptions and that there is an absence of an intercept term[47]. Failure to meet these assumptions may result in erroneous outcomes.

### Primary outcome measures

To examine the possible causative relationship between genetically predicted micronutrient levels and PCOS.

### Statistical analysis

We performed sensitivity analyses employing multiple approaches, such as the weighted median, simple mode, weighted mode, and MR-Egger regression[48–50]. Each method is based on distinct assumptions, and obtaining consistent results across these different approaches strengthens the evidence for causal inference[51]. The *Cochran's Q* test was conducted on the SNPs incorporated in the final MR analysis utilizing the mr_heterogeneity package in R version 4.3.2, enabling us to evaluate heterogeneity among individual genetic variants[52,53]. A $P$-value below 0.05 from the test signifies heterogeneity, indicating that variables such as age and gender may affect the association between the exposure and outcome. In instances of identified heterogeneity, we analyze the conclusive MR outcomes employing the IVW random effects model as the benchmark. In the absence of heterogeneity, the IVW fixed effects model is employed as the benchmark.

We evaluated horizontal pleiotropy via the MR Egger-intercept method and the MR-PRESSO test. The Egger-intercept approach plays a crucial role in determining whether genetic variants influence the outcome through mechanisms different from the exposure. A $P$-value below 0.05 signifies the existence of horizontal pleiotropy, implying that the chosen instrumental factors substantially influence the outcome via different mechanisms, hence contravening assumptions 2 and 3 in Fig. 4. A $P$-value exceeding 0.05 indicates that the exposure does not significantly influence the result via other routes. To enhance the validation of our findings, we performed a leave-one-out sensitivity analysis employing the IVW technique, sequentially omitting each SNP from the MR analysis to detect any influential spots[54]. We assessed the stability of our findings by analyzing the symmetry of the funnel plot. Finally, we detected outliers utilizing the MR-PRESSO approach and evaluated their influence on the overall outcomes.

## Conclusion

Based on the outcomes of the MR analysis, we believe that while the findings did not achieve statistical significance, they offer intriguing insights. Specifically, certain micronutrients may have both protective and risk-related effects on PCOS. This indicates that the incorporation of micronutrient supplementation in the clinical treatment of PCOS patients requires reassessment. Consequently, further clinical trials are essential to explore and validate the associations and underlying mechanisms between various micronutrients and PCOS.

## Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request, also could download them from the website https://gwas.mrcieu.ac.uk/.

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## Author contributions

Conceptualization, Y.B. and H.C; methodology, N.T. and H.Z; software, Y.B., H.C. and N.T.; formal analysis, X.W.; investigation, Y.B., H.C., X.W., H.Z., K.S., Z.X and Y.D.; data curation, Y.B. and Q.Z.; writing—original draft preparation, Y.B., H.C. and N.T.; writing—review and editing, X.Z.,X.Q and Q.Z.; visualization, Y.D and X.Q; project administration, X.Q. and Y.D.; All authors have read and agreed to the published version of the manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Institutional review board statement

Ethical review and approval were waived for this study due to we use a public database.

## Informed consent

Patient consent was waived due to we use a public database.

## Additional information

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