Circulating inflammatory and immune response proteins and endometrial cancer risk: a nested case-control study and Mendelian randomization analyses

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Summary

Background Inflammation and immune dysregulation are hypothesized contributors to endometrial carcinogenesis; however, the precise underlying mechanisms remain unclear.

Methods We measured pre-diagnostically 152 plasma protein biomarkers in 624 endometrial cancer case-control pairs nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Odds ratios

eBioMedicine
2024;108: 105341

Published Online 14 September 2024
https://doi.org/10.1016/j.ebiom.2024.105341

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(ORs) were estimated using conditional logistic regression, accounting for confounding and multiple comparisons. Proteins considered as associated with endometrial cancer risk were further tested in a two-sample Mendelian randomization (MR) analysis using summary data from the UK Biobank (n = 52,363) and the Endometrial Cancer Association Consortium (12,270 cases and 46,126 controls).

Findings In the EPIC nested case-control study, IL-6 [OR per NPX (doubling of concentration) = 1.28 (95% confidence interval (CI) 1.03–1.57)], HGF [1.48 (1.06–2.07)], PIK3AP1 [1.22 (1.00–1.50)] and CLEC4G [1.52 (1.00–2.32)] were positively associated; HSD11B1 [0.67 (0.49–0.91)], SCF [0.68 (0.49–0.94)], and CCL25 [0.80 (0.65–0.99)] were inversely associated with endometrial cancer risk; all estimates had multiple comparisons adjusted P-value > 0.05. In complementary MR analysis, IL-6 [OR per inverse-rank normalized NPX = 1.19 (95% CI 1.04–1.36)] and HSD11B1 [0.91 (0.84–0.99)] were associated with endometrial cancer risk.

Interpretation Altered IL-6 signalling and reduced glucocorticoid activity via HSD11B1 might play important roles in endometrial carcinogenesis.

Funding Funding for IIG_FULL_2021_008 was obtained from Wereld Kanker Onderzoek Fonds (WKOF), as part of the World Cancer Research Fund International grant programme; Funding for INCA_15849 was obtained from Institut National du Cancer (INCa).

Keywords: Endometrial cancer; Proteomics; Interleukin-6; HSD11B1; Mendelian randomisation

Research in context

Evidence before this study
Previous studies have associated several circulating inflammatory biomarkers with endometrial cancer risk. These studies have smaller sample sizes and have focused on smaller sets of inflammatory markers. The precise underlying mechanisms linking inflammation, immune response, and endometrial cancer remain unclear.

Added value of this study
In a large (n = 624 cases) nested case-control study, we investigated the association between 152 circulating inflammatory and immune response protein biomarkers and the risk of endometrial cancer. We complemented our study with Mendelian randomization analyses in non-overlapping study samples. We found that IL-6 was positively associated; and HSD11B1 was inversely associated with endometrial cancer risk in both nested case-control and Mendelian randomization analyses.

Implications of all the available evidence
Altered IL-6 signalling and reduced glucocorticoid activity via HSD11B1 may be involved in endometrial cancer development. These findings provide insight into the potential underlying biological mechanisms linking inflammation and altered immune response and endometrial cancer.

Introduction

The incidence of endometrial cancer has been rising over the past few decades, prompting a comprehensive exploration of its underlying drivers.[1] While gene mutations are essential for carcinogenesis, histological studies have revealed cancer-driver mutations are present in abundance even in normal endometrial tissues.[2,3] This observation suggests additional factors are critical in promoting the proliferation of dormant mutated cells and initiating endometrial carcinogenesis.[4] One such potential contributor to cancer development is inflammation and immune dysregulation. While emerging immunotherapies highlight the potential of modulating immune responses against cancer cells, immune dysregulation and inflammation could predispose to cancer development in normal tissues.[4,5] At least one-third of global endometrial cancer cases can be attributed to obesity, a condition characterized by chronic low-grade inflammation.[6] Inflammation also contributes to endometrial hyperplasia induced by excess oestrogens, a major cause of type I endometrioid cancer.[7]

Proteins are the major effectors of biological processes, making them desirable biomarkers to study cancer aetiology and targets for therapeutic interventions.[8] Previous studies, including analyses from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, have associated several circulating inflammation and immune response biomarkers measured pre-diagnostically with endometrial cancer risk, such as interleukin (IL)-6, tumour necrosis factor (TNF)-α, C-reactive protein (CRP), C-C motif chemokines

3 (CCL3), and C-X-C motif chemokines 3 (CXCL3).[9–12] However, findings were largely not reproduced among the studies.[9–12] The challenge in unravelling specific inflammation and immune response pathways involved in endometrial carcinogenesis persists, with the large number of cytokines to examine and the relatively small sample sizes (≤305 cases) available to date. Using multiplex protein panels, we aimed to extend the investigation for plasma inflammation and immune response biomarkers and their association with endometrial cancer risk in a large case-control study nested within the EPIC cohort. We complemented our investigations with proteogenomic Mendelian randomization (MR) analyses, leveraging the UK Biobank Pharma Proteomics Project (UKB-PPP) that has mapped protein quantitative trait loci (pQTL) to identify the associations between genetic variants and plasma protein levels.[13]

Methods

Methods for nested case-control study

Ethics

The study was approved by the ethics committee of the International Agency for Research on Cancer and all other centres (IEC 22-01). All participants provided written informed consent to participate.

Study population

The EPIC cohort comprises 519,978 participants (366,521 women) recruited from multiple centres across ten European countries.[14] Information on lifestyle, diet, medical and reproductive history, anthropometric measurements, and a blood sample were collected at recruitment, between 1992 and 2000.

Eligible participants for this study included women who provided a blood sample and had no cancer diagnosis (except non-melanoma skin cancer) at recruitment. Women who had hysterectomy or reported use of oral contraceptives or hormonal replacement therapy at blood collection were excluded. Women from Denmark, Sweden, Norway, and Greece were not included in the current analysis due to lack of available data.

Incident endometrial cancer cases were ascertained through record linkage with cancer registries or active follow-up. Women diagnosed with first primary epithelial invasive endometrial cancer were selected as cases. This included International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3), codes 8380, 8560, 8570, 8140, 8210, 8480, 8481, 8070, 8310, 8323, 8441, 8460, 8000, 8010, 8020, 8260, 8950, 8980. End of follow-up was defined as the latest date of ascertainment for both cancer incidence and vital status, ranging from June 2008 to December 2012. The median follow-up time of eligible participants was 14.9 years (5th–95th percentiles: 5.1–18.6 years). For each endometrial cancer case, one control was randomly selected using incidence density sampling. Controls were matched on factors at blood collection, including centre, age (±6 months), menopausal status (premenopausal, perimenopausal, or postmenopausal), fasting status (<3 h, 3–6 h, or >6 h), and time of the day (±1 h). A total of 624 cases and 624 controls were included in the analysis. Of these, 176 cases and 172 controls were included in previous EPIC studies.[9,10]

Laboratory measurements

We used the Olink Target 96 Inflammation and Target 96 Immune Response panels (Olink Bioscience AB, Uppsala, Sweden). Each panel included 92 proteins, totalling 184 proteins, with 4 proteins (IL-5, IL-6, IL-10, and CCL-11) assayed in both panels (Supplementary Table S1). Briefly, the assay uses pairs of antibodies labelled with DNA oligonucleotides to bind to their respective proteins in plasma samples. The binding brings antibodies into proximity, leading to hybridization of their oligonucleotides. The resulting double-stranded DNA is then amplified and quantified using quantitative real-time polymerase chain reaction (qPCR). An intensity normalization method was used to correct for plate variations. Each plate contained a randomized set of case-control pairs and plate-specific median values were centralized to the overall median value across all plates. Assay results were reported in Normalised Protein eXpression (NPX), a relative measure where one NPX unit represents a doubling of protein concentration. For each protein assay, measurements below its limit of detection (LOD) were flagged by the laboratory, and proteins with >75% measurements below LOD were excluded from the analysis. Measurements below LOD may still be biologically plausible but might not be linearly related to the reported NPX unit.

As part of sensitivity analyses, we further measured serum C-peptide, oestradiol, and oestrone. Serum C-peptide was measured in two phases: samples for 190 case-control pairs were previously measured in 2007 using an immunoradiometric assay by Immunotech (Marseille, France) and samples for 434 case-control pairs were measured in 2019 using an ELISA assay by Mercodia (Uppsala, Sweden). Oestrone and oestradiol were measured in 2024 using a platform consisting of an ultra-high-performance liquid chromatograph (Agilent 1290, Agilent, Santa Clara, CA) and a QTRAP 5500 mass spectrometer (SCIEX, Framingham, MA).

Statistical analyses

Baseline characteristics of cases and controls were described using mean and standard deviation (SD) or frequencies. The association between protein biomarkers and endometrial cancer risk was estimated using conditional logistic regression and reported as odds ratios (ORs) and corresponding 95% confidence intervals (CIs) per NPX (i.e., per doubling of protein concentration). To test for linearity, we modelled the

association between plasma proteins and endometrial cancer risk using restricted cubic splines with five knots and Harrell's recommended percentiles for knot locations.[15] A Wald test P-value < 0.05 for the restricted cubic spline terms were considered departure from linearity. Proteins with 50–75% samples below LOD were analysed as categorical variables (top 50% above LOD vs below LOD). We used the false discovery rate (FDR) method to account for multiple comparisons, which was estimated using a step-up procedure accounting for correlation between protein biomarkers tested.[16] We reported the adjusted P-value (Q-value) along with the raw P-value.

Potential confounders were identified *a priori* from a causal diagram (Supplementary Figure S1) and included in the regression model as covariates. A fully adjusted model was used as the primary analysis, which included body mass index (BMI: <25 kg/m^2, 25–<30 kg/m^2, ≥30 kg/m^2), physical activity (Cambridge index: inactive, moderately inactive, moderately active, active), smoking status at baseline (never, former, current), age at menarche (years), parous (yes, no), ever use of oral contraceptives (yes, no), and ever use of hormone replacement therapy (yes, no) as covariates. In addition, we reported estimates from 1) a univariable model conditional on matching variables only; 2) a minimally adjusted model without BMI, given the very strong association between BMI and endometrial cancer risk; 3) a fully adjusted model that further adjusted for C-peptide, given elevated insulin could either precede or mediate the effect of inflammation or immune response proteins.[12] Participants with missing data for covariates were assigned with the median or mode value. Missing values for each variable were less than 5% of the sample.

Complementary and sensitivity analyses

We further performed the following sensitivity analyses: 1) analysis excluding cases diagnosed within two years of blood collection, to examine potential bias due to reverse causation; 2) analysis restricted to endometrioid (type I) cancer cases (morphology codes 8140/3, 8210/3, 8380/3, 8480/3, 8481/3, 8560/3, 8570/3); 3) analysis restricted to post-menopausal women at recruitment; 4) analysis further adjusting for oestradiol and oestrone level in post-menopausal women at recruitment, given oestrogen's direct link with endometrial cancer risk in the absence of progesterone[17]; and 5) analysis replacing BMI with waist circumference, which might adjust for some aspect of central adiposity not captured by BMI. All sensitivity analyses used the same fully adjusted model as the primary analysis. In addition, we examined potential effect modification by BMI (≥25 kg/m^2 vs <25 kg/m^2), using likelihood ratio test to compare regression models with and without an interaction term between each plasma protein and BMI.

The main analysis was complemented with regression using clusters of correlated proteins, constructed using hierarchical clustering.[18] A cluster was represented by the first principal component derived from the proteins composing the cluster, and the squared Pearson correlation between each protein and the principal component was calculated. The dendrogram tree was cut at height 0.8, which gave an average Pearson correlation of ±0.82. We estimated the association between each protein cluster and endometrial cancer risk using the same primary regression model in the main analysis. We then performed L1-norm penalized (LASSO) logistic regression with 5-fold cross validation to select clusters associated with risk of endometrial cancer, while mutually adjusting for all other clusters. Matching and confounding variables were pre-specified (i.e., unpenalized) as covariates included in the model. The LASSO regression was applied on 1000 bootstrap samples, with each obtained by randomly sampling 624 matched case-control pairs with replacement from the original sample.[19] The proportion of bootstrap iterations for which the LASSO produces a non-null coefficient was used as a measure of our level of confidence for a protein cluster's association with endometrial cancer risk.

Analyses for nested case-control study was performed using Stata Statistical Software (Release 17), R version 4.1.2, and the following R packages: ClustOfVar (version 1.1) and dendextend (version 1.17.1).

Methods for Mendelian randomization analyses

For proteins that were associated with endometrial cancer risk in the nested case-control study and had available genome-wide association study (GWAS) data, we performed a two-sample MR analysis. Genetic variants associated with proteins were identified from the UKB-PPP GWAS summary data (n = 52,363 individuals, 95% European ancestries).[13] We selected variants within the gene region encoding each protein (*cis* variants). To increase statistical power, linkage disequilibrium clumping was performed with $r^2 < 0.01$ and $P < 5 \times 10^{-8}$ to select instrumental variables.[20] For proteins with no *cis* variants that reached the $P < 5 \times 10^{-8}$ threshold, we selected *trans* variants by clumping with a $r^2 < 0.001$ threshold. Instrumental variables for each protein were then extracted from the Endometrial Cancer Association Consortium (ECAC) GWAS summary data excluding UK Biobank participants (12,270 cases and 46,126 controls of European ancestry).[21] We used either inverse variance weighted (IVW) models for proteins instrumented with ≥2 variants or the Wald ratio for proteins instrumented with one variant to estimate the association between protein biomarkers and endometrial cancer risk.

A further two-sample *cis*-MR analysis was performed for IL-6 receptor (IL-6R), as extensive literature has demonstrated it is a suitable functional proxy for IL-6 signalling.[22–25] IL-6R was not included in the proteomic panels used in the nested case-control study. We

conducted a separate analysis for the missense *IL6R* variant Asp358Ala (rs2228145), which is known to increase conversion of the membrane-bound IL-6R to its soluble form through proteolytic cleavage, thereby decreasing classical signalling and potentially increasing trans-signalling of IL-6.[26]

As per previous IL-6R MR analyses,[23,27] we additionally performed 1) MR using *IL6R* variants weighted by their effect on CRP to proxy the downstream effect of IL-6 signalling and 2) MR using *CRP* variants to examine whether CRP might causally affect endometrial cancer risk. CRP-weighted *IL6R* and *CRP* variants were obtained from a meta-analysis of GWAS for CRP, including Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and UK Biobank (n = 575,531 of European ancestries).[28] Coefficients for CRP-weighted *IL6R* variants were obtained from the *IL6R* gene region in the CRP GWAS summary data, which corresponds to IL-6R's effect on CRP.

The unit for estimates obtained from the MR analyses are not comparable with those used in our nested case-control study, as GWAS summary data were reported in different units. Estimates obtained using the UKB-PPP GWAS data for plasma proteins correspond to ORs for endometrial cancer risk per inverse-rank normalized NPX increase in plasma protein. Estimates obtained using the CHARGE + UK Biobank meta-analysed GWAS data for CRP were inverted to represent ORs for endometrial cancer by decreased natural log-transformed CRP (i.e., an increase in plasma IL-6R). Given the differences in units, we interpreted only the direction and not the magnitude of ORs presented from MR analyses.

Colocalization and sensitivity analyses

For proteins instrumented using *cis* variants, we performed colocalization to assess the validity of the assumption that the two traits (plasma protein level and endometrial cancer risk) were affected by the same causal variant.[29] We first extracted a ±500 kb window around the sentinel *cis* variant identified by UKB-PPP GWAS.[13] The same region was then extracted from the ECAC GWAS data.[21] The linkage disequilibrium matrix was generated using the 1000 genomes reference panel (phase 3). Colocalization was performed using the single causal variant approach, with priors set at $p1 = 1 \times 10^{-4}$, $p2 = 1 \times 10^{-4}$, and $p12 = 5 \times 10^{-6}$.[30]

For proteins instrumented using *trans* variants, we performed sensitivity analysis using weighted-median, weighted-mode, MR-Egger, and MR-CAUSE. The MR-CAUSE method accounts for not only uncorrelated but also correlated horizontal pleiotropic effects that might be more prevalent for inflammatory biomarkers.[31] It compares a 'sharing model' that allows for horizontal pleiotropic effects but not causal effects and a 'causal model'. A negative expected log pointwise posterior density (ELPD) score and a one-sided P-value < 0.05 are supportive of a causal effect. The nuisance parameters were calculated using a random subset of 1,000,000 variants. We further performed 1) the Steiger test to examine causal direction between *trans* variants instrumented protein levels and endometrial cancer risk[32]; and 2) the leave-one-out analysis to examine whether observed associations were driven by specific variants.

MR analyses and colocalization were performed using R version 4.1.2 and the following R packages: ieugwasr (version 0.1.5), MendelianRandomization (version 0.9.0), TwoSampleMR (version 0.5.8), coloc (version 5.2.0), and cause (version 1.2.0).

Role of funders

The funders of the study played no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Nested case-control study

The distribution of demographic, reproductive, lifestyle, and clinical covariates among cases and matched controls are presented in Table 1. Cases and controls were on average 54 years old at blood collection. For cases, average age at diagnosis was 62.9 years. Of the 624 cases, 558 (89%) were type I endometrioid cancers and 66 (11%) were type II non-endometrioid cancers. Of the 184 protein assays, 29 had >75% samples below LOD and were excluded from the analysis. A total of 155 protein assays were included in the analysis, including three proteins (IL-6, IL-10, and CCL-11) assayed in both Target 96 Inflammation and Target 96 Immune Response panels (i.e., 152 unique proteins). There was high correlation (r >0.89) for the measurements of proteins assayed in both panels. Pearson correlation of proteins, both between and within the two assay panels, is presented as heat maps in Supplementary Fig. S2a–c.

Of the 155 protein biomarkers included in the analysis, 1) 30 from the univariable model; 2) 34 from the minimally adjusted model; 3) eight from the fully adjusted model including BMI; and 4) seven from the fully adjusted model including BMI and C-peptide were associated with endometrial cancer risk with a raw P-value < 0.05. After accounting for multiple comparisons, 1) 10 from the univariable model; and 2) eight from the minimally adjusted model were associated with endometrial cancer risk with a Q-value < 0.05. No protein was associated with endometrial cancer risk with a Q-value < 0.05 in the fully adjusted models. We thus considered MR analysis for proteins that were either 1) associated with endometrial cancer risk with a Q-value <0.05 in the univariable or minimally adjusted models or 2) associated with cancer risk with raw P-value < 0.05 in the fully adjusted models in the nested case-control study (Fig. 1). Results from the primary analysis and all

	N	Cases (N = 624)	Controls (N = 624)
Age at blood collection (years)[a]	1248	54.5 (8.0)	54.4 (8.0)
Age at diagnosis (years)	624	62.9 (8.3)	–
Time between blood collection and diagnosis (years)	624	8.5 (4.6)	–
Fasting status[a]	1239		
0–3 h		254 (41.0%)	251 (40.5%)
>3–6 h		96 (15.5%)	99 (16.0%)
>6 h		269 (43.5%)	270 (43.5%)
Age at menarche (years)	1248	12.7 (1.5)	13.1 (1.6)
Parous	1248	522 (83.7)	547 (87.7)
Age at first full-term pregnancy (years)[b]	1057	25.1 (4.0)	25.3 (4.2)
Number of full-term pregnancies[b]	1021	2.3 (1.0)	2.4 (1.2)
Ever use of oral contraceptives	1248	243 (38.9%)	285 (45.7%)
Menopausal status at blood collection[a]	1248		
Premenopausal		196 (31.4%)	196 (31.4%)
Postmenopausal		337 (54.0%)	337 (54.0%)
Perimenopausal[c]		91 (14.6%)	91 (14.6%)
Age at menopause (years)[d]	624	51.0 (4.1)	49.6 (4.2)
Ever use of hormone replacement therapy[d]	669	54 (16.2%)	45 (13.4%)
Smoking status	1245		
Never		417 (67.0%)	383 (61.5%)
Former		123 (19.8%)	128 (20.5%)
Current		82 (13.2%)	112 (18.0%)
Cambridge physical activity index	1237		
Inactive		195 (31.6%)	178 (28.7%)
Moderately inactive		214 (34.7%)	239 (38.5%)
Moderately active		113 (18.3%)	118 (19.0%)
Active		95 (15.4%)	85 (13.7%)
Alcohol at recruitment (g/day)	1248		
Non-drinker		155 (24.8%)	160 (25.6%)
>0–3		207 (33.2%)	187 (30.0%)
>3–12		149 (23.9%)	150 (24.0%)
>12		113 (18.1%)	127 (20.4%)
Educational level	1211		
Primary/no schooling		295 (49.1%)	321 (52.6%)
Technical/professional/secondary		213 (35.4%)	194 (31.8%)
Longer education		93 (15.5%)	95 (15.6%)
Height (cm)	1248	160.1 (6.7)	160.0 (6.8)
Weight (kg)	1248	72.3 (13.8)	66.6 (10.8)
Body Mass Index (kg/m^2)	1248	28.3 (5.5)	26.0 (4.3)
Body mass index (WHO categories)	1248		
Underweight (<18.5 kg/m^2)		1 (0.2%)	7 (1.1%)
Normal weight (18.5–24.9 kg/m^2)		196 (31.4%)	283 (45.4%)
Overweight (25–29.9 kg/m^2)		225 (36.1%)	236 (37.8%)
Obese (≥30 kg/m^2)		202 (32.4%)	98 (15.7%)
Waist circumference (cm)	1248	86.5 (12.5)	82.1 (10.9)
Waist circumference (WHO categories)	1248		
≤80 cm		216 (34.6%)	311 (49.8%)
>80 cm–88 cm		162 (26.0%)	160 (25.6%)
>88 cm		246 (39.4%)	153 (24.5%)
Hip circumference (cm)	1248	106.4 (11.0)	101.9 (8.8)
Waist/Hip Ratio	1248	0.8 (0.1)	0.8 (0.1)
Serum C-peptide (ng/ml)	1231	2.2 (1.5)	1.9 (1.3)
Prevalent diabetes	1111	25 (4.5%)	21 (3.8%)

[a]Matching factor. [b]Among parous women. [c]Perimenopausal defined as 9 or less periods during the year before recruitment. [d]Among postmenopausal women.

Table 1: **Baseline characteristics of endometrial cancer cases and matched controls, means (standard deviations) or number (percentages).**

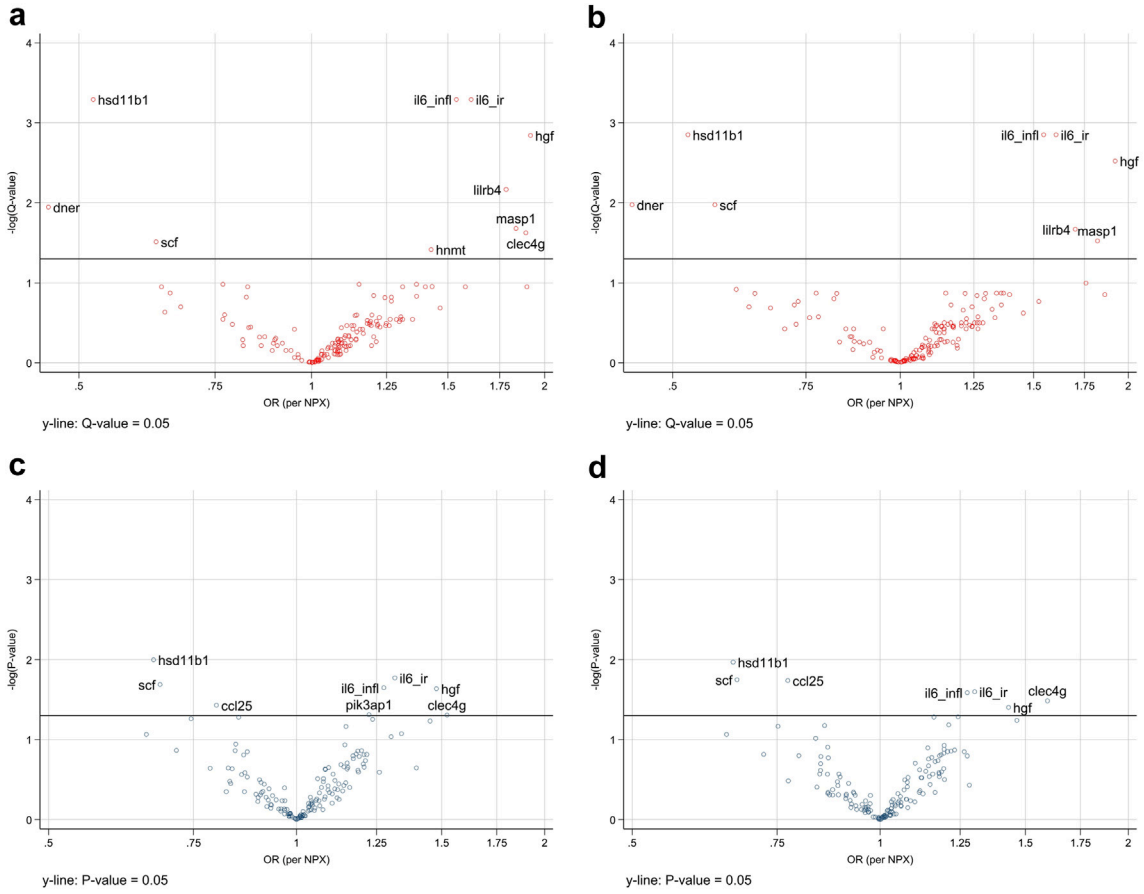

Fig. 1: The association between plasma protein levels and endometrial cancer risk in nested case-control study, estimated from (a) univariable regression, plotted against Q-values; (b) minimally adjusted model[1], plotted against Q-values; (c) fully adjusted model[2], plotted against P-values; (d) fully adjusted model[2] plus C-peptide, plotted against P-values. [1]Minimally adjusted model included physical activity, smoking status, age at menarche, parity, ever use of oral contraceptives, and ever use of hormone replacement therapy as covariates; matching factors (i.e., study centre, age, menopausal status, fasting status, and time of blood collection) were accounted using conditional logistic regression. [2]Fully adjusted model included all variables from minimally adjusted model plus BMI.

sensitivity analyses are available in Supplementary Tables S2 and S3.

In the primary analysis using a fully adjusted model, IL-6 [odds ratios (OR) per NPX (doubling of concentration) = 1.28 (95% confidence intervals (CI) 1.03–1.57)], HGF [1.48 (1.06–2.07)], PIK3AP1 [1.22 (1.00–1.50)] and CLEC4G [1.52 (1.00–2.32)] were positively associated with endometrial cancer risk, and HSD11B1 [0.67 (0.49–0.91)], SCF [0.68 (0.49–0.94)], and CCL25 [0.80 (0.65–0.99)] were inversely associated with risk, all estimates had Q-value > 0.05. We reported here estimates for IL-6 measured in the Inflammation panel, as it has less measurements below the LOD than the one in the Immune Response panel (8% vs. 12%). There was no evidence for departure from linearity for all plasma proteins analysed as continuous variables. There were 13 proteins with 50–75% samples below LOD and were analysed as categorical variables (Supplementary Table S3). One protein, FGF5, was inversely associated with endometrial cancer risk [top 50th percentile above LOD vs below LOD, OR = 0.69 (95% CI 0.49–0.98)].

Estimates from the sensitivity analysis excluding cases diagnosed within two years from baseline were comparable with those from primary analysis and did not suggest reverse causation. When the analysis was restricted to type I endometrioid cancers and their matched controls (n = 558 case-control pairs), the association with endometrial cancer was slightly stronger for HGF [OR = 1.57 (95% CI 1.08–2.28)] and PIK3AP1 [1.33 (1.07–1.65)] and slightly weaker for HSD11B1 [0.71 (0.52–0.98)]. When the analysis was restricted to post-menopausal women (n = 337 case-control pairs), estimates were stronger for HSD11B1 [0.47 (0.30–0.74)] and SCF [0.49 (0.31–0.78)] and estimates were attenuated for HGF [1.29 (0.84–1.97)], IL-6 [1.15 (0.90–1.48)], and PIK3AP1 [0.94 (0.71–1.25)]. In postmenopausal

women, further adjusting for oestradiol level at blood collection did not change the results, whereas further adjusting for oestrone level led to further attenuation in the estimates for HGF [1.23 (0.79–1.91)], IL-6 [1.10 (0.85–1.41)], and PIK3AP1 [0.90 (0.67–1.21)]. In the analysis that replaced BMI with waist circumference, results were similar to the primary analysis with slightly stronger associations. There was strong correlation between BMI and waist circumference (r = 0.87).

Results for analysis that explored potential effect modification by BMI are presented in Supplementary Table S4. Tests for interaction suggested possible effect modification by BMI (P interaction <0.05) for four proteins, for which the inverse association with endometrial cancer might be restricted to women with BMI ≥25 kg/m^2: HSD11B1 [OR = 0.46 (95% CI 0.32–0.67)] and CCL25 [0.60 (0.46–0.79)] were inversely associated with risk with Q-value < 0.05; and IL-10 [0.78 (0.61–0.99)] and CCL28 [0.55 (0.34–0.87)] with P-value < 0.05. In women with BMI <25 kg/m^2, there was limited evidence that HSD11B1 [1.01 (0.61–1.67)], CCL25 [1.15 (0.84–1.58)], IL-10 [1.21 (0.90–1.63)], and CCL28 [1.11 (0.64–1.91)] were associated with cancer risk.

We derived 44 clusters, including 31 clusters of correlated proteins with size ranging from 2 to 30 proteins, and 13 individual proteins (Fig. 2a). When examined individually, Cluster 3 [OR = 1.13 (95% CI 1.00–1.27)], Cluster 17 [0.89 (0.80–0.99)], and Cluster 27 [0.86 (0.76–0.97] were associated with endometrial cancer risk at P-value < 0.05. When examined using bootstrap LASSO regression, Cluster 3, Cluster 17, and Cluster 27 were associated with endometrial cancer risk in 91.1%, 90.4%, and 89.3% of the 1000 bootstrap iterations respectively (Fig. 2b).

Mendelian randomization analysis

Of the 11 proteins considered as associated with endometrial cancer risk in the observational analysis in EPIC (Fig. 1), two (SCF and HNMT) were not measured in the UKB-PPP GWAS; two (IL-6 and HSD11B1) did not have *cis* variants reaching the $P < 5 \times 10^{-8}$ threshold and were analysed using *trans* variants. Genetically instrumented IL-6 [OR = 1.19 (CI 1.04–1.36) by increase in inverse-rank normalized plasma protein NPX] was positively associated with endometrial cancer risk and HSD11B1 [0.91 (0.84–0.99)] was inversely associated with risk (Fig. 3, Supplementary Table S6). Genetically instrumented HGF, DNER, CCL25, MASP1, CLEC4G, LILRB4, and PIK3AP1 were not associated with endometrial cancer risk with most estimates close to the null value.

There was a suggestive positive association for IL-6R with endometrial cancer risk in both analysis using selected *cis* variants [OR = 1.02 (95% CI 0.99–1.06) per increase in inverse-rank normalized plasma protein NPX] and using the missense variant rs2228145 [1.03 (1.00–1.06)]. When IL-6R variants were weighted by their effect on CRP, the point estimates suggested a positive association with endometrial cancer risk, but estimates were imprecise [*cis* variants: OR = 1.30 (95% CI 0.91–1.85); missense variant rs2228145: 1.38 (0.99–1.92) by decrease in natural log-transformed CRP]. An instrument using *cis* CRP variants was not associated with endometrial cancer risk [OR = 1.07 (95% CI 0.89–1.29) by decrease in natural log-transformed CRP].

For all proteins instrumented using *cis* variants, there was no evidence for colocalization [i.e., the probability that the association with both plasma protein and endometrial cancer risk was at a shared causal variant (h4) <0.8 (Supplementary Table S7)].

For proteins instrumented using *trans* variants (IL6 and HSD11B1), direction of estimates from sensitivity analyses (weighted-median, weighted-mode, MR-Egger, and MR-CAUSE) were consistent with primary analysis (IVW method; Supplementary Table S8). In MR-CAUSE analysis, the ELPD score was negative but there was limited evidence to support the causal model over the sharing model for both IL-6 (P-value = 0.20) and HSD11B1 (P-value = 0.34). The leave-one-out analysis suggests that observed association for IL-6 and HSD11B1 were not driven by specific variants (Supplementary Figure S3). Scatter plots of results comparing MR-Egger with other methods did not suggest directional pleiotropic effects (Supplementary Figure S4). Results from Steiger test suggest genetically instrumented protein levels affecting endometrial cancer risk was the more likely causal direction (Supplementary Table S9).

Discussion

We explored the associations between 152 inflammation and immune response biomarkers and risk of endometrial cancer in 624 case-control pairs nested within the EPIC cohort. This study included the largest number of pre-diagnostic plasma protein biomarkers and double the number of endometrial cancer cases compared with previous studies.[9–12] Results from primary analysis suggested plasma IL-6, PIK3AP1 and CLEC4G might be positively associated with endometrial cancer risk, and HSD11B1, SCF, and CCL25 might be inversely associated with risk. In complementary MR analyses, IL-6 was positively associated with endometrial cancer risk, and HSD11B1 was inversely associated with risk, consistent with findings from the nested case-control analysis. Results from MR analysis for IL-6R suggest a positive association with endometrial cancer risk.

The complementary nested case-control and MR analyses in large non-overlapping study samples improved causal inference and triangulation of evidence for protein biomarkers and their association with endometrial cancer risk. Both observational and MR analyses were performed in study populations of

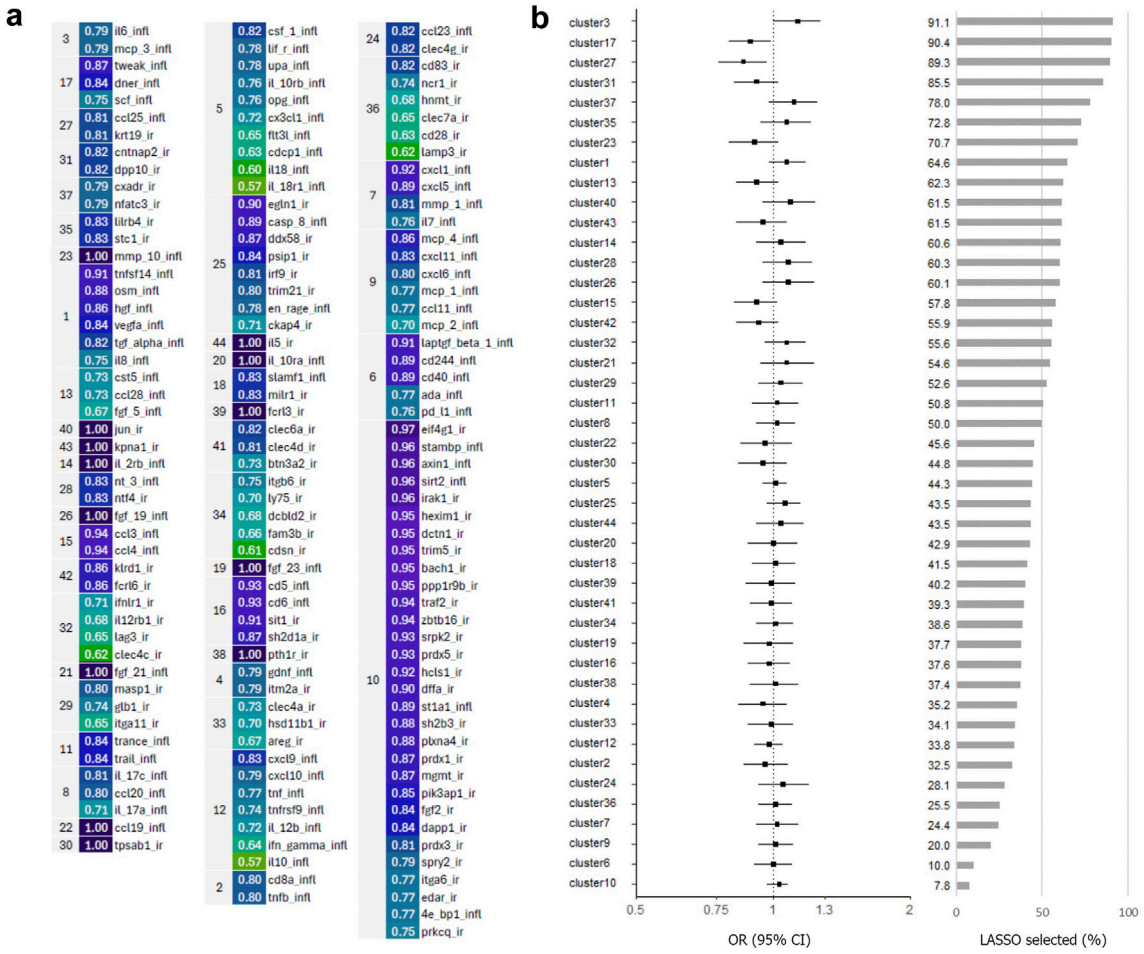

Fig. 2: (a) Composition of the 44 protein clusters, including 31 clusters of correlated proteins and 13 individual proteins. The heat map represents the Pearson correlation between each cluster centre (i.e., the 1st principal component) and their respective proteins; (b) Odds ratio (OR) and 95% confidence interval (CI) for the association between each cluster and endometrial cancer risk, estimated from the primary analysis model including body mass index, physical activity, smoking status, age at menarche, parity, ever use of oral contraceptives, and ever use of hormone replacement therapy as covariates. Clusters are ranked by the proportion out of 1000 bootstrap samples that were selected by LASSO regression.

predominantly European ancestries, the applicability of our findings in other ethnicities thus requires further investigation. The prospective data from the nested case-control study allowed us to establish temporality between biomarker measurements and endometrial cancer incidences, minimizing the risk of reverse causation in observed associations. We considered potential confounding by reproductive and lifestyle factors, adiposity, serum C-peptide, oestradiol (post-menopausal), and oestrone (postmenopausal) levels. However, residual confounding cannot be ruled out. The MR analysis leverages on the random allocation of genetic variants to reduce risk of reverse causation and confounding by factors that may not be accounted for in the traditional observational study.[33] There are several limitations to the MR analyses. Sex-specific GWAS for protein biomarkers were not available, although emerging evidence suggests the genetic regulation of plasma proteome is largely similar between sexes.[33] IL-6 and HSD11B1 were associated with endometrial cancer risk using *trans* variants as instruments, as both proteins had no *cis* variants associated with at $P < 5 \times 10^{-8}$. We thus performed additional analyses to examine the robustness of these findings. The Steiger test results suggest the observed associations were unlikely due to reverse causation. Results from MR-Egger regression did not indicate directional pleiotropy. However, there was limited evidence for causal effects in the MR-CAUSE analysis. Both MR-Egger and MR-CAUSE may be under-powered to detect horizontal pleiotropy in our study. We therefore cannot rule out that the associations observed for IL-6 and HSD11B1 were driven by other

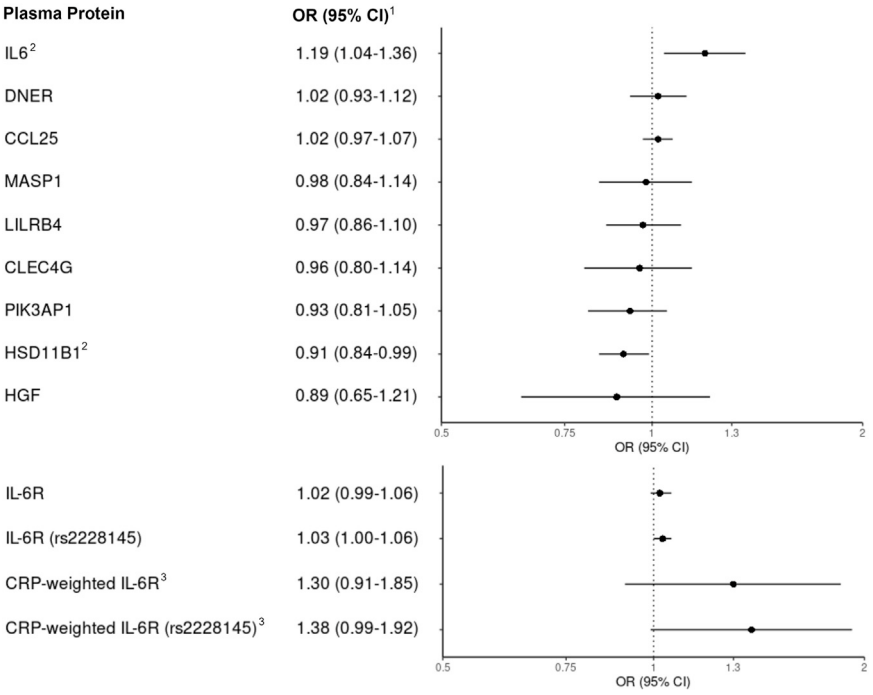

Fig. 3: The association between genetically instrumented plasma protein levels and endometrial cancer risk in Mendelian randomization analyses. [1]The OR (95% CI) estimated from MR analyses are not comparable with those estimated from the nested case-control study, as the units used for the GWAS are different. The UKB-PPP GWAS for plasma proteins were measured in inverse-rank normalized NPX. The CHARGE + UK Biobank GWAS for CRP was measured in natural log-transformed CRP. [2]No *cis* variant for IL-6 and HSD11B1 reached the $P < 5 \times 10^{-8}$ threshold. These estimates were obtained using *trans* variants selected using linkage disequilibrium clumping at $r < 0.001$. [3]CRP-weighted IL6R results were obtained using GWAS data for CRP (CHARGE + UKB). The estimates have been inverted to correspond to endometrial cancer risk for decrease in CRP, a proxy measure for increase in plasma IL-6R.

traits associated with the *trans* variants used to instrument these proteins. We complemented the MR analysis for IL-6 with IL-6R, which was not assayed in our case-control study but has been extensively studied in the MR literature. Previous MR studies have demonstrated that downregulation of IL-6 classical signalling modelled by IL-6R variants were associated with lower coronary heart disease[22,23] and reduced progression to critically-ill[24,25] leading to subsequent clinical trials with IL-6 receptor antagonists. However, we were not able to corroborate MR findings with colocalization.

The exploratory nature of our nested case-control study limited our certainty in drawing causal conclusions from observed associations, with no estimates surviving adjustment for multiple comparisons in our primary analysis; however, we were able to draw some parallels with previous nested case-control studies. In the Prostate, Lung, Colorectal and Ovarian (PLCO) cohort, VEGFA and SERPINE1were positively associated, and CCL3, IL-13, IL-21, IL-1β, and IL-23 were inversely associated with endometrial cancer risk; while positive associations for IL-6, TNFα, and CRP attenuated after adjusting for BMI.[11] VEGFA production is stimulated by IL-6, which could promote angiogenesis in solid tumors.[34] In a study nested in the Women's Health Initiative Observational Cohort (WHI-OS), CRP, but not IL-6 and TNFα, was associated with endometrial cancer risk.[12] CRP is a prominent downstream biomarker of IL-6 signalling, although our *cis*-MR results for CRP suggest it was not causally related to endometrial cancer risk. In previous EPIC analyses, TNFα, sTNFR1, and sTNFR2 were associated with endometrial cancer risk, whereas the associations for CRP, IL-6, and IL-1Rα were attenuated after adjusting for BMI.[9,10] TNFα has been shown to increase IL-6 and MCP1 (CCL2) levels in ovarian and breast cancer cells, contributing to inflammation and cancer cell proliferation.[35,36] In contrast, we observed a protein cluster (Cluster 17) including TWEAK (TNF-related weak inducer of apoptosis) that might be inversely associated with endometrial cancer risk, suggesting inhibited TNF-related apoptosis might contribute to the early-stage of cancer development. Differences in follow-up time (i.e., degree of bias due to reverse causation), study population (e.g., proportion of pre-/post-menopausal women), and sample sizes might also contribute to discrepancies in results observed across studies.

We observed a suggestive positive association between genetically instrumented IL-6R and risk of endometrial cancer. The missense *IL6R* variant Asp358Ala (rs2228145) is associated with increased conversion of membrane IL-6R to soluble IL-6R, and thereby downregulates IL-6 classical signalling and potentially upregulates trans-signalling.[26] Classical IL-6R signalling is important for immune response, and its blockade has been associated with opportunistic infections.[27,37] The downregulation of classical IL-6 signalling might thus compromise immunosurveillance against cancer growth. Similarly, studies on CLEC4G have documented immune dysregulation through the suppression of hepatic T cell immunity.[38–40] This suppression has been associated with decreased activity against the Hepatitis-B virus and immune evasion in colon cancer and melanoma cells, highlighting the link between compromised immunity and cancer development.[38–40] In addition, a recent publication described the role of hepatocytes in inhibiting T cell surveillance in extrahepatic tumours through STAT3 signalling, which could be activated by IL-6 or other cytokines.[41] These findings collectively underscore the complexity of signalling pathways involved in inflammation and immune response in cancer development.

IL-6 trans-signalling has a wide range of activities, including pro-inflammation.[42] It is more difficult to determine whether there is an upregulation of IL-6 trans-signalling, as the IL-6 and soluble IL-6R complex could be neutralized by circulating gp130 protein before reaching target cells.[42] Previous studies have reported that trans-signalling of IL-6 on endothelial and smooth cells led to secretion of MCP1 (also called CCL2).[37,42] In our nested case-control study, we observed a positive association between endometrial cancer risk and a protein cluster (Cluster 3) of IL6 and MCP3 (also called CCL7), which shares the closest structural homology and functions as MCP1 of the C-C motif chemokines.[43] MCP1 was also associated with endometrial cancer risk in similar magnitude as MCP3 but with wider confidence interval, suggesting a possible upregulation of IL-6 trans-signalling. In endometrial cancer, IL-6 can also promote tumour growth in a paracrine manner, by increasing aromatase expression and oestrogen production in the surrounding stromal cells.[44]

Plasma HSD11B1, an enzyme that converts cortisol and corticosterone into their active forms in tissues, was inversely associated with endometrial cancer risk in both observational and MR analyses. Notably, the inverse association between HSD11B1 and endometrial cancer risk was only observed in women with BMI $\geq$25 kg/m^2 in the nested case-control study. While increased HSD11B1 activity in adipose tissue and liver can contribute to insulin resistance development, a previous study in individuals with obesity has observed that the activity of HSD11B1 was inhibited.[45] Moreover, a restoration in HSD11B1 activity was observed in individuals who underwent significant weight loss.[46] These studies suggest that reduced HSD11B1 activity in obesity might be a compensatory mechanism to insulin resistance,[45,46] and might partly explain the elevated cortisol secretion, but normal or low plasma concentration observed in obesity.[47] It is thus possible that the association we observed for HSD11B1 captured some of the effect of insulin resistance on endometrial cancer risk. However, further adjustment for C-peptide did not change the estimates for HSD11B1, suggesting there may be other pathways linking reduced HSD11B1 activity and endometrial cancer risk. Glucocorticoids have been shown to counter the mitogenic effect of excess oestrogen in endometrial tissue.[48,49] Reduced local activation of cortisol and corticosterone might therefore lead to less inhibitory mechanism against oestrogen-induced endometrial cell proliferation, increasing the risk of carcinogenesis. As data on oestradiol was not available in our study, the link between HSD11B1, glucocorticoids, and sex hormones requires further investigation.

Other potential associations with endometrial cancer risk observed in our nested case-control study include an inverse association for CCL25, also observed only in women with BMI $\geq$25 kg/m^2. A study on chemokine expressions in adipose tissue macrophage populations in mice with obesity observed that CCL25 is predominately expressed in a population of anti-inflammatory adipose tissue macrophage (DN-ATM) that is likely important for tissue repair.[50] We also observed a positive association for PIK3AP1, which was stronger when the analysis was restricted to type I endometrioid cancers. This is consistent with the carcinogenic pathway of PTEN mutation and subsequent increased PIK3AP1 activation commonly observed in early phase of type 1 endometroid cancers.[51] SCF was inversely associated with endometrial cancer risk, individually and in protein Cluster 17, and has been shown to downregulate IL-6 and MCP1 (CCL2) production.[52] HGF, which was positively associated with endometrial cancer risk, can be upregulated by IL-6, and is also expressed in endometrial stromal cells and has been demonstrated to induce endometrial cancer cell growth.[53] These biomarkers may represent pathways in endometrial carcinogenesis and merit a more in-depth examination.

In conclusion, using a complementary approach of nested case-control and MR analyses, we identified circulating biomarkers that may represent potential inflammation and immune response pathways associated with endometrial cancer risk. In particular, altered IL-6 signalling and reduced glucocorticoid activity via the HSD11B1 enzyme might contribute to endometrial carcinogenesis.

Contributors

SEW: conceptualization, data curation, formal analysis, methodology, visualization, writing (original draft), writing (review & editing).

VV: conceptualization, funding acquisition, methodology, writing (review & editing).

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ND: methodology, writing (review & editing).
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TT: resources, writing (review & editing).
GS: resources, writing (review & editing).
RK: resources, writing (review & editing).
RTF: resources, writing (review & editing).
MBS: resources, writing (review & editing).
BB: resources, writing (review & editing).
SS: resources, writing (review & editing).
RT: resources, writing (review & editing).
CS: resources, writing (review & editing).
SP: resources, writing (review & editing).
MC: resources, writing (review & editing).
MS: resources, writing (review & editing).
AA: resources, writing (review & editing).
DRP: resources, writing (review & editing).
MG: resources, writing (review & editing).
RCT: resources, writing (review & editing).
KKT: resources, writing (review & editing).
AH: resources, writing (review & editing).
JY: methodology, writing (review & editing).
SR: conceptualization, funding acquisition, resources, writing (review & editing).
MJG: conceptualization, funding acquisition, resources, writing (review & editing).
LD: conceptualization, funding acquisition, data curation, resources, investigation, supervision, writing (review & editing).
All authors read and approved the final version of the manuscript.

Data sharing statement

The nested case-control study data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. EPIC data and biospecimens are available for investigators who seek to answer important questions on health and disease in the context of research projects that are consistent with the legal and ethical standard practices of the International Agency for Research on Cancer (IARC), WHO, and the EPIC centres. The primary responsibility for accessing the data, obtained in the frame of the present publication, belongs to the EPIC centres that provided them. Access to EPIC data can be requested to the EPIC Steering Committee, as detailed in the EPIC-Europe Access Policy (https://epic.iarc.who.int/access/). The UK Biobank Pharma Proteomics Project genome-wide association study (GWAS) summary data are publicly available from UK Biobank (https://doi.org/10.7303/syn51364943). The Endometrial Cancer Association Consortium (ECAC) GWAS summary data excluding UK Biobank participants were obtained directly from the first author of the GWAS (https://doi.org/10.1038/s41467-018-05427-7). They are available upon request from the corresponding author with the permission of the authors of the ECAC GWAS. The meta-analyzed Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and UK Biobank GWAS summary data (ID: GCST90029070) are publicly available from the NHGRI-EBI GWAS Catalog (https://ftp.ebi.ac.uk/pub/databases/gwas/).Statistical analysis codes used for this study are available on https://github.com/sabrinawang113/PROEC and https://github.com/sabrinawang113/PROECMR.

Declaration of interests

EJC is the president of the Peaches Womb Cancer Trust and the research advisory committee chair of the Eve Appeal, both roles are voluntary and unpaid. All other authors declare no conflicts of interest.

Acknowledgements

The coordination of EPIC-Europe is financially supported by International Agency for Research on Cancer (IARC) and by the Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London which has additional infrastructure support provided by the NIHR Imperial Biomedical Research Centre (BRC).

The national cohorts are supported by: Danish Cancer Society (Denmark); Ligue Nationale Contre le Cancer, Institut Gustave-Roussy, Mutuelle Générale de l'Education Nationale (MGEN), Institut National de la Santé et de la Recherche Médicale (INSERM), French National Research Agency (ANR, reference ANR-10-COHO-0006), French Ministry for Higher Education (subsidy 2102918823, 2103236497, and 2103586016) (France); German Cancer Aid, German Cancer Research Center (DKFZ), German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Federal Ministry of Education and Research (BMBF) (Germany); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy, Compagnia di SanPaolo and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS) - Instituto de Salud Carlos III (ISCIII), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology - ICO (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (C864/A14136 to EPIC-Norfolk; C8221/A29017 to EPIC-Oxford), Medical Research Council (MR/N003284/1, MC-UU_12015/1 and MC_UU_00006/1 to EPIC-Norfolk; MR/M012190/1 to EPIC-Oxford) (United Kingdom). Previous support has come from "Europe against Cancer" Programme of the European Commission (DG SANCO).

We thank the Norwegian Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands for their contribution and ongoing support to the EPIC Study; Bertrand Hémon for his support with the EPIC database; and all EPIC participants. The authors acknowledge the use of data and biological samples from EPIC-Utrecht (principal investigator: Roel Vermeulen) and EPIC-Norfolk (principal investigator: Nick Wareham).

EJC is funded by a National Institute for Health and Care Research (NIHR) Advanced Fellowship (NIHR300650) and the NIHR Manchester Biomedical Research Centre (NIHR203308).

TO'M is funded by a National Health and Medical Research Council of Australia (NHMRC) Emerging Leadership Fellowship (APP1173170).

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/World Health Organization.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105341.

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