Cigarette Smoking and Endometrial Cancer Risk: Observational and Mendelian Randomization Analyses

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ABSTRACT

Background: Current epidemiologic evidence indicates that smoking is associated with a lower endometrial cancer risk. However, it is unknown if this association is causal or confounded. To further elucidate the role of smoking in endometrial cancer risk, we conducted complementary observational and Mendelian randomization (MR) analyses.

Methods: The observational analyses included 286,415 participants enrolled in the European Prospective Investigation into Cancer and Nutrition and 179,271 participants in the UK Biobank, and multivariable Cox proportional hazards models were used. In two-sample MR analyses, genetic variants robustly associated with lifetime amount of smoking ($n = 126$ variants) and ever having smoked regularly ($n = 112$ variants) were selected and their association with endometrial cancer risk (12,906 cancer/108,979 controls from the Endometrial Cancer Association Consortium) was examined.

Results: In the observational analysis, lifetime amount of smoking and ever having smoked regularly were associated with a lower endometrial cancer risk. In the MR analysis accounting for body mass index, a genetic predisposition to a higher lifetime amount of smoking was not associated with endometrial cancer risk (OR per 1-SD increment: 1.15; 95% confidence interval: 0.91–1.44). Genetic predisposition to ever having smoked regularly was not associated with risk of endometrial cancer.

Conclusions: Smoking was inversely associated with endometrial cancer in the observational analyses, although unsupported by the MR. Additional studies are required to better understand the possible confounders and mechanisms underlying the observed associations between smoking and endometrial cancer.

Impact: The results from this analysis indicate that smoking is unlikely to be causally linked with endometrial cancer risk.

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Introduction

Endometrial cancer is one of the most common cancers in women globally with an estimated number of 417,367 new cases in 2020 (1). Exposure to unopposed estrogens is an established risk factor for endometrial cancer (2–6) and can, at least partially, explain the association of factors such as obesity, nulliparity, and later age at menopause with an increased endometrial cancer risk (7, 8). On the contrary, cigarette smoking has been consistently associated with a lower risk of developing endometrial cancer (9–11), including in a recent umbrella review of prospective and case-control studies (12). However, the biological mechanisms that underlie this association are unclear. Although it has been widely suggested that smoking may reduce risk via its antiestrogenic effects, smoking has not been clearly associated with lower circulating estradiol levels (13). Thus, it is unclear if the suggested inverse association of smoking with endometrial cancer risk found in many observational studies is causal or a result of bias from unknown or inaccurately measured confounders.

Mendelian randomization (MR) is a method that aims to establish potential causal associations triangulating evidence from other study designs because it is less prone to many sources of confounding and reverse causality (for instance participants may quit smoking due to comorbidities prior to cancer development), as genetic variants are randomly assigned and fixed at conception (14). To comprehensively examine the association between smoking and endometrial cancer risk, we conducted complementary observational and MR analyses. First, we investigated the association between smoking-related phenotypes (lifetime amount of smoking, and ever having smoked regularly) and subsequent endometrial cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) and UK Biobank. We then applied a two-sample MR to examine the potential causal association using genetic variants associated with lifetime amount of smoking (15) and ever having smoked regularly (16) from recent genome-wide association studies (GWAS), and tested whether these variants are related to endometrial cancer risk using data from the Endometrial Cancer Association Consortium (ECAC) (17).

Materials and Methods

Observational analyses

Study participants and data collection in EPIC and UK Biobank

EPIC is a multi-center cohort study including ∼520,000 adults recruited between 1992 and 2000 from ten European countries (n = 367,898 women, aged 35–75 years old at enrollment) (18). All participants provided written informed consent to participate in the EPIC study and the EPIC study was approved by the ethics committees of the International Agency for Research on Cancer (IARC) as well as every center. The UK Biobank is a prospective cohort of ∼502,000 adults from the general population across the UK (n = 273,383 women, aged between 40 and 69 years at recruitment) enrolled between 2006 and 2010 (19). This research has been conducted using the UK Biobank Resource under application number 41115.

In both cohorts, detailed information was collected on sociodemographics (including age, education, socioeconomic status), lifestyle factors (including smoking habits, physical activity, dietary intake, and alcohol consumption), reproductive, medical and anthropometric data at baseline. Participants provided information on current/former/never regular smoking status. Additional data on smoking intensity (number of cigarettes per day), smoking duration, and type of smoking (cigarettes, cigars, pipes) were also collected.

For the current analyses, we excluded the following individuals: men (n = 153,426 in EPIC, 229,122 in UK Biobank); women with prevalent cancer (excluding non-malignant skin cancer) at recruitment (identified by linkage to cancer registry data or active follow-up; n = 21,212 in EPIC, 21,217 in UK Biobank); women who had a history of hysterectomy (n = 35,970 in EPIC, 44,496 in UK Biobank); and women with no follow-up data (n = 2,431 in EPIC). In EPIC, we also excluded 7,541 participants with missing information on smoking status. In UK Biobank, 28,380 women who smoked occasionally in the past (as regular cigarette smoking was the focus of our study; n = 27,260 occasional smokers were excluded) or who had an unknown smoking status, were also excluded. Participants from EPIC-Greece were not included in this analysis (n = 14,248).

Ascertainment of endometrial cancer cases

The endpoint was first diagnosis of incident epithelial invasive endometrial cancer. Endometrial cancer was defined using the 10th Revision of the International Classification of Diseases (ICD-10: C54). Endometrial cancer cases were classified into type I and II using the histology codes from the International Classification of Diseases for Oncology (ICDO-3). Type I histologies (n = 1687 in EPIC, n = 606 in UK Biobank) included the following morphology codes: 8380, 8560, 8570, 8140, 8210, 8480, and 8481. Type II histologies (n = 94 in EPIC, n = 66 in UK Biobank) included codes: 8070, 8310, 8323, 8441, 8460). Cases with other or unknown histologies (n = 100 in EPIC, n = 61 in UK Biobank) were not classified into either type (codes: 8000, 8001, 8010, 8012, 8020, 8050, 8255, 8260, 8322, 8340, 8382, 8574, 8950, 8951, and 8980). *In situ* endometrial cancer cases (n = 8) or cases with nonspecific endometrial cancer morphology codes (i.e., 8246, 8263, 8461, 8503, 8572, 8800, 8890, 8896, 8930, 8931, 8933, and 9100) were excluded (n = 73 in EPIC, n = 19 in UK Biobank). Thus, the observational analyses included 286,415 participants enrolled in EPIC and 179,271 participants in UK Biobank.

Statistical analysis

HRs and 95% confidence intervals (CI) were estimated using Cox proportional hazards models. Age was the primary time variable in all models. Time at entry was the age at recruitment. Exit time was the age at whichever of the following came first: endometrial cancer diagnosis, death, or the last date at which follow-up was considered complete. Models were stratified by age at recruitment (in one-year categories in EPIC, and 5-year categories in the UK Biobank to account that baseline hazard may change by age group), and recruitment assessment center in EPIC or region of recruitment (10 regions) in UK Biobank. Two smoking-related phenotypes were calculated in our main analyses for comparability with the MR results: (i) lifetime amount of smoking, and (ii) ever having smoked regularly.

Lifetime amount of smoking was represented by the comprehensive smoking index which is a function of smoking status (never, former, and current smokers), smoking intensity (number of cigarettes per day), and smoking duration (Lifetime smoking = $[(1-0.5^{dur/t})(0.5^{tsc/t})ln(int+1)]$; where int = cigarettes per day, tss = time started smoking, tsc = time since cessation, dur = duration of smoking (either $age\text{-}tss$ for current smokers or $[age\text{-}tsc]\text{-}tss$ for former smokers) and t = is a constant estimating half-time). It is equal to zero in never smokers, quantifies smoking intensity as the number of cigarettes smoked per day, uses age of smoking initiation and age of smoking cessation to capture smoking duration in current and former smokers, and a half-life constant that models the exponentially decreasing effect of smoking at a given time on health outcomes (20). Half-life constant was set to 18 as it was shown in a previous study that this optimizes the model

fitting of lifetime smoking on lung cancer and all-cause mortality (15). Results for lifetime amount of smoking are presented per 1 SD (SD) increment (SD = 0.7 in EPIC and UK Biobank) which, as stated by Wootton and colleagues, is equivalent to an individual smoking 20 cigarettes/day for 15 years and stopping 17 years ago or an individual smoking 60 cigarettes/day for 13 years and stopping 22 years ago (15). Ever having smoked regularly was represented by a binary phenotype (ever versus never having regularly smoked), with regular ever smokers classified as those women who indicated they smoked on most/all days at baseline (current regular smokers) or smoked on most/all days in the past (former regular smokers). Never regular smokers included women who had never smoked or who just tried once or twice. Effect estimates from EPIC and UK Biobank were combined using a fixed-effects meta-analysis. As secondary analyses, we also assessed the effect of smoking-related characteristics such as age of smoking initiation, duration of smoking, number of cigarettes, and years since quitting smoking in former smokers.

All Cox regression models were adjusted for the following endometrial cancer risk factors that were determined *a priori:* ever use of oral contraceptives (OC), ever use of menopausal hormone therapy (MHT), parity, age at menopause, body mass index (BMI), physical activity, type-2 diabetes, and educational level measured at baseline (follow-up measurements of these confounders were unavailable for most participants). Additional adjustment for coffee consumption was considered since coffee has been shown to be inversely associated with endometrial cancer risk in previous observational studies (21), but there was no substantial change observed in the association of smoking-related phenotypes and endometrial cancer risk; hence this covariate was excluded from the final model. Deviations from proportionality were assessed using an analysis of Schoenfeld residuals (22), with no evidence of non-proportionality being detected.

We further assessed associations of lifetime amount of smoking and ever having smoked regularly with endometrial cancer across subgroups of menopausal status at diagnosis (pre-, post-), MHT use (no, yes), BMI (<25, 25–30, >30 kg/m^2), and country of recruitment (EPIC only). Interaction terms (multiplicative scale) between these variables and smoking-related phenotypes were modelled, and the statistical significance of the cross-product terms were evaluated using the Wald test (departure from a multiplicative relation suggests heterogeneity between strata). We used a competing risk approach to assess whether the risks associated with ever smoking and lifetime smoking differed by tumor subtype (23). This approach uses a data augmentation method to create a separate observation for each subject for each subtype and then stratifies on event type (23). Heterogeneity by subtype was assessed through a likelihood ratio test of a model estimating separate associations for each subtype and a model evaluating the same association for both subtypes.

Statistical tests were all two-sided and a P value <0.05 was considered statistically significant. Analyses were conducted using SAS software version 9.4.

Mendelian randomization analysis

Data on lifetime amount of smoking and ever having smoked regularly

For the smoking-related phenotypes (i.e., lifetime amount of smoking and ever having smoked regularly), we selected genetic variants for the MR analysis on the basis of a genome-wide significant association (i.e., p-value threshold for inclusion at $<5 \times 10^{-8}$) using data from the largest GWAS conducted to date. More specifically, we extracted the associations of each genetic variant with smoking-related phenotypes; beta-coefficients and their standard errors from univariable regression models (linear regression models for lifetime amount of smoking and logistic regression models for ever having smoked regularly) on each variant genetic variant in turn. For lifetime amount of smoking, we extracted genetic variants from a GWAS in the UK Biobank that included 462,690 participants that were adjusted for genotyping chip and sex (15). Variants reported in a GWAS meta-analysis of the GSCAN consortium including 1,232,091 individuals of European ancestry were used to select genetic variants related to the phenotype "ever having smoked regularly" (covariates used are not specified explicitly) (16). We pruned the list of reported variants using a R^2 linkage disequilibrium (LD) threshold of <0.001, resulting in 126 variants for lifetime amount of smoking and 112 for ever having smoked regularly. These genetic variants explained ∼0.4% and ∼2.0% of the variance in lifetime amount of smoking and ever having smoked regularly, respectively. Previous MR studies using these two smoking-related phenotypes have shown robust positive associations with lung cancer (15, 24), thereby demonstrating good face validity of the instruments.

GWAS data for endometrial cancer

For smoking-related variants we extracted the values (beta coefficients/standard errors) from the models of the association of smoking-related variants with endometrial cancer using GWAS data from the ECAC involving 121,885 participants of European ancestry (12,906 endometrial cancer cases and 108,979 controls) (17). This GWAS includes data from previously published GWAS (4,891 cases/11,573 controls), studies analysed with the iCOGS (2,381 cases/13,675 controls), and OncoArray genotyping chip (4,710 cases/19,438 controls), WHI (288 cases/1,440 controls), and UK Biobank studies (636 cases/62,853 controls) (17). Adjustment for principal components was performed in all studies within ECAC.

Statistical analysis

A random-effects inverse-variance weighted (IVW) model was used in our main analysis (25, 26). The MR-Egger regression (27) and the estimator from the weighted median approach (28) were used to investigate the potential violation of the MR assumptions (29, 30) and to account for potential horizontal pleiotropic effects on the causal estimates. An estimated intercept term from the MR-Egger regression that deviates from zero is indicative of directional (non-balanced horizontal) pleiotropy (27). The Cochran's Q statistic was calculated to quantify the heterogeneity in MR effect sizes obtained from each of the genetic variants used in the smoking related-phenotypes (31). Outlying variants were detected using the MR pleiotropy residual sum and outlier test (MR-PRESSO) (p-value threshold set at <0.05) (32). Multivariable IVW MR analyses (33), accounting for BMI, alcohol consumption and educational attainment were also considered to control for possible pleiotropic effects as high genetic correlations between these phenotypes have been previously reported (16, 34). Multivariable IVW MR analysis is a direct extension of the univariable approach where two or more exposures are analyzed simultaneously on the same regression model analyses (33). For multivariable MR analysis accounting for BMI, we used variants reported in a GWAS meta-analysis of the GIANT consortium and the UK Biobank with∼700,000 individuals of European ancestry (35), and obtained female-specific estimates retrieved from a previous GWAS of the GIANT consortium, with data available for 171,977 women (36). For alcohol consumption (quantified as drinks per week), we obtained genetic variants from a GWAS data on 537,349 participants (16). Multivariable MR analyses were also performed for educational attainment to account for possible pleiotropy between socioeconomic

status and smoking-related phenotypes including approximately 1.1 million European ancestry individuals from 71 cohorts (37). Variants that were associated with BMI, alcohol consumption and educational attainment at a genome-wide significance level and were independent of smoking-related variants (R^2 LD < 0.001) were also included in the multivariable MR models (Supplementary Tables S1 and S2). Missing regression coefficients of the association of any variant on BMI, alcohol consumption, educational attainment and on endometrial cancer were replaced by those of a suitable proxy genetic variant (minimum LD $R^2 = 0.8$) where available.

The MR statistical analyses were implemented in the Mendelian randomization R package (38).

Data availability

Data used in the MR analyses can be found in supplementary material. Researchers can apply to use the UK Biobank dataset by registering and applying at http://ukbiobank.ac.uk/register-apply/. For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at https://login.research4life.org/tacsgr0epic_iarc_fr/access/index.php.

Results

Observational analysis

After a mean follow-up time of 8.8 years among 286,415 women, 1,881 incident endometrial cancers were recorded in EPIC. After a mean follow-up time of 7.1 years among 179,271 women in UK Biobank, 733 incident endometrial cancer cases were recorded. The mean age at recruitment in EPIC was 50.2 (SD: 9.8) and 55.3 (SD: 8.1) years in UK Biobank. In EPIC, 60.2% had ever used OC and 22.4% MHT whereas the respective percentages in UK Biobank were 81.2% and 30.3%. Among EPIC participants, 44.1% had ever smoked regularly compared with 30.1% in UK Biobank (**Table 1**).

Association of lifetime amount of smoking with endometrial cancer risk

In the multivariable Cox regression models, lifetime amount of smoking was associated with lower endometrial cancer risk [HR per 1-SD increment, 0.87; 95% confidence interval (95% CI), 0.83–0.91] with no heterogeneity found across the two cohorts (HR, 0.87; 95% CI, 0.82–0.92 in EPIC; HR, 0.86; 95% CI, 0.79–0.93 in UK Biobank; $I^2 = 0$; **Fig. 1**). No heterogeneity was observed when stratifying by menopausal status ($P_{interaction} = 0.29$ in EPIC; $P_{interaction} = 0.90$ in UK Biobank), MHT use, histologic subtype, BMI (**Fig. 2A**) and in EPIC, by country of data collection ($P_{interaction} > 0.2$; Supplementary Table S3).

Association of ever having smoked regularly with endometrial cancer risk

Compared with never smoking, ever having smoked regularly was inversely associated with endometrial cancer risk (HR, 0.80; 95% CI, 0.74–0.87) with no heterogeneity found across the two cohorts (HR, 0.82; 95% CI, 0.74–0.90 in EPIC; HR, 0.75; 95% CI, 0.63–0.89 in UK Biobank; $I^2 = 0$; **Fig. 1**). Results of similar magnitude were found for ever versus never having smoked among former (HR, 0.83; 95% CI, 0.74–0.93 in EPIC; HR, 0.80; 95% CI, 0.67–0.96 in UK Biobank) and current smokers (HR, 0.80; 95% CI, 0.70–0.91 in EPIC; HR, 0.53; 95% CI, 0.36–0.79 in UK Biobank; Supplementary Table S4). No heterogeneity was observed in this association by menopausal status or any of the other factors assessed, with the exception of country of data collection in EPIC ($P_{interaction} = 0.03$; **Fig. 2B**; Supplementary Table S3).

Association of smoking-related characteristics with endometrial cancer risk

Other metrics of smoking, such as smoking duration, and number of cigarettes smoked per day were not associated with endometrial cancer risk in both current or former smokers. Age of smoking initiation was inversely associated with endometrial cancer risk in UK Biobank ($HR_{<16 \text{ versus } 26+ \text{ years}}$: 0.45; 95% CI, 0.23–0.87) but not in EPIC ($HR_{<16 \text{ versus } 26+ \text{ years}}$: 0.91; 95% CI, 0.67–1.24). In former smokers, time since smoking cessation was not associated with endometrial cancer risk in either study ($HR_{<10 \text{ versus } 20+ \text{ years}}$: 0.85; 95% CI, 0.66–1.09 in EPIC; $HR_{<10 \text{ versus } 20+ \text{ years}}$: 1.30; 95% CI, 0.89–1.90 in UK Biobank; Supplementary Table S4).

Mendelian randomization analyses

Mendelian randomization estimates for lifetime amount of smoking

In the IVW random effects model, genetic predisposition to higher lifetime amount of smoking was positively associated with risk of endometrial cancer (OR per 1-SD increment, 1.31; 95% CI, 1.06–1.62; $P = 0.01$; **Table 2**) with no evidence for heterogeneity (Cochran's Q $P = 0.18$). No outlying variants were detected using the MR-PRESSO test. However, the MR-Egger test showed evidence of pleiotropy (MR-Egger intercept P values = 0.02), and an inverse nonsignificant association was found for the MR-Egger model that accounted for pleiotropy (OR, 0.50; 95% CI, 0.21–1.16; $P = 0.11$). In addition, the weighted median approach showed little evidence of an association of lifetime amount of smoking and endometrial cancer risk (OR, 1.05; 95% CI, 0.77–1.43; $P = 0.75$). In the multivariable MR analyses that adjusted for BMI, no association was found between lifetime amount of smoking and endometrial cancer risk (OR, 1.15; 95% CI, 0.91–1.44; $P = 0.25$; **Fig. 1**; **Table 2**). Similar positive associations with the main IVW analysis were found in the multivariable MR analyses that accounted for alcohol consumption (OR, 1.40; 95% CI, 1.11–1.77; $P = 0.01$), while results were attenuated after controlling for educational attainment (OR, 1.04; 95% CI, 0.80–1.36; $P = 0.75$).

Mendelian randomization estimates for ever having smoked regularly

In the random-effects IVW model, we found no association between genetic predisposition to ever compared with never having smoked regularly and risk of endometrial cancer (OR, 1.05; 95% CI, 0.89–1.25; $P = 0.57$), with evidence for heterogeneity between the MR effect sizes obtained from each of the genetic variants used (Cochran's Q $P = 0.02$; **Table 2**). A similar null result was found for the MR Egger and weighted median approaches. There was no evidence of aggregated directional pleiotropy using MR-Egger ($P_{pleiotropy} = 0.77$). The multivariable analyses that adjusted for BMI also showed no association between ever having smoked regularly and endometrial cancer (OR, 0.99; 95% CI, 0.83–1.19; $P = 0.95$; **Fig. 1**; **Table 2**). Similar, nonsignificant associations were also found in the multivariable analyses that accounted for alcohol consumption and educational attainment.

Discussion

In observational analyses in EPIC and UK Biobank, we found that lifetime amount of smoking and ever having smoked regularly were inversely associated with endometrial cancer risk. These relationships were consistent across subgroups of menopausal status, MHT use, BMI, and histologic subtype. However, there was little evidence that smoking duration and smoking intensity were associated with endometrial cancer risk. In the MR analyses, although initial analyses suggested that lifetime amount of smoking was positively associated

Table 1. Characteristics of the European Prospective Investigation into Cancer (EPIC) ($N = 286,415$ participants) and UK Biobank study participants ($N = 179,271$ participants).

	EPIC ($N = 286,415$)	UK Biobank ($N = 179,271$)
N Endometrial cancer cases	1,881	733
Person-years	4,054,375	1,271,115
Age at recruitment (years)[a]	50.2 (9.8)	55.3 (8.1)
Body mass index (kg/m^2)[a]	24.7 (4.3)	26.9 (5.2)
Missing	0.0%	0.5%
Physical activity		
Inactive/low	20.4%	14.6%
Moderately inactive	34.2%	33.7%
Moderately active	28.0%	
Active/high	15.8%	29.7%
Missing	1.6%	22.0%
Education		
None or primary school completed	26.5%	
Technical/professional or secondary school	46.2%	
Longer education	23.7%	
None		14.9%
NVQ/HND/HNC		4.2%
O-level, CSE or equivalent		28.4%
A-level, college or university		45.4%
Other professionals		5.5%
Missing	3.6%	1.6%
Ever menopausal hormone therapy use		
Yes	22.4%	30.3%
Missing	7.1%	0.4%
Ever oral contraceptive use		
Yes	60.2%	81.2%
Missing	2.7%	0.4%
Parity		
None	15.3%	20.2%
≥1	80.3%	79.6%
Missing	4.4%	0.1%
Menopausal status		
Postmenopausal	43.7%	64.7%
Missing	0.0%	5.4%
Type 2 diabetes		
Yes	1.9%	3.2%
Missing	8.0%	0.0%
Lifetime amount of smoking[a,b]	0.4 (0.7)	0.3 (0.7)
Missing	14.1%	0.7%
Ever having smoked regularly		
Never	55.9%	69.9%
Ever	44.1%	30.1%
Smoking status		
Never	55.9%	69.9%
Former	23.7%	22.5%
Current	20.4%	7.7%
Number of cigarettes per day in current smokers		
1–15	63.8%	67.3%
16–25	28.0%	27.0%
≥26	4.4%	4.3%
Missing	3.9%	1.4%
Years since quitting smoking in former smokers		
≤10	36.8%	30.4%
>10–≤20	30.8%	26.7%
>20	28.5%	42.3%
Missing	3.9%	0.6%

[a]Mean and SD.
[b]Lifetime amount of smoking is a function of smoking status (never, former, and current smokers), smoking intensity (number of cigarettes per day), and smoking duration.

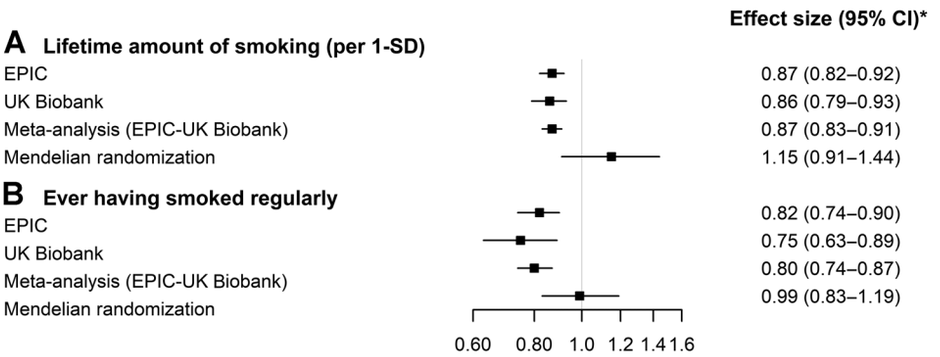

Figure 1.

Observational and Mendelian randomization estimates for (**A**) lifetime amount of smoking and (**B**) ever having smoked regularly and endometrial cancer risk. EPIC - observational analysis: Multivariable Cox regression model using age as the underlying time variable stratified by recruitment assessment center, and age category (1-year categories). Models adjusted for use of menopausal hormone therapy (ever, never, unknown); oral contraceptive use (ever, never, unknown); age of menopause (<50, 50–52, 53–55, >55 years, not applicable, unknown); parity (0, ≥1, unknown); body mass index (< 20, 20–<22.5, 22.5–<25, 25–<27.5, 27.5–<30, 30–<32.5, 32.5–<35, >35 kg/m^2); education (none or primary school completed, technical/professional or secondary school, longer education, unknown); physical activity (inactive, moderate inactive, moderate active, active, unknown); and type 2 diabetes (no, yes, unknown). UK Biobank - observational analysis: Multivariable Cox regression model using age as the underlying time variable stratified by region of recruitment assessment center, and age category (5-year categories). Models adjusted for use of menopausal hormone therapy (ever, never, unknown); oral contraceptive use (ever, never, unknown); age of menopause (<50, 50–52, 53–55, >55 years, not applicable, unknown); parity (0, ≥1, unknown); body mass index (<20, 20–<22.5, 22.5–<25, 25–<27.5, 27.5–<30, 30–<32.5, 32.5–<35, >35 kg/m^2); education (none, NVQ/HND/HNC, O-level, CSE or equivalent, A-level, college or university, other professionals, unknown); physical activity (low, moderate, high, unknown); and type 2 diabetes (no, yes, unknown). *HRs are reported for the observational analyses in EPIC and UK Biobank, whereas ORs from the multivariable analyses accounting for body mass index are reported in the Mendelian randomization analyses.

with endometrial cancer risk, there was evidence for pleiotropy and adjustment for BMI and educational attainment attenuated this association to the null. Ever having smoked regularly was also not associated with endometrial cancer risk in the Mendelian randomization analysis. Collectively, the observed inverse association of smoking with endometrial cancer risk was not supported by the MR analyses although alternative pleiotropic pathways cannot be ruled out.

The inverse association of smoking and endometrial cancer risk we observed in our observational analyses is not unexpected and many cohort studies have reported similar findings (12). However, it has been difficult to identify possible biological mechanisms that underlie this association. It has been proposed that smoking may reduce risk via its potential antiestrogenic effects (39) although, a pooled analyses of 13 studies has shown that postmenopausal women who smoked 15+ cigarettes/day actually had higher circulating estradiol levels compared with nonsmokers (13), suggesting that this pathway may not be relevant. It has also been suggested that cigarette smoking may increase risk via polyaromatic hydrocarbons from cigarette smoke that increase levels of anticarcinogenic metabolites of estradiol and suppress estrogen receptor function (40), although additional experimental studies are needed to confirm this hypothesis.

Smoking is associated with an earlier age at menopause (41), and thus may lower the risk of developing endometrial cancer due to a reduced exposure to endogenous circulating levels of ovarian-derived estrogens that characterise the menopause. However, adjustment for age at menopause had minimal impact on the smoking–endometrial cancer relationship and confirmed our previous findings in EPIC (10). These previous analyses in EPIC (involving 619 endometrial cancer cases) found an inverse association of smoking with endometrial cancer risk in postmenopausal women, and a positive association in women who were premenopausal at recruitment (limited to current smokers). However, these results were based on a relatively small number of cases ($n = 126$; ref. 10) and our current analyses that include 397 premenopausal endometrial cancer cases in EPIC and 102 in UK Biobank show no association.

We performed several statistical tests to test for potential violations of the MR assumptions. The first assumption (i.e., that the genetic variants are strongly associated with smoking-related phenotypes) was satisfied using genetic variants associated with smoking at a genome-wide significance level. To test for potential violation of the second and third MR assumptions (i.e., that the genetic variants are not associated with any confounder of the smoking-endometrial cancer association and are conditionally independent of endometrial cancer, given smoking and all confounders), we employed MR Egger, weighted median, MR-PRESSO, and multivariable approaches. Although we initially found a positive effect estimate for lifetime amount of smoking with endometrial cancer risk, other methods that account for pleiotropy (weighted median and multivariable MR approaches) found no association. Further, in multivariable MR analyses that accounted for possible pleiotropic effects with BMI and educational attainment, the association of smoking and endometrial cancer risk was attenuated to the null, suggesting the association is unlikely to be causal. Although we acknowledge that pleiotropy with additional phenotypes (apart from those included in the multivariable MR models such as age of first birth and high-density lipoprotein cholesterol) may still be present (i.e., the selected variants are not specific or sensitive to smoking related phenotypes) (16, 34), the genetic variants have shown robust associations with lung cancer (15, 24), colorectal and breast cancer risk (24, 42), oral/ oropharyngeal cancer (43), coronary artery disease (44) and diabetes (45), suggesting they are good proxies of smoking-related phenotypes. The MR-Egger results suggested an inverse non-significant association for lifetime amount on smoking and endometrial cancer risk although this result could be unreliable due to a low I^2_{GX} statistic (50% for lifetime amount of smoking), a metric that tests the suitability of this method (46); a multivariable version of this test would be similarly unreliable (47). In line with the findings of a previous MR study in UK Biobank (48), ever having smoked regularly was not associated with endometrial cancer risk.

We conducted comprehensive observational analyses in EPIC and UK Biobank with more than 4 times the number of endometrial

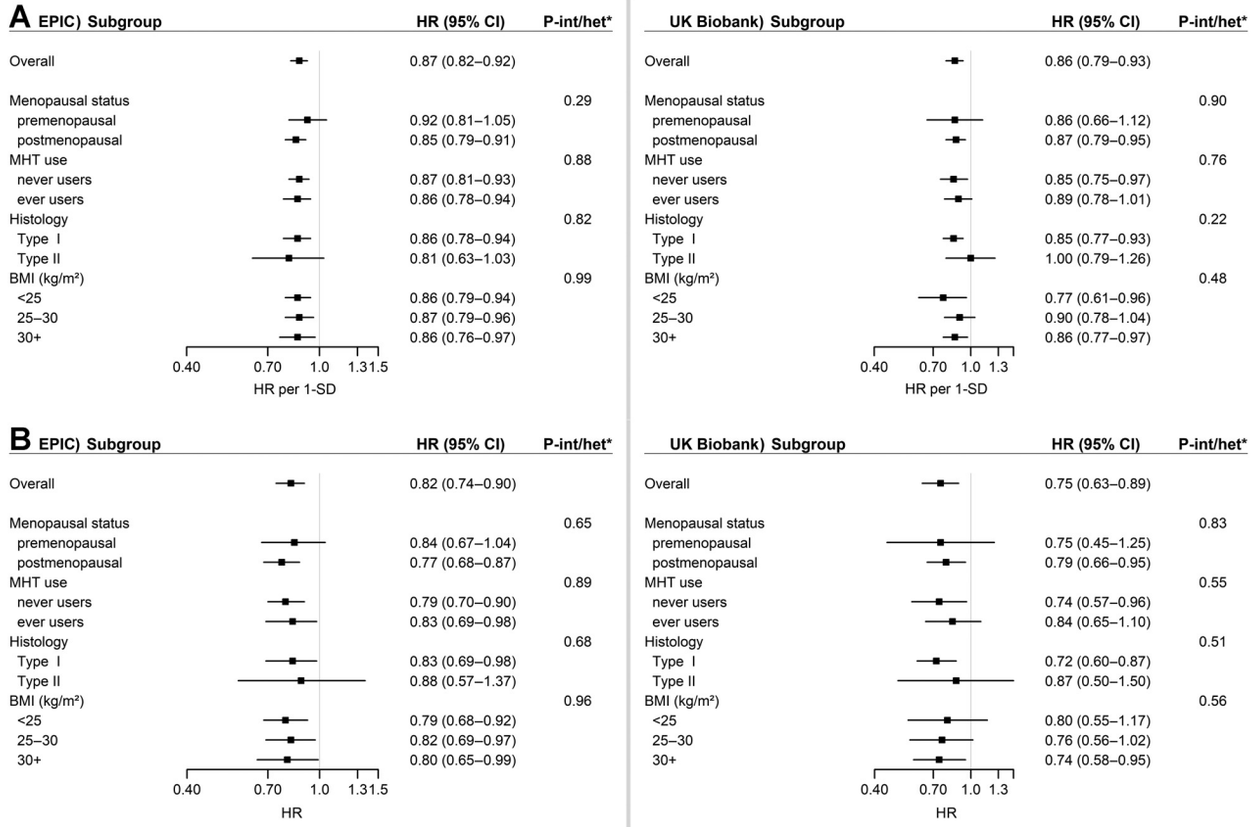

Figure 2.

Subgroup analyses of association between (**A**) lifetime amount of smoking (per 1-SD increment) and (**B**) ever having smoked regularly and endometrial cancer risk. EPIC - observational analysis: Multivariable Cox regression model using age as the underlying time variable stratified by recruitment assessment center, and age category (1-year categories). Models adjusted for use of menopausal hormone therapy (ever, never, unknown); oral contraceptive use (ever, never, unknown); age of menopause (<50, 50–52, 53–55, >55 years, not applicable, unknown); parity (0, ≥1, unknown); body mass index (<20, 20–<22.5, 22.5–<25, 25–<27.5, 27.5–<30, 30–<32.5, 32.5–<35, >35 kg/m^2); education (none or primary school completed, technical/professional or secondary school, longer education, unknown); physical activity (inactive, moderate inactive, moderate active, active, unknown); type 2 diabetes (no, yes, unknown). UK Biobank - observational analysis: Multivariable Cox regression model using age as the underlying time variable stratified by region of recruitment assessment center, and age category (5-year categories). Models adjusted for use of menopausal hormone therapy (ever, never, unknown); oral contraceptive use (ever, never, unknown); age of menopause (<50, 50–52, 53–55, >55 years, not applicable, unknown); parity (0, ≥1, unknown); body mass index (<20, 20–<22.5, 22.5–<25, 25–<27.5, 27.5–<30, 30–<32.5, 32.5–<35, >35 kg/m^2); education (none, NVQ/HND/HNC, O-level, CSE or equivalent, A-level, college or university, other professionals, unknown); physical activity (low, moderate, high, unknown); type 2 diabetes (no, yes, unknown). *$P_{interaction}$ for menopausal status, menopausal hormone therapy and body mass index; $P_{heterogeneity}$ for histology. Abbreviation: P-int/het, $P_{interaction}/P_{heterogeneity}$.

cancer cases compared to our previous study in EPIC and found an inverse association of smoking with endometrial cancer risk (10). We were also able to evaluate associations of smoking by histological subtypes of endometrial cancer with higher precision and no differential associations were found. There was little evidence of heterogeneity of the association of smoking and endometrial cancer risk by subgroups of menopausal status, MHT use, and BMI. Smoking-related characteristics such as smoking intensity and smoking duration do not seem to significantly alter endometrial cancer risk. A limitation of our MR study was that our use of summary-level data meant we were unable to assess the associations by menopausal status, and subgroups of other risk factors (e.g., BMI, exogenous hormone use). In addition, in our MR analyses, UK Biobank participants were included in both smoking-related phenotypes and endometrial cancer datasets, which might have introduced some bias in the MR estimates. However, only 4.9% of endometrial cancer cases (636 out of 12,906) within ECAC were from the UK Biobank, implying that any bias from participant overlap would be relatively small (49).

In conclusion, although we observed an inverse association of smoking and endometrial cancer risk, the Mendelian randomization analyses do not support a causal relationship, although alternative pleiotropic pathways cannot be ruled out. Additional observational studies are required to better understand the underlying confounding factors (if any) and further experimental studies are need to understand the possible mechanisms of carcinogenesis of endometrial cancer that may explain the estimated inverse association of smoking with endometrial cancer development.

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Table 2. Mendelian randomization estimates for the causal effect of (A) lifetime amount of smoking and (B) ever having smoked regularly on endometrial cancer risk.

Methods	OR[a] (95% CI)	*P*	*P* for pleiotropy or heterogeneity
(A) Lifetime amount of smoking			
IVW	1.31 (1.06–1.62)	0.01	0.18
MR-Egger	0.5 (0.21–1.16)	0.11	0.02
MR-Egger intercept		0.015 (0.002–0.027)	
Weighted median	1.05 (0.77–1.43)	0.75	NA
Multivariable IVW (BMI)	1.15 (0.91–1.44)	0.25	0.18
Multivariable IVW (alcohol)	1.40 (1.11–1.77)	0.01	0.24
Multivariable IVW (educational attainment)	1.04 (0.80–1.36)	0.75	0.20
(B) Ever having smoked regularly			
IVW	1.05 (0.89–1.25)	0.57	0.02
MR-Egger	0.96 (0.49–1.88)	0.89	0.77
MR-Egger intercept		0.002 (−0.012–0.017)	
Weighted median	1.02 (0.81–1.29)	0.88	NA
Multivariable IVW (BMI)	0.99 (0.83–1.19)	0.95	0.02
Multivariable IVW (alcohol)	1.20 (0.97–1.47)	0.09	0.01
Multivariable IVW (educational attainment)	1.02 (0.85–1.23)	0.80	0.02

[a]Per 1-SD increment for lifetime amount of smoking.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.
2. Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Manson JE, Li J, et al. A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. Cancer Epidemiol Biomarkers Prev 2008;17:921–9.
3. Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, et al. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer 2008;15:485–97.
4. Lukanova A, Lundin E, Micheli A, Arslan A, Ferrari P, Rinaldi S, et al. Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. Int J Cancer 2004;108:425–32.
5. Brinton LA, Trabert B, Anderson GL, Falk RT, Felix AS, Fuhrman BJ, et al. Serum estrogens and estrogen metabolites and endometrial cancer risk among postmenopausal women. Cancer Epidemiol Biomarkers Prev 2016;25:1081–9.
6. Zeleniuch-Jacquotte A, Akhmedkhanov A, Kato I, Koenig KL, Shore RE, Kim MY, et al. Postmenopausal endogenous oestrogens and risk of endometrial cancer: results of a prospective study. Br J Cancer 2001;84:975–81.
7. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. Cancer Epidemiol Biomarkers Prev 2002;11:1531–43.
8. Wu Y, Sun W, Liu H, Zhang D. Age at menopause and risk of developing endometrial cancer: a meta-analysis. Biomed Res Int 2019;2019:8584130.
9. International Agency for Research Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, France: IARC; 2004.
10. Al-Zoughool M, Dossus L, Kaaks R, Clavel-Chapelon F, Tjonneland A, Olsen A, et al. Risk of endometrial cancer in relationship to cigarette smoking: results from the EPIC study. Int J Cancer 2007;121:2741–7.
11. Zhou B, Yang L, Sun Q, Cong R, Gu H, Tang N, et al. Cigarette smoking and the risk of endometrial cancer: a meta-analysis. Am J Med 2008;121:501–8.
12. Raglan O, Kalliala I, Markozannes G, Cividini S, Gunter MJ, Nautiyal J, et al. Risk factors for endometrial cancer: an umbrella review of the literature. Int J Cancer 2019;145:1719–30.
13. Endogenous Hormones and Breast Cancer Collaborative Group, Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer KJ, et al. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. Br J Cancer 2011;105:709–22.
14. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22.
15. Wootton RE, Richmond RC, Stuijfzand BG, Lawn RB, Sallis HM, Taylor GMJ, et al. Evidence for causal effects of lifetime smoking on risk for depression and schizophrenia: a Mendelian randomisation study. Psychol Med 2020;50:2435–43.
16. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. Nat Genet 2019;51:237–44.
17. O'Mara TA, Glubb DM, Amant F, Annibali D, Ashton K, Attia J, et al. Identification of nine new susceptibility loci for endometrial cancer. Nat Commun 2018;9:3166.
18. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
19. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 2015;12:e1001779.
20. Leffondre K, Abrahamowicz M, Xiao Y, Siemiatycki J. Modelling smoking history using a comprehensive smoking index: application to lung cancer. Stat Med 2006;25:4132–46.
21. Zhao LG, Li ZY, Feng GS, Ji XW, Tan YT, Li HL, et al. Coffee drinking and cancer risk: an umbrella review of meta-analyses of observational studies. BMC Cancer 2020;20:101.
22. Schoenfeld D. Partial residuals for the proportional hazards regression model. Biometrika 1982;69:239–41.
23. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995;51:524–32.
24. Dimou N, Yarmolinsky J, Bouras E, Tsilidis KK, Martin RM, Lewis SJ, et al. Causal effects of lifetime smoking on breast and colorectal cancer risk: Mendelian randomization study. Cancer Epidemiol Biomarkers Prev 2021;30:953–64.
25. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37:658–65.
26. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. Eur J Epidemiol 2015;30:543–52.
27. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44:512–25.
28. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–14.
29. Greenland S. An introduction to instrumental variables for epidemiologists. Int J Epidemiol 2018;47:358.
30. Martens EP, Pestman WR, de Boer A, Belitser SV, Klungel OH. Instrumental variables: application and limitations. Epidemiology 2006;17:260–7.
31. Cochran WG. The combination of estimates from different experiments. Biometrics 1954;10:101–29.
32. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018;50:693–8.
33. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. Am J Epidemiol 2015;181:251–60.
34. Clarke TK, Adams MJ, Davies G, Howard DM, Hall LS, Padmanabhan S, et al. Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112, 117). Mol Psychiatry 2017;22:1376–84.
35. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. Hum Mol Genet 2018;27:3641–9.
36. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518:197–206.
37. Lee JJ, Wedow R, Okbay A, Kong E, Maghzian O, Zacher M, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. Nat Genet 2018;50:1112–21.
38. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. Int J Epidemiol 2017;46:1734–9.
39. Michnovicz JJ, Hershcopf RJ, Naganuma H, Bradlow HL, Fishman J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N Engl J Med 1986;315:1305–9.
40. van den Berg M, van Duursen MBM. Mechanistic considerations for reduced endometrial cancer risk by smoking. Curr Opin Toxicol 2019;52–9.
41. Zhu D, Chung HF, Pandeya N, Dobson AJ, Cade JE, Greenwood DC, et al. Relationships between intensity, duration, cumulative dose, and timing of smoking with age at menopause: A pooled analysis of individual data from 17 observational studies. PLoS Med 2018;15:e1002704.
42. Park HA, Neumeyer S, Michailidou K, Bolla MK, Wang Q, Dennis J, et al. Mendelian randomisation study of smoking exposure in relation to breast cancer risk. Br J Cancer 2021;25:1135–45.
43. Gormley M, Dudding T, Sanderson E, Martin RM, Thomas S, Tyrrell J, et al. A multivariable Mendelian randomization analysis investigating smoking and alcohol consumption in oral and oropharyngeal cancer. Nat Commun 2020;11:6071.
44. Levin MG, Klarin D, Assimes TL, Freiberg MS, Ingelsson E, Lynch J, et al. Genetics of smoking and risk of atherosclerotic cardiovascular diseases: a mendelian randomization study. JAMA Netw Open 2021;4:e2034461.
45. Yuan S, Larsson SC. A causal relationship between cigarette smoking and type 2 diabetes mellitus: A Mendelian randomization study. Sci Rep 2019;9:19342.

46. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int J Epidemiol 2016;45:1961–74.
47. Rees JMB, Wood AM, Burgess S. Extending the MR-Egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy. Stat Med 2017;36:4705–18.
48. Larsson SC, Carter P, Kar S, Vithayathil M, Mason AM, Michaelsson K, et al. Smoking, alcohol consumption, and cancer: a mendelian randomisation study in UK Biobank and international genetic consortia participants. PLoS Med 2020;17: e1003178.
49. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. Genet Epidemiol 2016;40: 597–608.