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ORIGINAL ARTICLE *Reproductive genetics*

Reduced cardiovascular risks in women with endometriosis or polycystic ovary syndrome carrying a common functional *IGF1R* variant

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STUDY QUESTION: Is the increased future cardiovascular risk seen in women with endometriosis or polycystic ovary syndrome (PCOS) mitigated by functional insulin-like growth factor-1 receptor (*IGF1R*) single-nucleotide polymorphism (SNP) rs2016347 as previously shown in women with hypertensive disorders of pregnancy?

SUMMARY ANSWER: This cohort study found that women with endometriosis or PCOS who carry a T allele of *IGF1R* SNP rs2016347 had a reduced future risk of developing cardiovascular disease (CVD) and associated risk factors, with risk reduction dependent on cohort era.

WHAT IS KNOWN ALREADY: Women with endometriosis or PCOS have been shown to have an increased future risk of CVD and associated risk factors with limited predictive ability.

STUDY DESIGN, SIZE, DURATION: This retrospective cohort study took place in the Nurses' Health Study 2 (NHS2), which enrolled 116 430 participants in 1989 who were followed through 2015. The study population was analyzed in its entirety, and subdivided into entry (pre-1989) and after entry (post-1989) exposure cohorts. All NHS2 participants were eligible for inclusion in the study, 9599 (8.2%) were excluded for missing covariates.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The NHS2 enrolled female registered nurses from 14 different states who ranged in age from 25 to 42 years at study entry. Data were collected from entry and biennial questionnaires, and analysis conducted from November 2020 to June 2021. Cox proportional hazard models were used to assess risk of CVD, hypertension (HTN), hypercholesterolemia (HC) and type 2 diabetes, both with and without genotyping for rs2016347.

MAIN RESULTS AND THE ROLE OF CHANCE: While women without endometriosis or PCOS, as a whole, demonstrated no impact of genotype on risk in either cohort, women with endometriosis carrying a T allele had a lower risk of CVD (hazard ratio (HR), 0.48; 95% CI, 0.27–0.86, $P = 0.02$) and HTN (HR, 0.80; 95% CI, 0.66–0.97, $P = 0.03$) in the pre-1989 cohort, while those in the post-1989 cohort had a decrease in risk for HC (HR, 0.76; 95% CI, 0.62–0.94, $P = 0.01$). Women with PCOS in the post-1989 cohort showed a significant protective impact of the T allele on HTN (HR, 0.44; 95% CI, 0.27–0.73, $P = 0.002$) and HC (HR, 0.62; 95% CI, 0.40–0.95, $P = 0.03$).

LIMITATIONS, REASONS FOR CAUTION: Data on specific endometriosis lesion locations or disease stage, as well as on PCOS phenotypes were lacking. In addition, data on systemic medical treatments beyond the use of oral contraceptives were missing, and these treatments may have confounded the results.

WIDER IMPLICATIONS OF THE FINDINGS: These findings implicate systemic dysregulation of the insulin-like growth factor-1 axis in the development of HTN, HC and clinical CVD in endometriosis and PCOS, suggesting a common underlying pathogenetic mechanism.

STUDY FUNDING/COMPETING INTEREST(S): The NHS2 infrastructure for questionnaire data collection was supported by National Institute of Health (NIH) grant U01CA176726. This work was also supported in part by NIH and National Cancer Institute grant U24CA210990; as well, research effort and publication costs were supported by the Elizabeth MA Stevens donor funds provided to the Buck Institute for Research on Aging. The authors declare they have no conflicts of interest.

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Introduction

Endometriosis and polycystic ovary syndrome (PCOS) are two of the most common conditions affecting women during their reproductive years. While differing in their pathogenesis, both conditions are associated with decreased fertility and increased later life risk of cardiovascular disease (CVD) as well as some of its precursor conditions: essential hypertension (HTN), hypercholesterolemia (HC) and type 2 diabetes (T2D). Interestingly, both of these reproductive disorders have also been linked to dysregulation of the insulin-like growth factor-1 (IGF-1) axis.

Endometriosis is a chronic estrogen-dependent disorder affecting up to 10% of US women in their reproductive years and is characterized by the presence of ectopic endometrial tissue which produces chronic pain, dysmenorrhea and infertility in 30% of cases (Burney and Giudice, 2012; Patel *et al.*, 2018; Bulun *et al.*, 2019). In addition, there is evidence of chronic systemic inflammation and vascular involvement with various studies reporting subclinical atherosclerosis, increased arterial stiffness, increased endothelial inflammatory markers and higher levels of low-density lipoprotein cholesterol (Melo et al., 2010; Kinugasa *et al.*, 2011; Santoro *et al.*, 2012, 2015; Tani *et al.*, 2015; Taskin *et al.*, 2019). Indeed, prior reports from the Nurses' Health Study 2 (NHS2) have shown that women with laparoscopically confirmed endometriosis experience significantly increased risks for later development of HTN, HC and ischemic cardiac and cerebrovascular diseases (Mu *et al.*, 2016, 2017). Potential dysregulation of the IGF-1 axis associated with endometriosis has been evidenced by increased IGF-1 levels in peritoneal washings, altered IGF-1 axis expression in ovarian endometriosis tissue, and higher pain levels in those with increased macrophage-derived IGF-1 expression (Kim *et al.*, 2000; Loverro *et al.*, 2001; Zhou *et al.*, 2016; Forster *et al.*, 2019).

PCOS is the most common endocrine disorder of women in their reproductive years and is characterized by hyperandrogenism, ovulatory dysfunction and cystic ovarian histology changes. The estimated US incidence of PCOS has ranged from 4% to 10%, in part due to changing diagnostic criteria over the past 30 years (El Hayek et al., 2016; Azziz, 2018). PCOS is associated with increased risk for CVD and its precursor conditions of HTN and HC, as well increased risk of T2D (Glueck et al., 2009; de Groot *et al.*, 2011; Mani *et al.*, 2013; Wekker *et al.*, 2020). PCOS tends to evolve through life with hyperandrogenism appearing in adolescence and largely resolving by age 40, and displays several different disease phenotypes each with distinct CVD risk profiles (Shroff *et al.*, 2007; Moran and Teede, 2009; Jovanovic *et al.*, 2010; Daskalopoulos *et al.*, 2015; Lizneva *et al.*, 2016; Tziomalos, 2016; Louwers and Laven, 2020).

Like the above two gynecologic diseases, conditions known as hypertensive disorders of pregnancy (HDP) have also been associated with increased later life risk of HTN and CVD and have similarly been linked to systemic dysregulation of the IGF-1 growth factor axis. Our prior population-based studies have shown that for women with HDP, later-life blood pressure as well as cancer risk, may be significantly reduced if they have also inherited a common functional variant of the IGF-1 receptor (*IGF1R*) gene (T allele for rs2016347, encoded within its 3 prime untranslated region (3′UTR)) that is known to reduce its transcript levels across multiple human tissues including coronary and peripheral arteries, heart muscle, pancreas and ovaries (Prebil *et al.*, 2014; Powell *et al.*, 2017; Powell *et al.*, 2020; GTEx portal 2021).

Materials and methods

Study population

The current retrospective cohort study was performed in the NHS2, which enrolled 116 430 registered female nurses in 1989 who ranged in age from 25 to 42 and resided in 14 different states. Extensive data were collected from original detailed entry questionnaires as well as subsequent biennial questionnaires through 2015; details describing the NHS2 and its numerous contributions to medical research are well-documented (Bao *et al.*, 2016; Chavarro *et al.*, 2016; Colditz *et al.*, 2016). This study excluded 9599 participants with missing covariates, leaving a final analytic sample of 106 831 women (flowcharts of study numbers are presented in Supplementary Figs S1 and S2). A subset of participants provided blood and buccal samples for genotyping providing information for our *IGF1R* single-nucleotide polymorphism (SNP) of interest, rs2016347. The NHS2 study protocol was approved by the Institutional Review Board (IRB) of the Brigham and Women's Hospital, which allowed participants' completion of questionnaires as implied consent.

Assessment of exposures and outcomes

In 1993, NHS2 participants were asked if they had 'physician-diagnosed endometriosis' and if yes, they were asked if it had been confirmed by laparoscopy. In a previously reported validation of self-reported endometriosis using medical records in NHS2 participants, 96% of those who reported laparoscopic confirmation were confirmed by medical records review, versus only 54% of participants who self-reported endometriosis without reporting laparoscopic confirmation (Missmer *et al.*, 2004). Consequently, 3304 participants who reported endometriosis without laparoscopic confirmation were censored at their report of endometriosis.

If the participant answered yes on the 1993 questionnaire, they were then asked during which time period the diagnosis occurred:

before 1989, and in 2-year subsequent ranges. The time of endometriosis diagnosis was taken at the midpoint of these ranges or was set for 1989 for those who reported the diagnosis as 'before 1989'. There were 12 804 women who reported laparoscopically confirmed endometriosis and comprised the exposure set. This endometriosis exposure group was analyzed in its entirety, and divided into two time period eras consisting of all those diagnosed prior to study entry (pre-1989 cohort), n = 5678, and those diagnosed after study entry (post-1989 cohort), n = 7126.

On NHS2 entry and follow-up questionnaires participants were asked if they had ever been diagnosed with PCOS, and midpoints of time intervals were used to set diagnosis dates. To minimize possible misclassification resulting from using self-reported PCOS alone, participants in our study had to also report at least one of the major symptoms of PCOS: hirsutism, infertility or significantly irregular menstrual cycles. Hirsutism was asked directly, infertility was defined as trying to become pregnant for 1 year without success, and significantly irregular menstrual cycles were defined as 'usually irregular' or 'always irregular' in high school or between ages 18–22. Using this stricter definition, 4661 of the 11 928 participants who had self-reported PCOS were censored at their report of PCOS, leaving 7267, or 6.8% of all participants meeting our study definition of PCOS. This included 9.2% with the symptom of hirsutism, 32.9% with infertility, 47.1% who solely reported significant menstrual irregularities and 10.8% with more than one of these symptoms.

Medical outcomes were self-reported except for myocardial infarction (MI) and T2D, which were validated using hospital or medical records. CVD was defined as the first event of confirmed MI, self-reported angiographically confirmed angina or self-reported coronary artery bypass graft/angioplasty/stent placement between enrollment and the last available questionnaire (2015), resulting in 2908 CVD cases.

Both HTN and HC were self-reported on the entry questionnaire in 1989 and biennially through 2015. A total of 46 936 women self-reported HTN and 63 291 women self-reported HC. Participants who self-reported T2D had their diagnosis confirmed using hospital/medical records yielding 8184 confirmed T2D cases.

DNA samples and genotyping

To obtain NHS2 genetic data, blood and buccal samples were collected from 29 611 and 29 392 participants, respectively from which DNA was extracted using Qiagen PureGene DNA Isolation Kits (Gentra Systems, Minneapolis, MN, USA). Genotyping of rs2016347 was determined from at least one of five different DNA analysis platforms (Lindström et al., 2017). Of these, four required imputing rs2016347 genotype while only OncoArray used direct genotyping. In four women with conflicting genotypes between OncoArray and an imputation method, OncoArray was favored. Two women had inconsistent results between two imputation methods and were dropped from the genetic analyses. Genotypes of rs2016347 among all NHS2 participants were in Hardy–Weinberg equilibrium ($P = 0.06$). After exclusions for missing covariates, 8170 participants with genotyping remained and comprised the study subset for the genetic analyses. Reported population T allele frequencies are 0.57 for European, 0.52 for East Asian and 0.30 for African. Of note, participants in our genetic analyses were 98% White and had an overall T allele frequency of 0.52, consistent with the 0.51 T allele frequency seen in White women of the California Teachers' Study (dbSNP 1000genomes 2020; Powell *et al.*, 2017).

Genetic model selection

In an effort to maximize statistical power and address potential multiple testing issues related to analyses of multiple genetic statistical models for each exposure, outcome, and cohort, we focused our analyses solely on the recessive genetic model. Prior published studies of the association of rs2016347 with disease outcomes demonstrated that the guanine/thymine (GT) genotype tracks most closely to the thymine/thymine (TT) genotype (Powell *et al.*, 2013; Prebil *et al.*, 2014; Winder *et al.*, 2014; de Groot *et al.*, 2016; Powell *et al.*, 2020). Therefore, as done in our prior population study examining the impact of rs2016347 on risk of CVD in women with HDP, our current analyses focused solely on the recessive genetic model, which compares participants carrying at least one T allele (TT and GT genotypes combined) with the reference group of guanine/guanine (GG) carriers (Powell *et al.*, 2020).

Statistical analysis

Cox proportional hazards analyses using time-on-study as the time scale were used to calculate hazard ratios (HRs) and 95% CI between each of the exposure cohorts and outcomes. Participants with outcomes prior to exposure were dropped from the relevant analyses. Individual person-years extended from study entry until final questionnaire completion, year of diagnosis with each outcome, lost-to-follow-up or death, whichever came first. Models examining the hazard of developing each outcome for endometriosis and PCOS were adjusted for age at study entry, ethnicity, BMI, smoking history, parity, age at menarche, family history of each outcome, history of uterine fibroids, gynecologic surgeries, oral contraceptive use in months, diet as Alternative Healthy Eating Index 2010 score and physical activity calculated in metabolic equivalents/week and were calculated for all participants with each exposure and then for the pre-1989 and post-1989 cohorts alone. The number of missing values for each covariate was relatively low with a missing value in only 9599 participants (8.2% of total) who were excluded from all analyses.

Assessment of the proportional hazards assumptions for large samples was evaluated using model-based analyses (Austin, 2018). All analyses were carried out using R version 4.0.1 'See Things Now' (R Core Team, 2020). The 'survival' and 'stats' packages were used to estimate HRs and 95% CIs (Therneau and Grambsch, 2000; Therneau, 2020). All graphics were created using 'ggplot2' (Wickham, 2016).

Results

Participant characteristics

Characteristics of study participants by exposure status are presented in Table I. For endometriosis, there were many minor differences consistent with previously reported characteristics (Parasar *et al.*, 2017). However, most notable was a difference in parity, 1.60 for those with endometriosis compared to 2.01 for those without, as well as a large

Table I Characteristics of study participants by exposure status.

Characteristic	Endometriosis exposure			PCOS exposure		
	Endo + (n = 12 804)	Endo – (n = 103 878)	*P*-value[a]	PCOS + (n = 7267)	PCOS – (n = 109 410)	*P*-value[a]
Age at entry, mean (SD)	34.4 (4.6)	34.4 (4.7)	0.879	34.7 (4.6)	34.3 (4.7)	<0.001
Ethnicity (%)			<0.001			<0.001
White	11 983 (93.7)	95 619 (92.3)		6779 (93.5)	100 818 (92.4)	
Black	171 (1.3)	1930 (1.9)		109 (1.5)	1992 (1.8)	
Asian	150 (1.2)	1922 (1.9)		57 (0.8)	2015 (1.8)	
Other	484 (3.8)	4153 (4.0)		309 (4.3)	4328 (4.0)	
BMI at entry, mean (SD)	23.9 (4.8)	24.1 (5.1)	<0.001	25.2 (6.0)	24.0 (5.0)	<0.001
Age at menarche (%)			<0.001			<0.001
<12	3564 (27.9)	25 105 (24.2)		1903 (26.3)	26 766 (24.5)	
12–13	7145 (56.0)	59 624 (57.6)		3871 (53.4)	62 893 (57.7)	
14+	2052 (16.1)	18 798 (18.2)		1471 (20.3)	19 379 (17.8)	
Parity, mean (SD)	1.60 (1.25)	2.01 (1.31)	<0.001	1.68 (1.28)	1.98 (1.31)	<0.001
OC use in months (%)			<0.001			<0.001
<6	1854 (14.5)	19 671 (18.9)		1030 (14.2)	20 493 (18.7)	
6–35	5074 (39.6)	38 525 (37.1)		3194 (44.0)	40 404 (36.9)	
36+	5876 (45.9)	45 682 (44.0)		3043 (41.9)	48 513 (44.3)	
Smoking history (%)			0.309			<0.001
Never	8254 (64.7)	67 390 (65.1)		4373 (60.4)	71 269 (65.4)	
Current or past	4550 (35.3)	36 488 (34.9)		2894 (39.6)	38 141 (34.6)	
Physical activity,[b] mean (SD)	22.3 (21.8)	23.0 (23.3)	0.001	22.5 (23.4)	23.0 (23.1)	0.076
Hysterectomy only (%)	1788 (14.0)	11 170 (10.8)	<0.001	867 (11.9)	12 091 (11.1)	0.022
BSO only (%)	180 (1.4)	1098 (1.1)	<0.001	111 (1.5)	1167 (1.1)	<0.001
Hysterectomy and BSO (%)	5772 (45.1)	14 149 (13.6)	<0.001	2556 (35.2)	17 364 (15.9)	<0.001

BSO, bilateral salpingo-oophorectomy; OC, oral contraceptive; PCOS, polycystic ovary syndrome.
[a]Hypothesis testing using the Chi-squared tests for categorical variables and *t*-tests for continuous variables.
[b]Physical activity in metabolic equivalents (METS) per week from recreation and leisure time.

difference between those receiving gynecologic surgeries: 45.1% with endometriosis receiving hysterectomy and bilateral salpingo-oophorectomy (BSO) versus 13.6% of women without.

Women with PCOS were older at study entry, more likely to be White, less likely to have been a smoker, used more oral contraceptives and had a lower parity than women without PCOS. They also had a slightly higher mean BMI at study entry, 25.16 vs 24.03; and most notably, there was also an increase in gynecologic surgeries, with 35.2% having had hysterectomy and BSO compared to 15.9% of women without PCOS.

Characteristics of study participants by cohort era are presented in Table II for endometriosis and PCOS. When comparing pre-1989 to post-1989 eras for women with endometriosis, those in the later cohort were younger at study entry, had a later age at menarche, higher parity, exercised more, smoked less and more underwent hysterectomy. As expected, the later cohort women with endometriosis had an older age at diagnosis and a shorter interval between diagnosis and outcome.

Women in the PCOS post-1989 cohort were slightly younger at study entry, less likely to have been a smoker and had a slightly lower BMI when compared with those in the pre-1989 cohort. Most significantly, they were diagnosed with PCOS at a mean age of 39.5 compared to a mean age at diagnosis of 29.3 in the pre-1989 cohort. This is at least partly due to the diagnosis coming at least 2 years after study entry in 1989 when their mean age would be 36.3, but may also reflect the infertility issues that arise in professional women who delay childbirth leading to a comprehensive work-up and possible PCOS diagnosis at a later age. Of note, the interval between their PCOS diagnosis and the studied outcomes was 5–9 years shorter than that of women in the pre-1989 cohort.

Subsequent cardiovascular risks in women diagnosed with endometriosis or PCOS

As shown in Fig. 1 and when not considering rs2016347 genotype status, women diagnosed with endometriosis had increased risk of developing CVD (HR, 1.45; 95% CI, 1.27–1.65, $P = <0.001$), HTN (HR, 1.11; 95% CI, 1.07–1.15, $P = <0.001$) and HC (HR, 1.17; 95% CI, 1.13–1.20, $P = <0.001$), but no significant increased risk of developing T2D relative to those without endometriosis (HR, 1.03, 95% CI,

Table II Characteristics of study participants by exposure cohort.

Characteristic	Endometriosis exposure			PCOS exposure		
	Pre-1989 (n = 5678)	Post-1989 (n = 7126)	*P*-value[a]	Pre-1989 (n = 4903)	Post-1989 (n = 2364)	*P*-value[a]
Age at entry, mean (SD)	35.6 (4.2)	33.4 (4.6)	<0.001	35.1 (4.5)	33.9 (4.6)	<0.001
Ethnicity (%)			0.073			0.331
White	5323 (93.9)	6660 (93.5)		4577 (93.6)	2202 (93.2)	
Black	73 (1.3)	98 (1.4)		69 (1.4)	40 (1.7)	
Asian	51 (0.9)	99 (1.4)		33 (0.7)	24 (1.0)	
Other	220 (3.9)	264 (3.7)		212 (4.3)	97 (4.1)	
BMI at entry, mean (SD)	23.9 (4.7)	23.9 (4.9)	0.488	25.3 (6.0)	24.9 (5.7)	0.017
Age at menarche (%)			0.001			0.323
<12	1664 (29.4)	1900 (26.8)		1298 (26.6)	605 (25.7)	
12–13	3147 (55.6)	3998 (56.3)		2582 (52.8)	1289 (54.7)	
14+	851 (15.0)	1201 (16.9)		1008 (20.6)	463 (19.6)	
Parity, mean (SD)	1.48 (1.23)	1.69 (1.27)	<0.001	1.66 (1.28)	1.71 (1.28)	0.076
OC use in months (%)			<0.001			<0.001
<6	687 (12.1)	1167 (16.4)		648 (13.2)	382 (16.2)	
6–35	2429 (42.8)	2645 (37.1)		2219 (45.3)	975 (41.2)	
36+	2562 (45.1)	3314 (46.5)		2036 (41.5)	1007 (42.6)	
Smoking history (%)			<0.001			0.009
Never	3533 (62.4)	4721 (66.5)		2897 (59.3)	1476 (62.6)	
Current or past	2145 (37.6)	2405 (33.5)		2006 (40.7)	888 (37.4)	
Physical activity,[b] mean (SD)	21.3 (20.2)	23.1 (23.0)	<0.001	22.2 (23.6)	23.1 (22.9)	0.094
Hysterectomy only (%)	697 (12.3)	1091 (15.3)	<0.001	571 (11.6)	296 (12.5)	0.298
BSO only (%)	86 (1.5)	94 (1.3)	0.391	73 (1.5)	38 (1.6)	0.776
Hysterectomy and BSO (%)	2539 (44.7)	3233 (45.4)	0.472	1745 (35.6)	811 (34.3)	0.295
Age at diagnosis, mean (SD)	<35.6 (4.2)[c]	41.9 (7.2)	<0.001	29.3 (5.1)	39.5 (5.5)	<0.001
Diagnosis interval (years), mean (SD)						
CVD	12.8 (7.3)	9.1 (6.4)	<0.001	16.7 (9.6)	10.0 (6.2)	<0.001
Hypertension	13.4 (6.7)	9.2 (6.3)	<0.001	17.1 (9.1)	10.0 (5.9)	<0.001
Hypercholesterolemia	11.2 (7.1)	8.1 (6.1)	<0.001	14.0 (8.9)	8.9 (6.0)	<0.001
Type 2 diabetes mellitus	16.5 (6.5)	10.1 (6.2)	<0.001	20.9 (7.7)	11.5 (6.8)	<0.001

BSO, bilateral salpingo-oophorectomy; CVD, cardiovascular disease; OC, oral contraceptive.
[a]Hypothesis testing using the Chi-squared tests for categorical variables and *t*-tests for continuous variables.
[b]Physical activity in metabolic equivalents (METS) per week from recreation and leisure time.
[c]No information is available as to the diagnosis year of participants diagnosed with endometriosis prior to 1989, thus diagnosis year was set to year of study entry (1989).

0.96–1.11, $P = 0.43$). When comparing these HR risks between the two endometriosis cohorts, there were minimal HR differences except for CVD risk, which was not significantly increased in the post-1989 cohort (HR, 1.05, 95% CI, 0.87–1.27, $P = 0.49$).

Also shown in Fig. 1, for all women with PCOS there was an increase in the risk for all outcomes: CVD (HR, 1.71; 95% CI, 1.52–1.93, $P < 0.001$), HTN (HR, 1.09; 95% CI, 1.05–1.13, $P < 0.001$), HC (HR, 1.16; 95% CI, 1.12–1.20, $P < 0.001$) and T2D (HR, 1.34; 95% CI, 1.25–1.45, $P < 0.001$). HR risks for CVD and HC were very similar in the two PCOS cohorts, but differed for HTN (HR, 1.15; 95% CI, 1.10–1.20, $P < 0.001$) in the pre-1989 cohort relative to HTN (HR, 0.94, 95% CI, 0.88–1.01, $P = 0.94$) in the post-1989 cohort, as well as for HC where it was increased (HR, 1.30; 95% CI, 1.25–1.35, $P < 0.001$) in the pre-1989 cohort but decreased (HR, 0.86; 95% CI, 0.80–0.92, $P < 0.001$) in the post-1989 cohort.

Association of *IGF1R* rs2016347 genotype with study exposures and outcomes

The association of rs2016347 genotype with each study exposure and outcome is presented in Table III. When looking at the Chi-squared-tests, the recessive model (TT/GT genotype compared to GG reference group) was not associated with endometriosis, PCOS, CVD, HTN, HC or T2D. Cox proportional hazard models also did not show any effect of genotype on studied outcomes in women without endometriosis or PCOS and are presented in Supplementary Fig. S3.

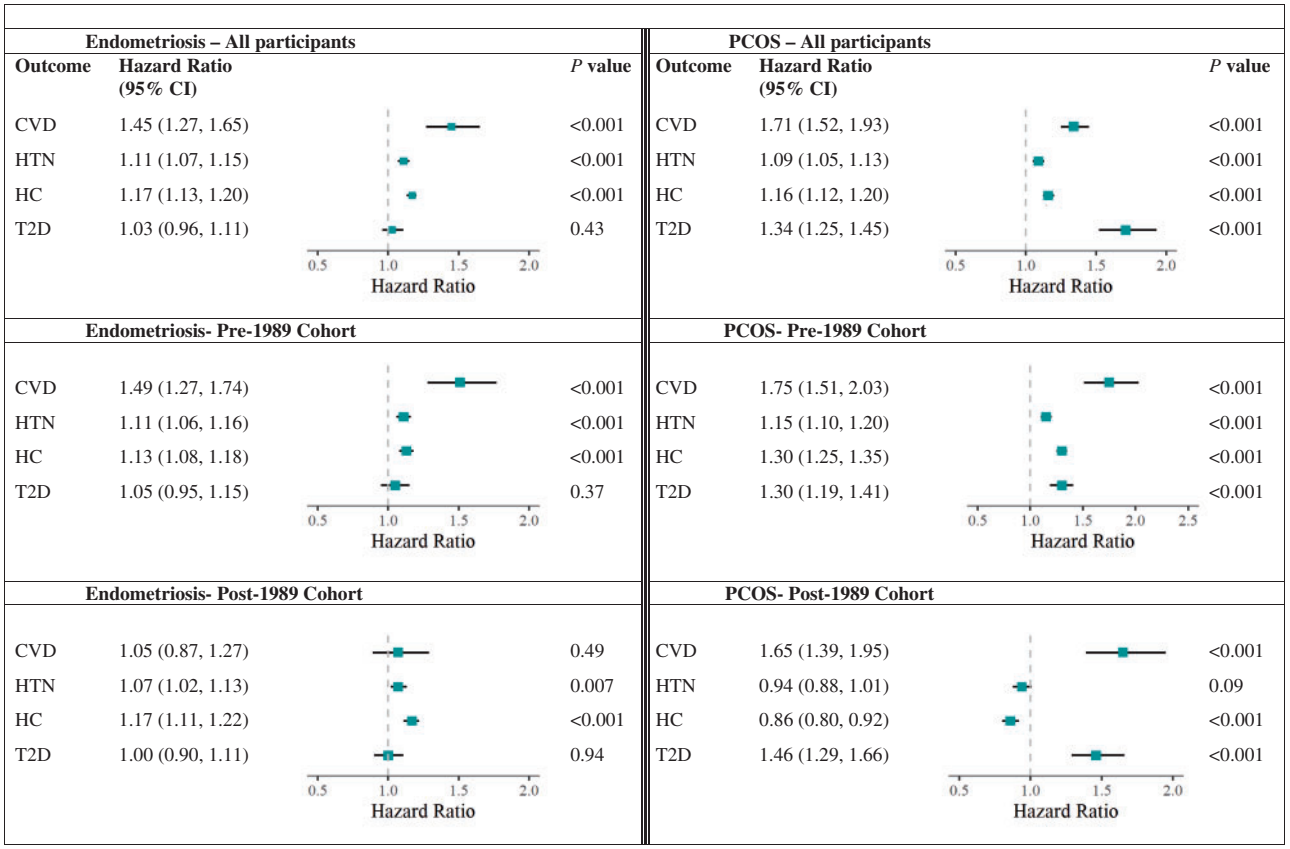


Figure 1. Outcome risk for endometriosis and polycystic ovary syndrome (PCOS) for all participants and by cohort. Result plotted as box with line representing 95% CI. Number of outcomes: CVD = 2120, HTN = 46 532, HC = 62 568, T2D = 8145. CVD, cardiovascular disease; HC, hypercholesterolemia; HTN, hypertension; T2D, type 2 diabetes.

Cardiovascular risks for endometriosis and PCOS cohorts stratified by rs2016347 genotype

The results of cardiovascular outcome risks stratified by rs2016347 genotype status for NHS2 women diagnosed with either endometriosis or PCOS are presented in Figs 2 and 3, respectively. For all women with endometriosis, those carrying at least one T allele (recessive model) had a lower risk of HC (HR, 0.86; 95% CI, 0.76–0.98, $P = 0.02$), but no significant change in their risk of CVD, HTN or T2D. However, when looking by cohort and as shown in Fig. 2, women with endometriosis in the pre-1989 cohort carrying at least one T allele had a decreased risk for CVD (HR, 0.48; 95% CI, 0.27–0.86, $P = 0.02$) and HTN (HR, 0.80; 95% CI, 0.66–0.97, $P = 0.03$) while those in the post-1989 cohort had a decrease in their risk for HC (HR, 0.76; 95% CI, 0.62–0.94, $P = 0.01$). The interaction term (Endometriosis*T allele) *P*-values were 0.01, 0.03 and 0.04, respectively.

While analysis of all NHS2 women with PCOS did not reveal any significant protective impact by carrying the rs2016347 T allele on either CVD, HTN, HC or T2D risk, cohort analysis as shown in Fig. 3 suggested otherwise. Whereas no significant impact of rs2016346 genotype on cardiovascular outcome risks was apparent for women with PCOS in the pre-1989 cohort, those diagnosed with PCOS in the post-1989 cohort showed a significant protective impact of the T allele on HTN (HR, 0.44; 95% CI, 0.27–0.73, $P = 0.002$) and HC (HR, 0.62; 95% CI, 0.40–0.95, $P = 0.03$). The interaction term (PCOS*T allele) *P*-values were 0.03 and 0.11, respectively.

Due to small sample sizes and results of prior rs2016347 studies, the authors chose to concentrate on the impact of carrying a T allele in their analyses. Results demonstrating the impact of the GT genotype and the TT genotype each compared to the reference GG genotype for all models are presented in Supplementary Tables SI and SII. In most of the analyses with larger sample sizes, the GT genotype does track closely to the TT genotype, however, it is possible that larger numbers may have supported the use of an additive model.

Discussion

This study reveals a lower later-life risk of developing CVD-related outcomes, including HTN, HC and clinical CVD events, in women with a diagnosis of endometriosis or PCOS that also inherit the

Table III Distribution of rs2016347 genotypes in study exposures and outcomes.

	GG genotype No. (%)	GT/TT genotype No. (%)	*P*-value[a]
Exposure			
Endometriosis +	619 (23.6)	2092 (76.4)	0.45
Endometriosis −	1287 (22.8)	4172 (77.2)	
PCOS +	192 (24.0)	607 (76.0)	0.62
PCOS −	1714 (23.3)	5657 (76.7)	
Outcome			
Cardiovascular disease +	53 (20.9)	200 (79.1)	0.36
Cardiovascular disease −	1853 (23.4)	6064 (76.6)	
Hypertension +	848 (23.0)	2727 (77.0)	0.54
Hypertension −	1058 (23.0)	3537 (77.0)	
Hypercholesterolemia +	1254 (23.8)	4012 (76.2)	0.16
Hypercholesterolemia −	652 (22.5)	2252 (77.5)	
Type 2 diabetes +	153 (23.0)	511 (77.0)	0.86
Type 2 diabetes −	1753 (23.4)	5753 (76.6)	

GG, guanine/guanine; GT, guanine/thymine; PCOS, polycystic ovary syndrome; TT, thymine/thymine.
[a]*P*-values are for the Chi-square test for independence between genotype and exposure/outcome for recessive genetic model.

functionally blunted T allele variant of *IGF1R* gene, rs2016347. Consistent with prior population studies demonstrating the protective effect of inheriting a T allele on later-life blood pressure and cancer risk in women with HDP exposure, the present study offers new evidence implicating systemwide dysregulation of the IGF-1 axis in the later-life CVD risk experienced by women exposed during their reproductive years to the seemingly unrelated gynecologic disorders, endometriosis and PCOS. Despite presumed differences in their pathogenetic bases, these epidemiologic findings suggest a shared pathogenetic link that involves dysregulation of the IGF-1 growth factor receptor system.

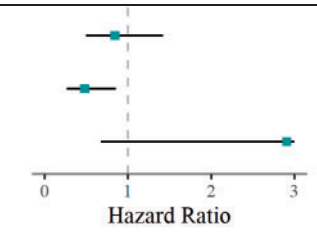
Our current non-genetic findings (i.e. independent of rs2016347 genotype) confirm prior NHS2 and non-NHS2 population studies showing that both endometriosis and PCOS predispose to increased risks of developing HTN, HC and CVD; as well, PCOS but not endometriosis also predisposes to T2D (Fig. 1). For endometriosis, our latest findings are consistent with prior NHS2 studies (Tobias, 2015; Mu *et al.*, 2016, 2017). Outcomes following self-reported PCOS have not previously been reported from the NHS2 study population due to potential misclassification issues; but the additional inclusion of a known PCOS-associated symptom as a diagnostic requirement served to mitigate potential misclassification.

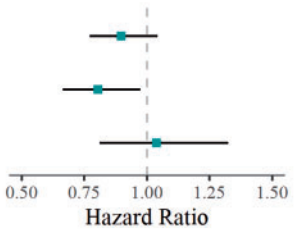
Our sub-analysis of all NHS2 women having endometriosis or PCOS by their diagnostic cohort era is novel and was motivated by known changes in diagnostic techniques and disease classification criteria occurring over the 50 years from when many participants in the NHS2 reached the age of 18 until the end of their follow-up period. In addition, participants in the two cohorts also differ in characteristics such as parity, smoking history and oral contraceptive usage, which may contribute to the varying impact of genotype on CVD risk. Lesions in women with endometriosis are generally thought to progress over time, and women diagnosed after age 26 have more advanced stages of this disease (Burney and Giudice, 2012; Stochino-Loi *et al.*, 2020). In addition to our observed T allele association with decreased risk of CVD (HR, 0.48; 95% CI, 0.27–0.86, $P = 0.02$) and HTN (HR, 0.80; 95% CI, 0.66–0.97, $P = 0.03$) in women with endometriosis restricted to the pre-1989 cohort, there was strong evidence of interaction for both findings, $P = 0.01$ and 0.03, respectively. This suggests that women in the pre-1989 cohort may have been diagnosed in a milder stage of their disease, which could make them more sensitive to the protective impact of inheriting the rs2016347 T allele, as observed.

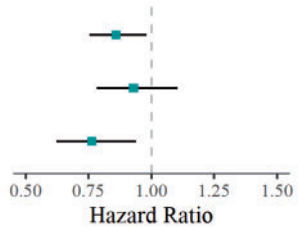
PCOS diagnosis and classification have gone through multiple changes since the NHS2 began, including the establishment of National Institutes of Health (NIH) criteria in April 1990, establishment of the Rotterdam criteria in 2003, and implementation of the Androgen Excess and PCOS Society criteria in 2006 (Azziz *et al.*, 2009; Lizneva *et al.*, 2016). In addition, women with PCOS are now known to have distinct phenotypes based on their diagnostic criteria and demonstrate clinical differences as they age, with less reproductive symptoms but more metabolic manifestations that enhance future cardiovascular risk (Głuszak et al., 2012; Aziz *et al.*, 2015; Bellver *et al.*, 2018; Louwers and Laven, 2020). These age-related changes could feasibly account for the observed decrease in HTN and HC risks seen in women with PCOS in the post-1989 cohort that carry the T allele of rs2016347. In that post-1989 cohort, the reduced risk for HTN (HR, 0.44; 95% CI, 0.27–0.73, $P = 0.002$) showed an interaction *P*-value of 0.03, while the reduced risk for HC (HR, 0.62; 95% CI, 0.40–0.95, $P = 0.03$) showed an interaction *P*-value of 0.11.

Clearly, more research is needed to posit a mechanistic explanation for these epidemiologic findings. Tantalizing molecular and cellular observations relating to the IGF-1 axis provide disease-specific clues but no complete or unifying mechanistic explanation. Expression of *IGF1R* at the post-transcriptional level in endometrial tissue is decreased in women with endometriosis, resulting from the subcellular expression of a long noncoding RNA on let-7 microRNA (Ghazal *et al.*, 2015). Similarly, in PCOS the expression of *IGF1R* has been shown to be decreased in the cumulus cells of mature oocytes (Kenigsberg *et al.*, 2009; Haouzi *et al.*, 2012). Also of interest, *IGF1R* is downregulated in early-onset pre-eclampsia by overexpression of microRNA-320a (Liao *et al.*, 2021). At the cellular level, endothelial dysfunction has been frequently reported in association with endometriosis and PCOS as well as with pre-eclampsia; and this suggests they share systemwide vascular system dysregulation predisposing to their common later-life risk of developing CVD (Diamanti-Kandarakis *et al.*, 2006; Powe *et al.*, 2011; Santoro *et al.*, 2012; Bañuls *et al.*, 2017). A key subcellular determinant of endothelial function is nitric oxide (NO) production, and notably, *IGF1R* expression may play a significant role here with reduced endothelial expression enhancing NO bioavailability; likewise, mouse models have confirmed that decreased *IGF1R* expression increases NO bioavailabilty (Abbas *et al.*, 2011; Imrie *et al.*, 2012; Cyr *et al.*, 2020). *IGF1R* expression also regulates immune and inflammatory cell responses, with inhibition reducing systemic inflammation in mouse models (Erlandsson *et al.*, 2017; Li *et al.*, 2018). The studied SNP is in tight linkage disequilibrium with two other functional SNPs in the 3′UTR of IGF1R, two of which create miRNA binding sites, and studies are underway to

Cohort	N	Hazard Ratio (95% CI)		P value[a]	P value for interaction[b]
Cardiovascular Disease					
All	76	0.84 (0.50, 1.42)		0.52	0.61
Pre-1989	51	0.48 (0.27, 0.86)		0.01	0.01
Post-1989	25	2.91 (0.68, 12.46)		0.15	0.17
Hypertension					
All	971	0.90 (0.77, 1.04)		0.16	0.13
Pre-1989	564	0.80 (0.66, 0.97)		0.03	0.03
Post-1989	407	1.04 (0.81, 1.32)		0.77	0.98
Hypercholesterolemia					
All	1209	0.86 (0.75, 0.98)		0.02	0.12
Pre-1989	725	0.93 (0.78, 1.10)		0.40	0.98
Post-1989	484	0.76 (0.62, 0.94)		0.01	0.04
Type 2 Diabetes					
All	197	1.35 (0.93, 1.95)		0.11	0.04
Pre-1989	108	1.24 (0.77, 2.02)		0.38	0.14
Post-1989	89	1.54 (0.86, 2.74)		0.15	0.09







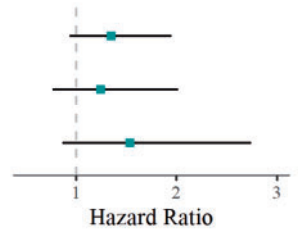
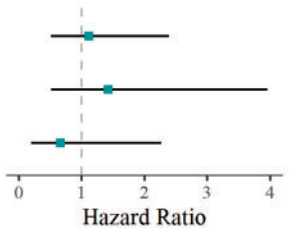


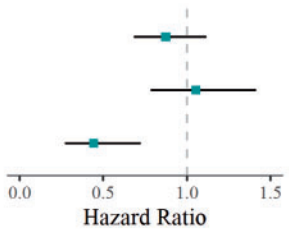
Figure 2. Outcome risk for endometriosis by rs2016347 genotype and cohort. Result plotted as box with line representing 95% CI. [a]Outcomes for recessive genetic model of *IGF1R* SNP rs2016347. [b]*P*-value for interaction term endometriosis*T allele (TT or GT genotype).

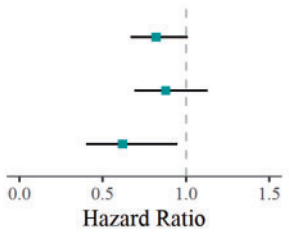
determine which of these SNPs play key physiologic roles. These mechanistic links may explain our epidemiologic observations of mitigated CVD risks in women whose reproductive disorders put them at increased CVD risk but who otherwise inherit a functionally blunted *IGF1R* gene variant.

This analysis has several limitations. We did not have data on specific endometriosis lesion locations or disease stage, as well as on PCOS phenotypes. In addition, we did not have information on systemic medical treatments of the underlying conditions beyond the use of oral contraceptives, and these treatments may have confounded the results, although it is unclear how this would have impacted the genetic analyses. There was also a relatively low number of NHS2 participants that had been genotyped, which resulted in our analyses for CVD and T2D being underpowered. In addition, the relatively low number of genotyped participants could have biased the results in an undefinable manner. Although NHS2 inclusion of only registered nurses may have improved self-reported symptom and disease accuracy, it does raise issues of

Cohort	N	Hazard Ratio (95% CI)	P value[a]	P value for interaction[b]
Cardiovascular Disease				
All	38	1.11 (0.52, 2.39)	0.79	0.83
Pre-1989	22	1.42 (0.51, 3.96)	0.50	0.61
Post-1989	16	0.66 (0.19, 2.26)	0.51	0.82
Hypertension				
All	338	0.87 (0.68, 1.12)	0.28	0.23
Pre-1989	242	1.05 (0.78, 1.42)	0.73	0.92
Post-1989	96	0.44 (0.27, 0.73)	0.002	0.03
Hypercholesterolemia				
All	491	0.82 (0.67, 1.01)	0.07	0.09
Pre-1989	365	0.88 (0.69, 1.13)	0.32	0.26
Post-1989	126	0.62 (0.40, 0.95)	0.03	0.11
Type 2 Diabetes				
All	90	1.30 (0.78, 2.16)	0.31	0.48
Pre-1989	54	1.30 (0.56, 3.05)	0.54	0.65
Post-1989	36	1.54 (0.86, 2.74)	0.15	0.58







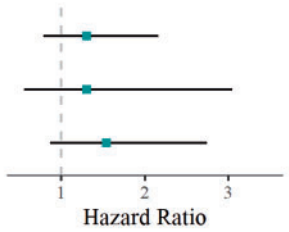


Figure 3. Outcome risk for PCOS by rs2016347 genotype and cohort. Result plotted as box with line representing 95% CI. [a]Outcomes for recessive genetic model of *IGF1R* SNP rs2016347. [b]*P*-value for interaction term endometriosis*T allele (TT or GT genotype).

generalizability, as the study population for the genetic analyses was 98% White, were all highly educated, and had a relatively low incidence of obesity.

Conclusions

Previously described cardiovascular risk increases in women with endometriosis and PCOS, two of the most common disorders of women of reproductive age, are shown to be significantly reduced in women who carry a common T allele variant of *IGF1R* (SNP rs2016347), with the magnitude of risk reduction dependent on cohort eras that reflect different diagnostic criteria, mean ages at diagnosis, and changing medical definition of disease stages and phenotypes. The inherited T allele has been shown to reduce *IGF1R* gene expression in key tissues, and this may offset pathogenetic alterations in the IGF-1 axis suspected to link these two early-life gynecologic conditions with increased later-life CVD risk.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

NHS2 data are available to researchers by external collaborator request. The data underlying this article will be shared on reasonable request to the corresponding author. All code needed to reproduce the analyses is available on Github at https://github.com/sfuller2/endo-analysis.

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Authors' roles

M.J.P.: study design and coordination, data access, manuscript preparation. S.F.: data and statistical analysis, manuscript draft preparation. E.P.G.: study design, study methodology, critical revision of manuscript. C.C.B.: study design, interpretation of data, manuscript preparation. All authors read and approved the manuscript for submission.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

Abbas A, Imrie H, Viswambharan H, Sukumar P, Rajwani A, Cubbon RM, Gage M, Smith J, Galloway S, Yuldeshava N *et al.* The insulin-like growth factor-1 receptor is a negative regulator of nitric oxide bioavailability and insulin sensitivity in the endothelium. *Diabetes* 2011;**60**:2169–2178.

Austin PC. Statistical power to detect violation of the proportional hazards assumption when using the Cox regression model. *J Stat Comput Simul* 2018;**88**:533–552.

Aziz M, Sidelmann JJ, Faber J, Wissing M-LM, Naver KV, Mikkelsen A-L, Nilas L, Skouby SO. Polycystic ovary syndrome: cardiovascular risk factors according to specific phenotypes. *Acta Obstet Gynecol Scand* 2015;**94**:1082–1089.

Azziz R. Polycystic ovary syndrome. *Obstet Gynecol* 2018;**132**:321–336.

Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE *et al.*; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* 2009;**91**:456–488.

Bañuls C, Rovira-Llopis S, Martinez de Marañon A *et al.* Metabolic syndrome enhances endoplasmic reticulum, oxidative stress and leukocyte-endothelium interactions in PCOS. *Metabolism* 2017;**71**:153–162.

Bao Y, Bertoia ML, Lenart EB, Stampfer MJ, Willett WC, Speizer FE, Chavarro JE. Origin, methods, and evolution of the three Nurses' Health Studies. *Am J Public Health* 2016;**106**:1573–1581.

Bellver J, Rodríguez-Tabernero L, Robles A, Muñoz E, Martínez F, Landeras J, García-Velasco J, Fontes J, Álvarez M, Álvarez C *et al.*; Group of interest in Reproductive Endocrinology (GIER) of the Spanish Fertility Society (SEF). Polycystic ovary syndrome throughout a woman's life. *J Assist Reprod Genet* 2018;**35**:25–39.

Bulun SE, Yilmaz BD, Sison C, Miyazaki K, Bernardi L, Liu S, Kohlmeier A, Yin P, Milad M, Wei J *et al.* Endometriosis. *Endocr Rev* 2019;**40**:1048–1079.

Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* 2012;**98**:511–519.

Chavarro JE, Rich-Edwards JW, Gaskins AJ, Farland LV, Terry KL, Zhang C, Missmer SA. Contributions of the Nurses' Health Studies to reproductive health research. *Am J Public Health* 2016;**106**:1669–1676.

Colditz GA, Philpott SE, Hankinson SE. The impact of the Nurses' Health Study on population health: prevention, translation, and control. *Am J Public Health* 2016;**106**:1540–1545.

Cyr AR, Huckaby LV, Shiva SS, Zuckerbraun BS. Nitric oxide and endothelial dysfunction. *Crit Care Clin* 2020;**36**:307–321.

Daskalopoulos G, Karkanaki A, Piouka A, Prapas N, Panidis D, Gkeleris P, Athyros VG. Excess metabolic and cardiovascular risk is not manifested in all phenotypes of polycystic ovary syndrome: implications for diagnosis and treatment. *Curr Vasc Pharmacol* 2015;**13**:788–800.

dbSNP, 1000Genomes rs2016347. NIH National Center for Biotechnology Information, 2020. https://www.ncbi.nlm.nih.gov/snp/rs2016347#frequency_tab (May 2021, date last accessed).

de Groot PC, Dekkers OM, Romijn JA, Dieben SW, Helmerhorst FM. PCOS, coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis. *Hum Reprod Update* 2011;**17**:495–500.

de Groot S, Charehbili A, van Laarhoven HWM, Mooyaart AL, Dekker-Ensink NG, van de Ven S, Janssen LGM, Swen JJ, Smit VTHBM, Heijns JB *et al.*; Dutch Breast Cancer Research Group. Insulin-like growth factor 1 receptor expression and IGF1R 3129G > T polymorphism are associated with response to neoadjuvant chemotherapy in breast cancer patients: results from the NEOZOTAC trial (BOOG 2010-01). *Breast Cancer Res* 2016;**18**:3.

Diamanti-Kandarakis E, Alexandraki K, Piperi C *et al*. Inflammatory and endothelial markers in women with polycystic ovary syndrome. *Eur J Clin Invest* 2006;**36**:691–697.

El Hayek S, Bitar L, Hamdar LH, Mirza FG, Daoud G. Poly cystic ovarian syndrome: an updated overview. *Front Physiol* 2016;**7**:124.

Erlandsson MC, Töyrä Silfverswärd S, Nadali M, Turkkila M, Svensson MND, Jonsson I-M, Andersson KME, Bokarewa MI. IGF-1R signalling contributes to IL-6 production and T cell dependent inflammation in rheumatoid arthritis. *Biochim Biophys Acta Mol Basis Dis* 2017;**1863**:2158–2170.

Forster R, Sarginson A, Velichkova A, Hogg C, Dorning A, Horne AW, Saunders PTK, Greaves E. Macrophage-derived insulin-like growth factor-1 is a key neurotrophic and nerve-sensitizing factor in pain associated with endometriosis. *FASEB J* 2019;**33**: 11210–11222.

Ghazal S, McKinnon B, Zhou J, Mueller M, Men Y, Yang L, Mueller M, Flannery C, Huang Y, Taylor HS *et al*. H19 lncRNA alters stromal cell growth via IGF signaling in the endometrium of women with endometriosis. *EMBO Mol Med* 2015;**7**:996–1003.

Glueck CJ, Morrison JA, Goldenberg N, Wang P. Coronary heart disease risk factors in adult premenopausal white women with polycystic ovary syndrome compared with a healthy female population. *Metabolism* 2009;**58**:714–721.

Głuszak O, Stopińska-Głuszak U, Glinicki P, Kapuścińska R, Snochowska H, Zgliczyński W, Dębski R. Phenotype and metabolic disorders in polycystic ovary syndrome. *ISRN Endocrinol* 2012; **2012**:569862.

GTEx portal. *Variant Page*. The Broad Institute of MIT and Harvard, Version 8, 2021. https://www.gtexportal.org/home/snp/rs2016347 (April 2021, date last accessed).

Haouzi D, Assou S, Monzo C, Vincens C, Dechaud H, Hamamah S. Altered gene expression profile in cumulus cells of mature MII oocytes from patients with polycystic ovary syndrome. *Hum Reprod* 2012;**27**:3523–3530.

Imrie H, Viswambharan H, Sukumar P, Abbas A, Cubbon RM, Yuldasheva N, Gage M, Smith J, Galloway S, Skromna A *et al*. Novel role of the IGF-1 receptor in endothelial function and repair: studies in endothelium-targeted IGF-1 receptor transgenic mice. *Diabetes* 2012;**61**:2359–2368.

Jovanovic VP, Carmina E, Lobo RA. Not all women diagnosed with PCOS share the same cardiovascular risk profiles. *Fertil Steril* 2010; **94**:826–832.

Kenigsberg S, Bentov Y, Chalifa-Caspi V, Potashnik G, Ofir R, Birk OS. Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients. *Mol Hum Reprod* 2009;**15**:89–103.

Kim JG, Suh CS, Kim SH, Choi YM, Moon SY, Lee JY. Insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and IGFBP-3 protease activity in the peritoneal fluid of patients with and without endometriosis. *Fertil Steril* 2000;**73**:996–1000.

Kinugasa S, Shinohara K, Wakatsuki A. Increased asymmetric dimethylarginine and enhanced inflammation are associated with impaired vascular reactivity in women with endometriosis. *Atherosclerosis* 2011;**219**:784–788.

Li J, Dong R, Yu J, Yi S, Da J, Yu F, Zha Y. Inhibitor of IGF1 receptor alleviates the inflammation process in the diabetic kidney mouse model without activating SOCS2. *Drug Des Devel Ther* 2018;**12**: 2887–2896.

Liao G, Chen D, Li J, Hu S. Clinical significance of microRNA-320a and insulin-like growth factor-1 receptor in early-onset preeclampsia patients. *Eur J Obstet Gynecol Reprod Biol* 2021;**263**:164–170.

Lindström S, Loomis S, Turman C, Huang H, Huang J, Aschard H, Chan AT, Choi H, Cornelis M, Curhan G *et al*. A comprehensive survey of genetic variation in 20,691 subjects from four large cohorts. *PLoS One* 2017;**12**:e0173997.

Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril* 2016;**106**:6–15.

Louwers YV, Laven JSE. Characteristics of polycystic ovary syndrome throughout life. *Ther Adv Reprod Health* 2020;**14**: 2633494120911038.

Loverro G, Maiorano E, Napoli A, Selvaggi L, Marra E, Perlino E. Transforming growth factor-beta 1 and insulin-like growth factor-1 expression in ovarian endometriotic cysts: a preliminary study. *Int J Mol Med* 2001;**7**:423–429.

Mani H, Levy MJ, Davies MJ, Morris DH, Gray LJ, Bankart J, Blackledge H, Khunti K, Howlett TA. Diabetes and cardiovascular events in women with polycystic ovary syndrome: a 20-year retrospective cohort study. *Clin Endocrinol (Oxf)* 2013;**78**:926–934.

Melo AS, Rosa-e-Silva JC, Rosa-e-Silva AC, Poli-Neto OB, Ferriani RA, Vieira CS. Unfavorable lipid profile in women with endometriosis. *Fertil Steril* 2010;**93**:2433–2436.

Missmer SA, Hankinson SE, Spiegelman D, Barbieri RL, Marshall LM, Hunter DJ. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. *Am J Epidemiol* 2004;**160**:784–796.

Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. *Hum Reprod Update* 2009;**15**: 477–488.

Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Forman JP, Missmer SA. Association between endometriosis and hypercholesterolemia or hypertension. *Hypertension* 2017;**70**:59–65.

Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Missmer SA. Endometriosis and risk of coronary heart disease. *Circ Cardiovasc Qual Outcomes* 2016;**9**:257–264.

Parasar P, Ozcan P, Terry KL. Endometriosis: epidemiology, diagnosis and clinical management. *Curr Obstet Gynecol Rep* 2017;**6**: 34–41.

Patel BG, Lenk EE, Lebovic DI, Shu Y, Yu J, Taylor RN. Pathogenesis of endometriosis: interaction between endocrine and inflammatory pathways. *Best Pract Res Clin Obstet Gynaecol* 2018;**50**:50–60.

Powe CE, Levine RJ, Karumanchi SA. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. *Circulation* 2011;**123**: 2856–2869.

Powell M, Benz C, Ereman R, Prebil LA, Jamshidian F, Rose S. Abstract P3-07-03: Insulin-like growth factor-1 receptor variant associated with decreased breast cancer risk in women with pregnancy-induced hypertension. Abstracts: Thirty-Sixth Annual CTRC-AACR San Antonio Breast Cancer Symposium—Dec 10-14, 2013; San Antonio, TX. *Cancer Res* 2013;**73**(24 Suppl): P3-07-03.

Powell MJ, Dufault SM, Gunderson EP, Benz CC. Cancer and cardiovascular risk in women with hypertensive disorders of pregnancy carrying a common IGF1R variant. *Mayo Clin Proc* 2020;**95**: 2684–2696.

Powell MJ, Von Behren J, Neuhausen S, Reynolds P, Benz CC. Functional IGF1R variant predicts breast cancer risk in women with preeclampsia in California Teachers Study. *Cancer Causes Control* 2017;**28**:1027–1032.

Prebil LA, Ereman RR, Powell MJ, Jamshidian F, Kerlikowske K, Shepherd JA, Hurlbert MS, Benz CC. First pregnancy events and future breast density: modification by age at first pregnancy and specific VEGF and IGF1R gene variants. *Cancer Causes Control* 2014;**25**:859–868.

R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2020.

Santoro L, D'Onofrio F, Campo S, Ferraro PM, Tondi P, Campo V, Flex A, Gasbarrini A, Santoliquido A. Endothelial dysfunction but not increased carotid intima-media thickness in young European women with endometriosis. *Hum Reprod* 2012;**27**:1320–1326.

Santoro L, D'Onofrio F, Flore R, Gasbarrini A, Santoliquido A. Endometriosis and atherosclerosis: what we already know and what we have yet to discover. *Am J Obstet Gynecol* 2015;**213**: 326–331.

Shroff R, Syrop CH, Davis W, Van Voorhis BJ, Dokras A. Risk of metabolic complications in the new PCOS phenotypes based on the Rotterdam criteria. *Fertil Steril* 2007;**88**:1389–1395.

Stochino-Loi E, Millochau JC, Angioni S, Touleimat S, Abo C, Chanavaz-Lacheray I, Hennetier C, Roman H. Relationship between patient age and disease features in a prospective cohort of 1560 women affected by endometriosis. *J Minim Invasive Gynecol* 2020;**27**:1158–1166.

Tani A, Yamamoto S, Maegawa M, Kunimi K, Matsui S, Keyama K, Kato T, Uemura H, Kuwahara A, Matsuzaki T *et al*. Arterial stiffness is increased in young women with endometriosis. *J Obstet Gynaecol* 2015;**35**:711–715.

Taskin O, Rikhraj K, Tan J, Sedlak T, Rowe TC, Bedaiwy MA. Link between endometriosis, atherosclerotic cardiovascular disease, and the health of women midlife. *J Minim Invasive Gynecol* 2019;**26**: 781–784.

Therneau T. *A Package for Survival Analysis in S. Version 2.38*. 2020. https://CRAN.R-project.org/package¼survival (6 June 2020, date last accessed).

Therneau T, Grambsch P. *Modeling Survival Data: Extending the Cox Model*. New York, NY: Springer-Verlag New York, 2000.

Tobias DK, Gaskins AJ, Missmer SA, Hu FB, Manson JE, Buck Louis GM, Zhang C, Chavarro JE. History of infertility and risk of type 2 diabetes mellitus: a prospective cohort study. *Diabetologia* 2015; **58**:707–715.

Tziomalos K. Cardiovascular risk in the different phenotypes of polycystic ovary syndrome. *Curr Pharm Des* 2016;**22**:5547–5553.

Wekker V, van Dammen L, Koning A, Heida KY, Painter RC, Limpens J, Laven JSE, Roeters van Lennep JE, Roseboom TJ, Hoek A *et al*. Long-term cardiometabolic disease risk in women with PCOS: a systematic review and meta-analysis. *Hum Reprod Update* 2020;**26**:942–960.

Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer International Publishing, 2016.

Winder T, Giamas G, Wilson PM, Zhang W, Yang D, Bohanes P, Ning Y, Gerger A, Stebbing J, Lenz H-J *et al*. Insulin-like growth factor receptor polymorphism defines clinical outcome in estrogen-receptor positive breast cancer patients treated with tamoxifen. *Pharmacogenomics J* 2014;**14**:28–34.

Zhou Y, Zeng C, Li X, Wu P-L, Yin L, Yu X-L, Zhou Y-F, Xue Q. IGF-I stimulates ERβ and aromatase expression via IGF1R/PI3K/AKT-mediated transcriptional activation in endometriosis. *J Mol Med (Berl)* 2016;**94**:887–897.