Polycystic Ovary Syndrome and Risk of Type 2 Diabetes, Coronary Heart Disease, and Stroke

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Polycystic ovary syndrome (PCOS) has been associated with diabetes and cardiovascular disease; however, whether the relationship is causal is uncertain. We conducted a two-sample Mendelian randomization study to investigate the associations of PCOS with type 2 diabetes, coronary heart disease (CHD), and stroke. Association between PCOS and diabetes risk was examined in European and Asian cohorts, both sex specific and sex combined. Causal effects of PCOS on risks of CHD and stroke were evaluated in European cohorts. Stroke was analyzed as any stroke as well as four subtypes of stroke (ischemic, large artery, cardioembolic, small vessel). We found no association of genetically predicted PCOS with risk of diabetes, CHD, or stroke. This suggests that PCOS in and of itself does not increase the risk of these outcomes. Other features of PCOS (obesity, elevated testosterone, low sex hormone binding globulin) may explain the association between PCOS and cardiometabolic diseases. In light of these results, efforts to prevent cardiometabolic complications in PCOS should focus on women with high-risk features rather than all women with PCOS.

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age. PCOS has been associated with significant adverse health conditions including obesity, diabetes, dyslipidemia, cardiovascular disease (CVD), sleep apnea, depression, and nonalcoholic fatty liver disease. A key question is whether these associations represent causal relationships. Such knowledge is critical to efforts to prevent morbidity in women with PCOS. The fact that PCOS is a syndrome with multiple features complicates efforts to establish causality between PCOS and adverse outcomes because individual features may contribute differentially to outcomes.

Observational epidemiologic studies do not establish causality because the relationship between two conditions may be driven by confounding factors or reverse causality. To avoid these pitfalls, investigators have used genetics to address questions of causality. In Mendelian randomization (MR), a risk factor or exposure is represented by genetic variants for that factor, which are then used in instrument variable analysis to yield unconfounded evidence to support causality of the exposure with an outcome of interest. Completion of genome-wide association studies (GWAS) of PCOS in Asian and European origin cohorts (1–5) has made MR possible for analysis of the relationship between PCOS and various traits and diseases. Recent MR studies suggested that obesity, age of menopause, insulin resistance/hyperinsulinemia, sex hormone binding globulin (SHBG) levels, and depression may be causal risk factors for PCOS (2,3).

A major focus in PCOS concerns its relationship with cardiometabolic diseases. Experts in the field generally agree that PCOS is a risk factor for type 2 diabetes, largely based on a substantial literature documenting that insulin resistance is frequent in women with PCOS. While obese women with PCOS consistently have greater insulin resistance than BMI-matched control subjects, some studies but not others find that this is also the case for nonobese PCOS (6,7). A meta-analysis of euglycemic-hyperinsulinemic clamp studies concluded that women with PCOS are nearly 30% less insulin sensitive than control subjects, independent of BMI but exacerbated by higher BMI and lower SHBG (8). Several observational studies have found higher rates of diabetes in women with PCOS versus unaffected control subjects. A meta-analysis of these studies found

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increased prevalent impaired glucose tolerance (odds ratio [OR] 3.26, 95% CI 2.17–4.90) and diabetes (OR 2.87, 95% CI 1.44–5.72) in women with PCOS (9). Whether PCOS also predisposes to coronary heart disease (CHD) and stroke is less certain, given the lack of large long-term studies following women with PCOS into older age, during which CVD events mainly occur. Meta-analyses of available case-control and cohort studies suggest that PCOS may increase the risk of CHD (OR 1.44, 95% CI 1.13–1.84) and stroke (OR 1.36, 95% CI 1.09–1.70), though risk estimates decrease with adjustment for BMI (10,11). Given the high clinical relevance of these questions, we carried out two-sample MR analyses to determine whether genetically increased risk of PCOS leads to an increased risk of type 2 diabetes, CHD, or stroke.

RESEARCH DESIGN AND METHODS

Instrumental Variables (Genetic Variants Associated With PCOS)

In a GWAS meta-analysis of PCOS consisting of 10,074 PCOS case and 103,164 control subjects of European ancestry, 14 independent single nucleotide polymorphisms (SNPs) were reported to be associated with PCOS risk at the genome-wide significance level ($P < 5 \times 10^{-8}$) (2). Of the 14 SNPs, all were included to construct the genetic instrument for PCOS except SNP rs853854, because it is a palindromic SNP (A/T) with an effect allele frequency close to 50% (12). We included another independent SNP (rs2349415), which was initially identified in Chinese PCOS GWAS (4) and was significantly associated with PCOS in the European PCOS meta-analysis (2). Therefore, 14 SNPs in total were included in our instrument for PCOS in Europeans (Table 1).

SNPs associated with PCOS in Asians were selected from two PCOS GWAS conducted in cohorts of Han Chinese ancestry (1,4). The GWAS by Chen et al. (1) consisted of 4,082 PCOS case and 6,687 control subjects and identified three independent SNPs strongly associated with PCOS. The GWAS of Shi et al. (4) including 10,480 case and 10,579 control subjects discovered 10 novel PCOS–associated SNPs. In total, 13 independent SNPs were used as instrumental variables for PCOS in Asians (Table 2).

We applied the *F* statistic to measure the strength of the instrument variables, with values >10 reflecting strong instruments (13). The 14-SNP instrument for PCOS in Europeans had an *F* statistic of 39.5, and the 13-SNP instrument for PCOS in Asians had an *F* statistic of 66.6.

Outcome Data Sources (GWAS of Diabetes, CHD, and Stroke)

Summary-level data, both sex combined and sex stratified, for type 2 diabetes GWAS in Europeans were obtained from the DIAbetes Meta-ANalysis of Trans-Ethnic association studies (DIAMANTE) consortium, which included 74,124 case and 824,006 control subjects of European ancestry (14). We obtained GWAS summary data on type 2 diabetes in Asians from the Asian Genetic Epidemiology Network (AGEN) consortium with 77,418 case and 356,122 control subjects of East Asian ancestry (15).

Genetic association data on CHD were acquired from the CHD GWAS meta-analysis of the UK Biobank (UKBB) with the Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus the Coronary Artery Disease (C4D) Genetics (CARDIoGRAMplusC4D) consortium, which encompassed 34,541 CHD case and 261,984 control subjects from UKBB and 88,192 case and 162,544 control subjects from CARDIoGRAMplusC4D, of whom ~90% were of European origin (16).

Summary statistics on stroke and stroke subtypes used in the current study were from the MEGASTROKE consortium including 40,585 case and 406,111 control subjects of European ancestry (17). We evaluated five sets of stroke subtypes, including any stroke, any ischemic stroke, large artery stroke, cardioembolic stroke, and small vessel stroke. Details of the studies included in our analysis are shown in Table 3.

Statistical Analysis

Associations of PCOS with risks of CHD and stroke were examined in European cohorts only. Association of PCOS with diabetes risk was evaluated in both European and Asian cohorts, both sex stratified and sex combined. MR analyses in Europeans were conducted with the 14-SNP instrument for PCOS in Europeans, while MR analyses in Asians were carried out with the 13-SNP instrument for PCOS in Asians.

Primary MR analyses were conducted with the inverse variance weighted (IVW) method with random effects (18). For each SNP, the ratio estimate of the causal effect of an exposure (herein, PCOS) on an outcome is the ratio of the effect of the SNP on the outcome over the effect of the SNP on the exposure. In IVW, the overall estimate is generated via IVW meta-analysis of the ratio estimates of all variants in the set of instrument variables. Given that the IVW method may be affected by directional pleiotropy (where a genetic variant affects the outcome through a pathway other than the exposure), we performed sensitivity analyses using MR-Egger (19) and weighted median methods (20) to check for robustness of the estimates from the IVW method. MR-Egger can detect and correct for the bias due to directional pleiotropy because it allows a nonzero intercept and provides a consistent estimate of the causal effect under the InSIDE (Instrument Strength Independent of Direct Effect) assumption (the genetic associations with the exposure are independent of the direct effects of the genetic variants on the outcome) (19). The weighted median approach can provide a consistent causal effect estimate as long as at least 50% of the information contributing to the analysis comes from valid instrumental variables (20). Furthermore, we tested the heterogeneity of the causal estimates using Cochran *Q* test

Table 1—PCOS SNPs used to construct the main instrument variable in Europeans

Chr:position	SNP	Effect allele	Other allele	EAF	β	SE	Nearest gene	*P*	*F* statistic
2:43561780	rs7563201	A	G	0.451	−0.108	0.017	*THADA*	3.68E−10	39.50
2:213391766	rs2178575	A	G	0.151	0.166	0.022	*ERBB4*	3.34E−14	57.66
5:131813204	rs13164856	T	C	0.729	0.124	0.019	*IRF1/RAD50*	1.45E−10	40.95
8:11623889	rs804279	A	T	0.262	0.128	0.018	*GATA4/NEIL2*	3.76E−12	48.09
9:5440589	rs10739076	A	C	0.308	0.110	0.020	*PLGRKT*	2.51E−08	31.01
9:97723266	rs7864171	A	G	0.428	−0.093	0.017	*C9orf3*	2.95E−08	30.84
9:126619233	rs9696009	A	G	0.068	0.202	0.031	*DENND1A*	7.96E−11	42.19
11:30226356	rs11031005	T	C	0.854	−0.159	0.022	*ARL14EP/FSHB*	8.66E−13	51.03
11:102043240	rs11225154	A	G	0.094	0.179	0.027	*YAP1*	5.44E−11	43.16
11:113949232	rs1784692	T	C	0.824	0.144	0.023	*ZBTB16*	1.88E−10	40.49
12:56477694	rs2271194	A	T	0.416	0.097	0.017	*ERBB3/RAB5B*	4.57E−09	34.22
12:75941042	rs1795379	T	C	0.240	−0.117	0.020	*KRR1*	1.81E−09	36.25
16:52375777	rs8043701	A	T	0.815	−0.127	0.021	*TOX3*	9.61E−10	37.46
2:49247832	rs2349415	T	C	0.343	0.076	0.017	*FSHR*	9.59E−06	19.65

Chr, chromosome; EAF, effect allele frequency.

Table 2—PCOS SNPs used to construct the main instrument variable in East Asians

Chr:position	SNP	Effect allele	Other allele	EAF	β	SE	Nearest gene	*P*	*F* statistic
2:43638838	rs13429458	A	C	0.81	0.401	0.040	*THADA*	1.73E−23	99.75
2:48978159	rs13405728	A	G	0.754	0.343	0.037	*LHCGR*	7.55E−21	87.72
2:49201612	rs2268361	C	T	0.504	0.139	0.020	*FSHR*	9.89E−13	50.87
2:49247832	rs2349415	T	C	0.181	0.174	0.025	*FSHR*	2.35E−12	49.17
9:97648587	rs4385527	G	A	0.781	0.174	0.030	*C9orf3*	5.87E−09	33.88
9:97741336	rs3802457	G	A	0.904	0.261	0.035	*C9orf3*	5.28E−14	56.62
9:126525212	rs2479106	G	A	0.222	0.293	0.033	*DENND1A*	8.12E−19	78.47
11:102070639	rs1894116	G	A	0.194	0.239	0.024	*YAP1*	1.08E−22	96.12
12:56390636	rs705702	G	A	0.245	0.239	0.023	*RAB5B/SUOX*	8.64E−26	110.25
12:66224461	rs2272046	A	C	0.907	0.357	0.038	*HMGA2*	1.95E−21	90.4
16:52347819	rs4784165	G	T	0.325	0.140	0.021	*TOX3*	3.64E−11	43.8
19:7166109	rs2059807	G	A	0.301	0.131	0.023	*INSR*	1.09E−08	32.67
20:52447303	rs6022786	A	G	0.339	0.122	0.020	*SUMO1P1*	1.83E−09	36.15

Chr, chromosome; EAF, effect allele frequency.

Table 3—Characteristics of the outcome data sources used for MR analyses

Trait	No. of case subjects	No. of control subjects	Consortium	Population	Year
Diabetes in Asian (all subjects)	77,418	356,122	AGEN	Asian	2020
Female	27,370	135,055	AGEN	Asian	2020
Male	28,027	89,312	AGEN	Asian	2020
Diabetes in European (all subjects)	74,124	824,006	DIAMANTE	European	2018
Female	30,053	434,336	DIAMANTE	European	2018
Male	41,846	383,767	DIAMANTE	European	2018
CHD	122,733	424,528	UKBB plus CARDIoGRAMplusC4D	Majority European	2018
Any stroke	40,585	406,111	MEGASTROKE	European	2018
Any ischemic stroke	34,217	406,111	MEGASTROKE	European	2018
Large artery stroke	4,373	406,111	MEGASTROKE	European	2018
Cardioembolic stroke	7,193	406,111	MEGASTROKE	European	2018
Small vessel stroke	5,386	406,111	MEGASTROKE	European	2018

(21). We used R 3.6.3 software and the R package TwoSampleMR (22) for the analyses.

We also carried out a series of sensitivity analyses wherein subsets of the PCOS SNPs were used as instrument variables. In the first such analysis, an instrument based on three SNPs (rs804279, rs7864171, rs11031005) associated at genome-wide significance with PCOS diagnosed by the National Institutes of Health (NIH) criteria (5) was used in MR analyses of diabetes, CHD, and stroke in Europeans. We also examined association of the PCOS instrument variable SNPs with potential confounding phenotypes including BMI, waist-to-hip ratio, testosterone, and SHBG. Summary results from GWAS were used to characterize the association of PCOS SNPs with BMI and waist-to-hip ratio with adjustment for BMI (23). Conservatively, we used a P value cutoff of $<1 \times 10^{-4}$ to flag for sensitivity analyses two European PCOS SNPs associated with BMI, one European PCOS SNP associated with waist-to-hip ratio, and two Asian PCOS SNPs associated with waist-to-hip ratio (Supplementary Table 1). We examined whether PCOS SNPs were in linkage disequilibrium ($r^2 > 0.2$) with genome-wide significant signals for total and bioavailable testosterone and SHBG (24). We found that 3 of the 14 European PCOS SNPs and 3 of the 13 Asian PCOS SNPs were linked to SNPs for total or bioavailable testosterone (Supplementary Table 2). Therefore, we conducted the following sensitivity analyses using the IVW, MR-Egger, and weighted median methods. In Europeans, we examined instrument variables *1*) excluding the three SNPs associated with adiposity traits, *2*) excluding the three SNPs associated with testosterone, and *3*) excluding all six of these SNPs. The outcomes for these analyses were diabetes, CHD, and stroke. In Asians, we examined instrument variables *1*) excluding the two SNPs associated with waist-to-hip ratio, *2*) excluding the three SNPs associated with testosterone, and *3*) excluding all five of these SNPs, each with the outcome of diabetes.

Data and Resource Availability

Summary-level data of diabetes GWAS that were used in this study are available at the AGEN consortium website, https://blog.nus.edu.sg/agen/summary-statistics/t2d-2020/, and the DIAGRAM consortium website, https://diagram-consortium.org/. CHD summary GWAS data are accessible at https://data.mendeley.com/datasets/gbbsrpx6bs/1, and stroke summary data are available at the MEGASTROKE consortium website https://megastroke.org/download.html upon reasonable request.

RESULTS

Causal effect estimates of PCOS on diabetes, CHD, and stroke traits are displayed in Tables 4 and 5. According to primary MR analyses by the IVW method, genetically predicted PCOS is not significantly associated with the risk of diabetes, CHD, or any stroke traits. The analyses by weighted median and MR-Egger methods showed results similar to those by IVW. In all cases, the MR-Egger intercepts were not different from zero, indicating absence of directional pleiotropy. The result of the weighted median method found that genetically predicted PCOS is inversely associated with diabetes in European females (OR 0.88, 95% CI 0.82–0.96, $P = 0.003$); however, the IVW method found no significant association (OR 0.95, 95% CI 0.88–1.02, $P = 0.16$). In addition, the Cochran Q test detected substantial heterogeneity for diabetes in European women (Table 4). Thus, there is insufficient evidence to support the association between genetically predicted PCOS and diabetes in European women.

Additional sensitivity analyses also showed no effect of genetically predicted PCOS to increase the outcomes examined. These included MR in Europeans for the outcomes of diabetes, CHD, and stroke with use of an instrument composed of three SNPs associated with PCOS diagnosed by the NIH criteria (Supplementary Table 3). Results similar to our primary results for diabetes, CHD, and stoke in Europeans were generated in MR with an 11-SNP instrument composed of SNPs not associated with BMI or waist-to-hip ratio adjusted for BMI (Supplementary Table 4), with an 11-SNP instrument composed of SNPs not associated with testosterone (Supplementary Table 5), and with an 8-SNP instrument composed of SNPs not associated with adiposity traits or testosterone (Supplementary

Table 4—Associations between genetically predicted PCOS and risk of type 2 diabetes, CHD, and stroke with use of the IVW method

	IVW		Cochran *Q* statistic	
Trait	OR (95% CI)	*P*	Test statistic	*P*
Diabetes in Asian (all)	0.98 (0.96–1.01)	0.13	23.48	0.02
Female	0.98 (0.95–1.02)	0.33	11.40	0.50
Male	0.99 (0.95–1.02)	0.45	14.45	0.27
Diabetes in European (all)	0.97 (0.92–1.01)	0.16	29.79	0.005
Female	0.95 (0.88–1.02)	0.16	29.38	0.006
Male	0.98 (0.93–1.03)	0.42	15.66	0.27
CHD	1.00 (0.96–1.04)	0.88	24.42	0.03
Any stroke	0.98 (0.93–1.02)	0.33	10.21	0.68
Any ischemic stroke	0.98 (0.93–1.03)	0.40	8.26	0.83
Large artery stroke	0.88 (0.78–1.00)	0.06	12.16	0.51
Cardioembolic stroke	0.92 (0.83–1.02)	0.10	13.88	0.38
Small vessel stroke	1.10 (0.95–1.27)	0.21	18.59	0.14

Table 6). No effect on diabetes in Asians was observed for an 11-SNP instrument composed of SNPs not associated with waist-to-hip ratio adjusted for BMI, for a 10-SNP instrument composed of PCOS SNPs not associated with testosterone, or for an 8-SNP instrument composed of SNPs not associated with waist-to-hip ratio or testosterone (Supplementary Table 7).

DISCUSSION

Our MR analyses suggest that PCOS per se does not have a causal relationship with type 2 diabetes, CHD, or stroke. That genetic risk of PCOS was not associated with increased risk of diabetes was unexpected considering the body of observational studies linking PCOS to impaired glucose tolerance and diabetes. Of 40 such studies cataloged in a systematic review, one-half were deemed low quality (9). After exclusion of low-quality studies, meta-analysis of 12 studies found positive association of PCOS with diabetes (OR 2.87, 95% CI 1.44–5.72), though with substantial heterogeneity. However, the median number of women with PCOS in these 12 studies was only 92. Considering a 5–10% prevalence rate of diabetes in PCOS (only premenopausal women were included in these studies), it is evident that the number of women included with both PCOS and diabetes was quite low. The robustness of a meta-analysis depends on its component studies. Among the few studies reporting risk of diabetes that had substantial numbers of women with PCOS and were judged to be of good or fair quality, results were mixed (25–28).

Though our results suggest that PCOS does not cause diabetes, several common features of PCOS do appear to cause diabetes, which may explain the epidemiologic association. Adiposity is one such feature. Several studies have documented that women with PCOS have increased BMI compared with women without PCOS (29). For example, in a large health system study, women with PCOS had a fourfold increased odds of having BMI >30 kg/m^2 (25). Ample physiologic, epidemiologic, and MR evidence exists implicating increased adiposity as a causal risk factor for diabetes (30). Thus, the frequent finding of increased BMI in women with PCOS may explain much of the association between PCOS and diabetes, especially in studies that did not account for BMI. In the meta-analysis discussed above, subgroup analyses restricted to studies where women with PCOS and control subjects were matched on BMI found no association of PCOS with diabetes (seven studies [OR 1.13, 95% CI 0.83–1.54]) (9). However, some studies that did attempt to match for or statistically adjust for BMI reported positive association between PCOS and diabetes (25,31,32). A retrospective observational study found a higher incidence of diabetes in women with PCOS versus age- and BMI-matched control subjects (hazard ratio 1.75, 95% CI 1.51–2.03), which was also observed in the stratum with BMI <25 kg/m^2 (hazard ratio 1.39, 95% CI 1.09–1.99) (33). These data suggest that additional factors beyond BMI contribute to diabetes risk in PCOS.

Hyperandrogenemia is another potential diabetogenic feature of PCOS. Observational studies have yielded conflicting results in this regard (26,34,35). A recent MR study has illuminated differential effects of testosterone on adverse outcomes in men and women (24). This study first greatly expanded the number of SNPs associated with testosterone, bioavailable testosterone, and SHBG by conducting GWAS for these traits in $>425{,}000$ individuals from the UKBB. While the genetic architecture of testosterone was very different between men and women, largely the same variants were associated with SHBG in both sexes. After conducting cluster analyses to identify loci with primary effects on testosterone or SHBG, the investigators used these as instrument variables in two-sample MR analyses using sex-stratified GWAS for metabolic and oncologic outcomes. While manifesting a protective effect in men, increased circulating testosterone in women had a causal effect on risk of diabetes. Higher SHBG was protective against diabetes in both sexes (discussed below). Thus, it appears that while PCOS overall does not increase diabetes risk, one of its defining features, elevated

Table 5—Associations between genetically predicted PCOS and risk of type 2 diabetes, CHD, and stroke with use of MR-Egger and weighted median methods

	MR-Egger				Weighted median	
Trait	OR (95% CI)	*P*	Intercept	P_{Inter}	OR (95% CI)	*P*
Diabetes in Asian (all)	0.96 (0.90–1.03)	0.29	0.005	0.58	0.99 (0.96–1.02)	0.42
Female	0.96 (0.88–1.04)	0.29	0.01	0.44	0.99 (0.94–1.03)	0.53
Male	0.99 (0.90–1.09)	0.83	−0.001	0.94	0.98 (0.93–1.02)	0.34
Diabetes in European (all)	1.00 (0.81–1.24)	1.00	−0.004	0.75	0.95 (0.91–1.01)	0.08
Female	0.97 (0.70–1.35)	0.87	−0.003	0.88	0.88 (0.82–0.96)	0.003
Male	1.02 (0.82–1.25)	0.88	−0.005	0.73	0.97 (0.91–1.04)	0.40
CHD	0.91 (0.77–1.06)	0.24	0.01	0.24	0.99 (0.95–1.04)	0.76
Any stroke	1.06 (0.87–1.29)	0.58	−0.01	0.43	1.00 (0.94–1.07)	0.90
Any ischemic stroke	1.04 (0.83–1.30)	0.73	−0.01	0.59	0.99 (0.92–1.06)	0.72
Large artery stroke	1.02 (0.60–1.74)	0.95	−0.02	0.60	0.91 (0.77–1.08)	0.29
Cardioembolic stroke	1.07 (0.69–1.66)	0.77	−0.02	0.50	0.94 (0.82–1.08)	0.38
Small vessel stroke	0.88 (0.48–1.62)	0.69	0.03	0.48	1.09 (0.92–1.29)	0.33

P_{Inter}, intercept *P* value.

testosterone, does have a causal link with diabetes in women. Of note, the study described above also studied testosterone as an exposure and PCOS as an outcome, with MR suggesting a causal relationship (24). That both genetically increased BMI and testosterone are associated with both PCOS and diabetes suggests that the association between PCOS and diabetes may be mediated by these factors (Fig. 1), as supported by negative results of sensitivity analyses wherein PCOS instrument variable SNPs associated with testosterone were excluded.

Another feature of PCOS that may influence diabetes risk is reduced SHBG levels. Numerous epidemiologic and MR studies have associated lower SHBG with higher risk of diabetes in women and men, strongly suggesting a causal relationship (24,36,37). Low SHBG is a well-established feature of PCOS and is thought to arise from the effect of insulin resistance or hyperinsulinemia on the liver (38). MR suggests that low SHBG may be a causal factor for PCOS itself (2,3). Thus, low SHBG not only exacerbates hirsutism by increasing free androgens but also appears to influence risk of both PCOS and diabetes (Fig. 1). A recent health care registry study of women selected to be free of comorbidities (including PCOS) found that higher testosterone and lower SHBG were associated with incident diabetes, suggesting that these relationships apply to women in general (39).

Lack of genetic association between PCOS and CHD and stroke was less surprising than the results with diabetes, given that these have not been consistently associated with PCOS. While there is a large body of literature finding increased CVD risk factor burden in PCOS (e.g., dyslipidemia, hypertension), as well as subclinical atherosclerosis (e.g., increased carotid intima-media thickness, increased coronary artery calcium), whether this translates into increased CVD events is uncertain, as fewer studies have addressed the latter question (40). Similar to diabetes, increased adiposity frequently present in PCOS may influence CVD risk, as MR studies strongly suggest that increased BMI is causal for CHD but not stroke (30) (Fig. 1). Several prior meta-analyses (10,11,41) addressing whether PCOS was associated with increased CHD and/or stroke were heavily influenced by large cohort studies where the underlying condition was irregular menses, rather than formally diagnosed PCOS (42,43). This is problematic because other disorders featuring irregular menses, such as hypothalamic amenorrhea, may also be associated with CVD risk (44). These studies were not included in a recent systematic review and meta-analysis of 16 studies, 12 of which were population based (45). This meta-analysis found association of PCOS with a composite outcome consisting of coronary artery disease, CVD, myocardial infarction, angina, heart failure, and ischemic heart disease; that each component of the outcome was represented by few studies limited the ability to explore them individually. The composite result was influenced by type of study, with population-based studies manifesting the association in premenopausal but not postmenopausal women. However, even this systematic review was affected by the extremely low number of events in published reports, which represents the most significant challenge in the evidence base regarding the risk of CVD in PCOS, as discussed below.

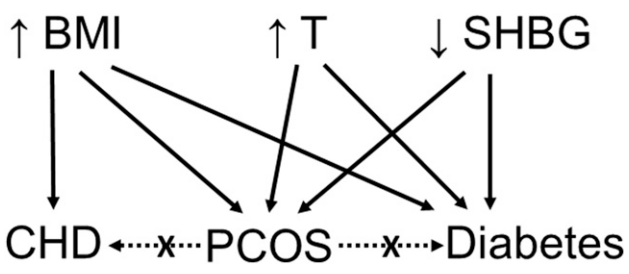

Figure 1—Relationships among risk factors, PCOS, and cardiometabolic events suggested by MR. Each solid arrow represents a positive genetic correlation documented by MR, indicating possible causal relationships. The relationship between increased testosterone and PCOS applies only to women. These relationships may explain the epidemiologic associations among PCOS and CHD and diabetes. The dotted arrows represent the negative MR results of the current study. T, testosterone.

A key consideration in comparing epidemiologic studies with the current MR analysis is the age of studied participants. As PCOS is a disorder mainly affecting women of reproductive age, most observational studies have included young women. For example, all 40 of the studies in a systematic review of PCOS and diabetes and 12 of 16 studies in a systematic review of PCOS and CVD were conducted in adolescents or premenopausal women (9,45). In women of reproductive age, the prevalence of CHD and cerebrovascular disease is quite low; the prevalence of each was found to be 0.2% in women aged 15–44 years (25). Most events of type 2 diabetes and especially CHD occur in postmenopausal women. Thus, though GWAS for diabetes and CHD span subjects of all ages, the majority are older individuals. Given this, our results could be interpreted to indicate that PCOS does not appear to be causal for diabetes or CHD typically occurring in older individuals, leaving open the question of whether it is causal in younger women. To this point, some observational studies have suggested that PCOS may increase risk of diabetes or CVD in younger women but not in older women (45,46). Furthermore, as women with PCOS advance in age, the syndrome may improve or even regress entirely with reduction in ovarian size and androgen production (47). Considering the potential causal role of androgens in cardiometabolic risk (24), this improvement might result in reduced risk of diabetes and CHD with aging (48). In a study of 200 women with PCOS aged 45 years and older and 200 age-matched control subjects, the prevalence of diabetes, metabolic syndrome, dyslipidemia, and calculated 10-year CVD risk was similar (49). While these observations are consistent with our MR results, what is clearly needed is a large, prospective study following

women from youth through menopause and beyond, with detailed characterization of risk factors and incident diabetes and CHD.

Another possible explanation of our negative results is that PCOS as currently defined and implemented in GWAS may represent a heterogenous collection of underlying pathophysiologies. If some of these but not others are causal for diabetes or CVD, grouping them together may reduce the power to detect genetic association between PCOS and cardiometabolic outcomes. The largest European GWAS for PCOS, which was the source of our instrument variables, was liberal in how PCOS was diagnosed, allowing not only NIH (14.6% of included cases) and Rotterdam criteria (34.0%) but also self-reported diagnosis (51.4%) (2). This may have affected the specificity of the European PCOS instrument, which reflects PCOS identified by various diagnostic criteria that may differ in accuracy. The Asian GWAS used Rotterdam criteria, which allow multiple phenotype patterns to result in a diagnosis of PCOS. To generate a more specific instrument for PCOS, we examined the three genome-wide significant signals from the one European GWAS that used NIH criteria for PCOS (5). MR using these three SNPs yielded essentially the same results as the 14 SNPs examined in Europeans (Supplementary Table 3). Future GWAS efforts with large sample sizes of different subphenotypes of PCOS may facilitate MR efforts geared toward linking PCOS subphenotypes with adverse outcomes.

Our study has several strengths. We conducted state-of-the-art two-sample MR analysis using robust GWAS loci for PCOS and the cardiometabolic outcomes. Unlike observational studies in PCOS, our analysis represented a large number of individuals with diabetes, CHD, and stroke (though numbers for large artery stroke, cardioembolic stroke, and small vessel stroke individually were lower). We analyzed two race groups and obtained similar results, an important feature given the prior suggestion from meta-analyses that the risk of diabetes in PCOS might be greater in Asians than in Europeans (9). The availability of sex-stratified genetic data on diabetes was another advantage. Unfortunately, sex-stratified summary data from GWAS for CHD were not available at the time of our study. Following best practices, we used different methods of MR analysis that are affected differently by genetic confounding or pleiotropy. Though MR-Egger did not detect positive or negative pleiotropy, we cannot rule out balanced pleiotropy. The robustness of our results is supported by the sensitivity analyses in which we excluded SNPs associated with adiposity traits or testosterone from the instrument variable. We believe that the inverse association between PCOS and diabetes in European women in weighted median analysis is a chance effect related to heterogeneity of the diabetes data (most pronounced in European women) (Table 4), as such association was not seen in Asian women or observed in European women in results from the other MR methods; however, we cannot rule out violation of at least one of the instrumental variable assumptions. As long as core assumptions are met (instrument variables strongly represent the exposure, are not associated with confounders, and are associated with outcome through the exposure and not through other mediators), MR can be used with GWAS data to strongly suggest (but not prove) causal relationships between an exposure (here, PCOS) and outcomes. Though *F* scores indicated that our instrument variables were strong, it is possible that the relatively low number of SNPs could have contributed to the lack of association. These analyses, including the nearly significant inverse association with large artery stroke (Table 4), will be revisited once investigators discover additional susceptibility SNPs for PCOS.

If confirmed with a greater number of SNPs and in larger cohorts, the current results would have important implications for how clinicians counsel and manage patients with PCOS, especially regarding the risk of diabetes. Currently, patients with PCOS are often informed that they are at increased risk of future diabetes. In many cases, measures to prevent diabetes are instituted, typically lifestyle modification and/or metformin treatment. While there is strong evidence that these measures prevent diabetes in people with prediabetes, whether they do so in the case of PCOS is uncertain. Therefore, especially for pharmacologic methods, it is imperative that efforts to prevent diabetes be recommended to those PCOS patients at highest risk rather than exposure of all patients to potential adverse effects (e.g., gastrointestinal disturbance, vitamin B12 deficiency with metformin). While the current study does not support PCOS per se as an indication for cardiometabolic preventive strategies, other MR studies (24,30) highlighted increased diabetes risk in people who are overweight or obese and women with hyperandrogenemia. A synthesis of these MR studies implies that women with PCOS with these features are the most appropriate for focused diabetes prevention efforts. Should this notion be supported by large, prospective studies of women with PCOS, we would be better positioned to provide risk counseling. Normal-weight women with PCOS who have normal androgen levels would not need to experience the stress of being told they are at increased risk of diabetes, given that PCOS in and of itself does not genetically increase the risk of diabetes; however, they should be counseled to avoid weight gain that could confer this risk. This study is an example of how genetic data can lead to personalized medicine.

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