Letter to the Editor

Polycystic Ovary Syndrome is not Associated with Offspring Birth Weight: A Mendelian Randomization Study*

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Polycystic ovary syndrome (PCOS) is a common metabolic and hormonal disorder afflicting approximately 5%–20% of all women of reproductive age[1]. PCOS is characterized with hyperandrogenism, oligo-anovulation, and a polycystic ovarian morphology. The syndrome features heterogeneous manifestations, such as hirsutism, menstrual dysfunction, and obesity. Women with PCOS are at higher risk of developing multiple metabolic comorbidities and subsequent cardiovascular complications even beyond childbearing age. Several studies have suggested that neonates born to PCOS mothers differ from the reference population and show wide disparities in anthropometrics, such as birth weight[2,3]. Recent evidence also indicated associations between endocrine- and metabolic-related changes and offspring birth weight. For example, glycemic traits, including fasting glucose, 2 h glucose, and HbA1C, may affect offspring birth weight[4]. Considering the strong relationship between insulin resistance and metabolic changes in PCOS, hypothesizing the potential effects of PCOS on offspring birth weight is reasonable. Previous observational studies revealed that controlling all possible confounders and giving unbiased estimates when investigating the causal effect of PCOS on birth weight are difficult. Given that birth weight has long been postulated to determine an individual's predisposition to adult diseases[5], identifying the influence of maternal-origin exposures on birth weight is essential to unravel the precise etiophysiology of PCOS.

Mendelian randomization (MR) has been widely and successfully applied as a robust methodological approach to obtain causal inferences; it mainly utilizes genome-wide association studies (GWAS) and summary statistics of single nucleotide polymorphisms. MR utilizes genetic variants associated with potentially modifiable exposures as ideal instrumental variables to derive conclusions on the causality of health outcomes. An independent assortment of alleles in Mendelian inheritance resembles the randomized allocation of participants[6]. In contrast to conventional methods, MR is less prone to confounding or measurement errors or reverse causation[6]. This study was conducted to assess the possible causal effect of maternal PCOS on offspring birth weight using a two-sample MR design.

First, we obtained summary statistics of PCOS from the largest GWAS meta-analysis available; this dataset included 10,074 cases and 103,164 controls of European descent collected by the International PCOS Consortium[7]. This study incorporated seven cohorts based on the NIH database (2,540 cases; 15,020 controls), Rotterdam Criteria (2,669 cases; 17,035 controls), and self-reported diagnosis (5,184 cases; 82,759 controls). Day et al.[7] demonstrated minimal differences in genetic architecture across three diagnostic criteria for PCOS regardless of its clinical heterogeneity; hence, we utilized overall effect estimates derived from a meta-analysis of all available participants. Effect estimates were adjusted for age and presented as log-odds changes per additional effect allele.

Data on offspring birth weight were obtained from the Early Growth Genetics Consortium and UK Biobank. Warrington et al.[8] performed a European-ancestry meta-analysis on birth weight. They partitioned the genetic contributions of direct fetal and indirect maternal effects by establishing a structural equation model utilizing the available phenotype information of individuals in two generations and the genotype information of older individuals; they then generalized this equation to

doi: 10.3967/bes2021.023

*This study was supported by the Public Communication of Science and Technology Program [2020A1414050018] by the Department of Science and Technology of Guangdong Province.

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datasets with offspring or own birth weight alone[8]. Finally, we obtained summary statistics for partitioned maternal effects on birth weight (n = 257,734). Birth weights were transformed into Z-scores and adjusted for gestational duration and sex[8], and effect estimates for birth weight were presented as changes in standard deviation, which approximated one as birth weights were Z-score transformed.

We initially obtained 14 genome-wide significant SNPs for PCOS ($P < 5 \times 10^{-8}$) and examined their linkage independence using the European population as a reference panel. The linkage disequilibrium threshold was set to $r^2 = 0.2$ within a distance of 100 kb. Then, we inspected pleiotropic effects, which refers to the polygenic effects of one variant on multiple traits or pathways, through look-up in the GWAS catalog. Vertical pleiotropy, which would not bias MR assumptions, incorporated associations with hyperandrogenism, oligomenorrhea, polycystic ovarian morphology, testosterone, follicle-stimulating hormone, luteinizing hormone, and ovarian volume. Horizontal pleiotropy refers to associations with unrelated diseases, such as respiratory conditions and cancers, and was examined through MR-Egger tests and funnel plots as described below. Thus, our selected SNPs conformed to MR assumptions; specifically, instrumental variables should be (1) unrelated to any measured or unmeasured confounding factors, (2) associated with intermediate exposures, and (3) linked to outcomes only *via* exposure variables (Figure 1). All corresponding loci for these instrumental SNPs were identified in the outcome GWAS datasets. Therefore, no proxies were required. We further harmonized signs of effect size in summary statistics for PCOS and birth weight by aligning the effect alleles and making allowances for the forward strand. We then removed palindromic SNPs with non-inferable forward strands. Finally, we merged the exposure and outcome datasets for subsequent analysis.

To assess the causal effect of maternal PCOS on offspring birth weight, we performed two-sample MR using R 3.6.1 software and the TwoSampleMR package[6]. To compute overall estimates, we adopted three MR methods, namely, the inverse-variance weighted method, weighted median estimator, and MR-Egger regression. The first MR method generally gives a consistent estimate of causality and was used for the primary analysis. However, inverse-variance weighted estimation may be biased in the presence of invalid instrumental variables with horizontal pleiotropy[9]. The two methods are less statistically well-powered but more robust to horizontal pleiotropy. The weighted median estimator pools the effects of individual variants efficiently under the prerequisite that over 50% of instrumental variables are valid. MR-Egger regression assumes that pleiotropic associations are independent and balanced. The intercept and slope of the MR-Egger regression equation provides an exploration of the pleiotropy and causality estimate adjusted for the pleiotropy of the data[6]. To explore whether the MR estimates are disproportionately influenced by certain SNPs alone, we performed

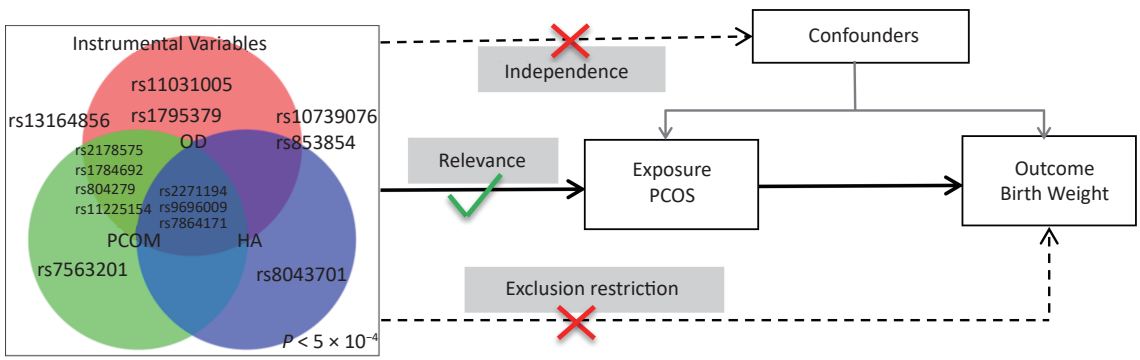

Figure 1. Schematic of key assumptions in this Mendelian randomization study. First, the relevance assumption is satisfied by selecting instrumental SNPs associated with polycystic ovary syndrome at genome-wide significance ($P < 5 \times 10^{-8}$). The associations of these SNPs ($P < 5 \times 10^{-4}$) with three primary components, namely ovulatory dysfunction, hyperandrogenism (HA), and polycystic ovarian morphology (PCOM), are presented in the Venn diagram. Secondly, the independence assumption is satisfied since there exists no confounding factors interfering with gamete formation. Finally, potential horizontal pleiotropic effects violating the exclusion–restriction assumption are inspected.

leave-one-out analysis and constructed the corresponding funnel plot. We also examined the heterogeneity of the data, which was quantified by Cochran's Q statistics. Finally, we calculated statistical power of the MR analysis to detect associations using the mRnd web-tool[10].

In total, 13 genome-wide significant SNPs associated with PCOS[7] formed our independent set of instrumental variables (Table 1). These SNPs did not show linkage disequilibrium (r^2 > 0.8). One SNP (rs853854) showing genome-wide significance (P = 2.36 × 10^{-9}) in the meta-analysis of PCOS was not taken into account in our analysis. Because of the palindromic nature and intermediate allele frequency (A/T, 0.499/0.501) of this SNP, its ambiguous strand hindered further harmonization for the effect allele. Besides associations with three main PCOS-related traits (Figure 1), rs11031005 was significantly associated with follicle-stimulating hormone serum levels (P = 1.39 × 10^{-10}). Five other loci (i.e., rs2178575, rs804279, rs9696009, rs1784692, and rs11225154) were associated with oligomenorrhea (P < 5×10^{-8}). Given that the influence of PCOS on offspring is likely to implicate endocrine pathways, loci with vertical pleiotropy should not be removed. All 13 SNPs identified were valid instruments with F ranging from 31.0 to 57.6; none of these SNPs were weak instruments (F < 10). The SNPs obtained collectively explained 4.8% of the variance of PCOS. According to mRnd power estimates[12], we had adequate power (> 80%) to identify effect estimates beyond ± 0.025.

In general, none of the three MR methods employed in this work supported the supposition that per unit increases in the log-odds of maternal predisposition to PCOS would decrease the Z-score of offspring birth weight (Supplementary Table S1 available in www.besjournal.com). Primary causality estimates obtained through inverse-variance weighted analysis (β = −0.013; P = 0.26) showed directional consistency according to the weighted median (β = −0.023; P = 0.14) and MR-Egger methods (β = −0.038; P = 0.54). Disregarding their statistical insignificance, our MR results suggest a negative correlation between maternal PCOS and offspring birth weight.

MR-Egger inspection of potential horizontal pleiotropy showed a regression intercept of 0.0031 [95% confidence interval (*CI*), −0.012 to 0.018; P = 0.69], which suggests no evidence of horizontal

Table 1. Characteristics of instrumental single nucleotide polymorphisms utilized in the Mendelian randomization analysis

SNP	Chr:Pos	Nearest gene	Effect allele	Association with maternal PCOS				Association with offspring BW			
				EAF	β	Standard error	P-value	EAF	β	Standard error	P-value
rs7563201	2:43561780	*THADA*	A	0.4507	−0.1081	0.0172	3.68×10^{-10}	0.4963	0.0093	0.0044	0.0331
rs2178575	2:213391766	*ERBB4*	A	0.1512	0.1663	0.0219	3.34×10^{-14}	0.1620	−0.0045	0.0058	0.4444
rs13164856	5:131813204	*IRF1/RAD50*	T	0.7291	0.1235	0.0193	1.45×10^{-10}	0.7128	0.0009	0.0047	0.8479
rs804279	8:11623889	*GATA4/NEIL 2*	A	0.2616	0.1276	0.0184	3.76×10^{-12}	0.2679	−0.0029	0.0048	0.5498
rs10739076	9:5440589	*PLGRKT*	A	0.3078	0.1097	0.0197	2.51×10^{-8}	0.3000	−0.0034	0.0049	0.4902
rs7864171	9:97723266	*C9orf3*	A	0.4284	−0.0933	0.0168	2.95×10^{-8}	0.4190	−0.0005	0.0044	0.9038
rs9696009	9:126619233	*DENND1A*	A	0.0679	0.2020	0.0311	7.96×10^{-11}	0.0622	0.0004	0.0088	0.9869
rs11031005	11:30226356	*ARL14EP/FSHB*	T	0.8537	−0.1593	0.0223	8.66×10^{-13}	0.8543	0.0037	0.0061	0.5413
rs11225154	11:102043240	*YAP1*	A	0.0941	0.1787	0.0272	5.44×10^{-11}	0.0778	−0.0139	0.0082	0.0872
rs1784692	11:113949232	*ZBTB16*	T	0.8237	0.1438	0.0226	1.88×10^{-10}	0.8283	0.0078	0.0057	0.1700
rs2271194	12:56477694	*ERBB3/RAB 5B*	A	0.4160	0.0971	0.0166	4.57×10^{-9}	0.4285	0.0052	0.0043	0.2239
rs1795379	12:75941042	*KRR1*	T	0.2398	−0.1174	0.0195	1.81×10^{-9}	0.2231	0.0064	0.0051	0.2075
rs8043701	16:52375777	*TOX3*	A	0.8150	−0.1273	0.0208	9.61×10^{-10}	0.8264	−0.004	0.0058	0.4860

Note. EAF was derived from normal controls in the PCOS-association study and from whole participants in the offspring BW-association study. BW = birth weight; Chr:Pos = chromosome and position according to the GRCh37/hg19 genome assembly; EAF = effect allele frequency; PCOS = polycystic ovary syndrome.

pleiotropy. Cochran's Q test demonstrated no evident heterogeneity in our primary results (Q statistic, 13.21; *P* = 0.35) obtained using the inverse-variance weighted model to assess causal effects. The leave-one-out analysis plot shown in Figure 2 reveals that single elimination of each instrumental SNP has no apparent influence on the overall MR estimates. The relatively symmetric funnel plot illustrated in Figure 2 demonstrates the absence of bias arising from the disproportionate effects of certain variants. Taken together, our overall MR results are robust and convincing.

To the best of our knowledge, this MR study is the first to explore the causality between PCOS and offspring birth weight. However, despite the adequate statistical power and low confounding bias of our analyses, we failed to identify evidence supporting a causal effect. Whether a genetically predicted higher risk of PCOS will cause a decrease in offspring birth weight has yet to be verified by further well-designed epidemiological studies.

Few studies have examined the effect estimate of PCOS on birth weight as a continuous variable, but several large observational cohorts examining the effect of the syndrome on small-for-gestational-age deliveries have been published. A recent retrospective cohort study[3] explored whether PCOS presents an independent risk factor for neonatal outcomes but demonstrated no difference in the proportion of women who gave birth to small-for-gestational-age infants between women with PCOS and those in the reference group (*OR*-adjusted = 0.97, 95% *CI*: 0.82–1.15, *P* = 0.72); this cohort study included the largest inpatient dataset (PCOS cases, *n* = 14,882; reference group, *n* = 9,081,906) of the American population published thus far. PCOS mothers from another prospective multicenter cohort[2] in the Netherlands seemed to be at higher risk of birthing small-for-gestational-age infants than the reference group (*OR*-adjusted = 3.76; 95% *CI:* 1.69–8.35) regardless of hyperandrogenic status. de Wilde et al.[2] pointed out the comparable incidence of large-for-gestational-age infants between PCOS (16/188, 9%) and the reference group (335/2889, 12%; *P* = 0.14) of naturally conceived singleton women, although a significant higher incidence of gestational diabetes was noted in the former (23% *vs.* 5%, *P* < 0.001). Glucose regulation disturbance and insulin resistance are well-known changes in PCOS[1], and gestational diabetes is a well-recognized risk factor for macrosomia. The effect of PCOS on neonatal birth weight, however, may be different from that of gestational diabetes.

Differences in genetic architecture underlying PCOS across religion and race have been extensively studied. Taking genome-wide significant loci for

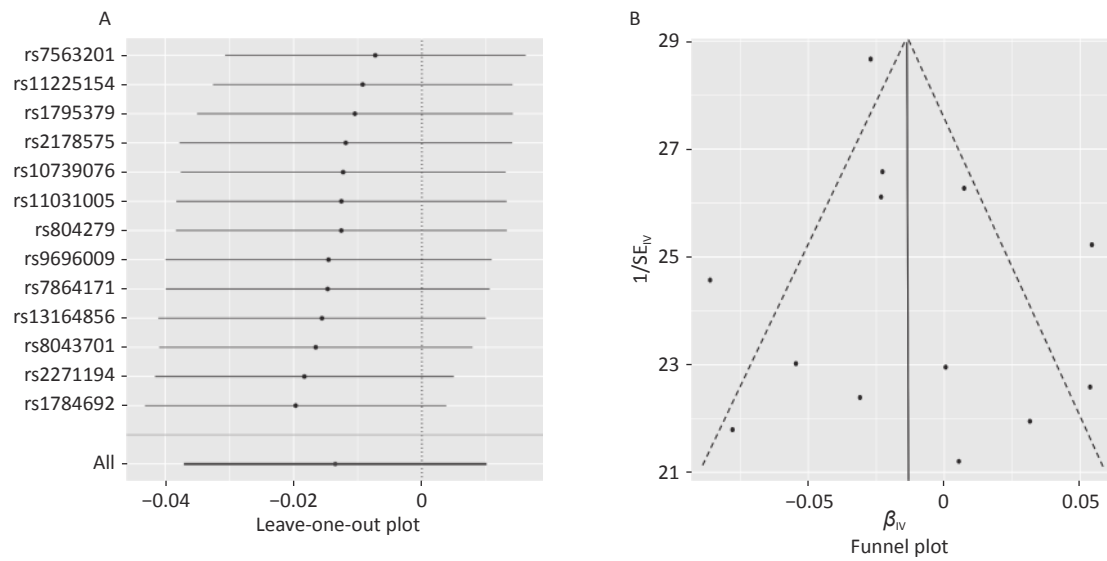

Figure 2. Leave-one-out (A) and funnel plots (B) determined by sensitivity analyses. On the left, each point delineates the Mendelian randomization (MR) estimate excluding that particular variant. The leave-one-out plot suggests that the association determined is not disproportionately affected by a certain instrumental variable. On the right, scattering points represent the effect estimated using each instrumental variable. The vertical line denotes the overall estimate obtained by MR analysis. The relatively symmetric distribution observed indicates the absence of directional horizontal pleiotropy.

PCOS in the European and Asian populations as an example, only one third of the loci identified in Europeans could be replicated in the Chinese Han population (Supplementary Figure S1 available in www.besjournal.com). Thus, we cannot rule out a possible effect of PCOS on the birth weight of offspring in populations other than Europeans and caution should be exercised when generalizing our conclusions. Furthermore, PCOS is a heterogeneous disorder consisting of three primary components, namely, ovulatory dysfunction, hyperandrogenism, and polycystic ovarian morphology that involve complex interactions between polygenic and environmental effects. Nevertheless, overall estimates for PCOS per effect allele across three different diagnostic groups show negligible differences in heterogeneity[7], except for one SNP near GATA4/NEIL2 (rs804279, Het $P = 2.6 \times 10^{-5}$). Without individual-level genotype-phenotype data, we could not conduct stratified analyses based on NIH or Rotterdam Criteria alone. We also failed to perform comprehensive estimates of the possible effect of individual PCOS-related traits on offspring birth weight. The GWAS datasets utilized in the main MR analysis incorporated associations between index SNPs at each genome-wide-significant locus for PCOS and the three major components; nevertheless, these summary statistics are insufficient for multivariable MR[5], which is the optimal approach to elucidate the causative effects of individual PCOS-related traits. Thus, we could not preclude such effects in the current study.

Our study presents several limitations. First, we could not examine the influence of non-linear effects, such as U-shaped associations; this limitation is present in all two-sample MR studies. Second, because the datasets used in our analyses were restricted, we could not conduct stratified analyses accounting for body mass index or explore the effects of PCOS on small-for-gestational age using birth weight data from normal delivery cohorts. Third, we failed to construct a comprehensive and sophisticated model considering the influences of paternal genotype and fetal-placental interaction, which may distort our MR estimates. Finally, the instrumental SNPs and outcome statistics originated from European-ancestry studies and a ≥ 1% overlap was present in the sample size of contributing cohorts[4,7]; these factors may add bias to the MR estimates to some extent.

In conclusion, we conducted the first MR study investigating the causality between maternal PCOS and offspring birth weight. Our findings suggest no evidence of the negative effect of PCOS on offspring birth weight.

We gratefully acknowledge Dr. Felix Day, Dr. Nicole Warrington and the Early Growth Genetics Consortium for sharing GWAS summary statistics on PCOS (https://www.repository.cam.ac.uk/handle/1810/289950) and birth weight (http://egg-consortium.org/birth-weight-2019.html). WU Peng Fei received a stipend from the China Scholarship Council.

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Received: May 6, 2020;

Accepted: September 21, 2020

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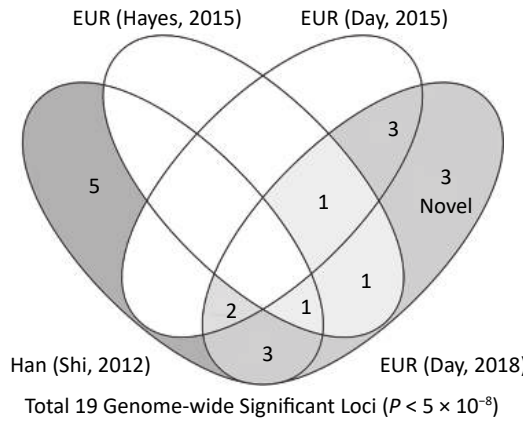
Supplementary Table S1. Effects of polycystic ovary syndrome on offspring birth weight estimated by Mendelian randomization (MR)

Approach	Beta	SD	*P*-value
Inverse-variance weighted analysis	−0.013	0.012	0.26
Weighted median	−0.023	0.016	0.14
MR-Egger slope	−0.038	0.060	0.54

Note. The MR-Egger intercept representing horizontal pleiotropy is not shown here. MR results represent estimated causal differences in SD changes of offspring birth weight per 1-SD higher log-odds of maternal polycystic ovary syndrome. SD = standard error.



Supplementary Figure S1. Venn diagram showing 19 genome-wide significant loci for polycystic ovary syndrome identified primarily in the European and Chinese Han populations. References: (1) Shi Y. et al. Nat Genet 2012. pmid:22885925. (2) Hayes MG. et al. Nat Commun 2015. pmid:26284813. (3) Day F. et al. Nat Commun 2015. pmid:26416764. (4). Day F. et al. PLoS Genet 2018. pmid:30566500.