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Causal effects of lifetime smoking on breast and colorectal cancer risk: Mendelian randomization study

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Abstract

Background—Observational evidence has shown that smoking is a risk factor for breast and colorectal cancer. We used Mendelian randomization (MR) to examine causal associations between smoking and risks of breast and colorectal cancer.

Methods—Genome-wide association study summary data were used to identify genetic variants associated with lifetime amount of smoking (n=126 variants) and ever having smoked regularly (n=112 variants). Using two-sample MR, we examined these variants in relation to incident breast (122,977 cases/105,974 controls) and colorectal cancer (52,775 cases/45,940 controls).

Results—In inverse-variance weighted models, a genetic predisposition to higher lifetime amount of smoking was positively associated with breast cancer risk [odds ratio [OR] per 1-standard deviation (SD) increment: 1.13 (95% confidence interval [CI]: 1.00-1.26); P: 0.04]; although heterogeneity was observed. Similar associations were found for estrogen receptor-positive and estrogen receptor-negative tumors. Higher lifetime amount of smoking was positively associated with colorectal cancer [OR per 1-SD increment: 1.21 (95% CI: 1.04-1.40); P: 0.01], colon cancer [OR: 1.31 (95% CI: 1.11-1.55); P: <0.01], and rectal cancer [OR: 1.36 (95% CI: 1.07-1.73); P: 0.01]. Ever having smoked regularly was not associated with risks of breast [OR: 1.01 (95% CI: 0.90-1.14); P: 0.85] or colorectal cancer [OR: 0.97 (95% CI: 0.86-1.10); P: 0.68].

Conclusions—These findings are consistent with prior observational evidence and support a causal role of higher lifetime smoking amount in the development of breast and colorectal cancer.

Impact—The results from this comprehensive MR analysis indicate that lifetime smoking is a causal risk factor for these common malignancies.

Keywords

smoking; breast cancer; colorectal cancer; Mendelian randomization

Introduction

Breast and colorectal cancer are two of the most common cancers globally with a combined estimated number of 4 million new cases and 1.5 million deaths in 2018 (1). Smoking is the most common cancer risk factor globally, with an estimated 18% of all cancers attributable to smoking (2). Tobacco smoke is the main source of exposure to aromatic amines (i.e. aniline, and o-anisidine), with strong evidence of carcinogenic effects in animals (3). Epidemiological studies have generally found smoking to be associated with higher risks of breast and colorectal cancer (4,5). However, the potential causality in humans of these relationships is uncertain due to the modest strength of the reported relative risks (often in the order of 1.07 to 1.20 for current versus never smokers (4,5)) and the possibility of residual confounding from other established risk factors for these malignancies, such as elevated adults body mass index (BMI) and alcohol consumption.

Mendelian randomization (MR) is an increasingly used analytical method to investigate causal inference between a given exposure and outcome. MR uses genetic variants robustly associated with the exposure of interest as instrumental variables to facilitate the estimation of causal effect of an exposure on an outcome (6). MR analyses should be largely free of the sources of confounding and reverse causality that are characteristic of conventional observational epidemiological studies, as genetic variants are randomly assigned, and fixed, at conception.

We used a two-sample MR framework to examine the association of smoking with breast and colorectal cancer. We constructed two genetic instruments for smoking: (a) lifetime amount of smoking (7) and, (b) ever having smoked regularly (8). We then investigated the associations of these genetic instruments with risks of breast cancer (122,977 cases and 105,974 controls) and colorectal cancer (52,775 cases and 45,940 controls).

Materials and methods

Data on 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 $<5 \times 10^{-8}$) with lifetime amount of smoking (capturing information on smoking duration, intensity, and cessation) and, ever having smoked regularly (binary phenotype with ever vs never smoking status). Variants associated with lifetime amount of smoking were selected from a genome-wide association study (GWAS) of 462,690 individuals in the UK Biobank, from which a score was constructed based on combined information on smoking intensity (number of cigarettes per day), smoking duration, and ever/never regular smoking status (7). Information on the calculation of lifetime amount of smoking score, quality control, and selection of the participants was detailed in the published GWAS (7). Briefly, this score is zero in non

smokers, uses age at initiation and age at cessation to quantify smoking duration in current and former smokers, number of cigarettes smoked per day to measure smoking intensity, and a half-life constant which captures the exponentially decreasing effect of smoking at a given time on health outcomes. For the ever having smoked regularly instrument, we extracted variants from a GWAS meta-analysis of the GSCAN consortium for a total of 1,232,091 individuals of European ancestry (8). It is a binary phenotype, coding participants reporting ever being a regular smoker in their life (current or former) as ever having smoked regularly, while any participant who reported never being a regular smoker in their life was coded as never having smoked regularly. More specifically, ever smokers regularly were classified as those who had smoked over 100 cigarettes over the course of their life, and/or had smoked every day for at least a month, and/or had ever smoked regularly. Information about pipes/cigar/chew, or other non-cigarette forms of tobacco use is not included. The selected genetic variants were pruned based on a R^2 linkage disequilibrium (LD) <0.001 , resulting in 126 independent variants for lifetime amount of smoking and 112 for ever having smoked regularly. The genetic instruments explained $\sim 0.4\%$ and $\sim 2.0\%$ of the variance in lifetime amount of smoking and ever having smoked regularly, respectively.

GWAS data for breast and colorectal cancer

The association of smoking-related genetic variants with breast cancer risk was obtained using data from the Breast Cancer Association Consortium (BCAC), involving 122,977 cases (105,974 controls), of which, 69,501 were estrogen receptor-positive (ER+) cases and 21,468 were estrogen receptor-negative (ER-) cases (9) (Supplementary Table 1). For colorectal cancer, we used summary data from a meta-analysis of 98,715 participants (52,775 colorectal cancer cases and 45,940 controls) within the ColoRectal Transdisciplinary Study (CORECT), the Colon Cancer Family Registry (CCFR), and the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) (10) (Supplementary Table 1). All smoking-related genetic variants had an imputation score above 0.6 and 0.3 in breast and colorectal cancer GWAS, respectively.

Statistical analysis

For our main analysis, we used a random-effects inverse-variance weighted (IVW) method (11,12). A series of statistical tests were conducted to investigate potential violation of the MR assumptions (13,14), and to assess the possible influence of horizontal pleiotropy on the causal estimates, including MR-Egger regression (15) and the estimator from the weighted median approach (16). We calculated the Cochran's Q statistic that quantifies the heterogeneity in effect sizes attributed to the selected genetic variants. We also estimated the intercept term from the MR-Egger regression, with a deviation from zero being indicative of directional (non-balanced horizontal) pleiotropy (15). The MR pleiotropy residual sum and outlier test (MR-PRESSO) was performed to identify, and exclude, any outlying variants (p-value threshold set at <0.05) (17). Leave-one-variant out analyses were conducted to assess the influence of individual variants on the observed associations. We also conducted multivariable MR analyses, adjusting for BMI and alcohol consumption, established confounders of the smoking-cancer association (4,5). For BMI, we used data from a recent GWAS of the GIANT consortium and the UK Biobank including $\sim 700,000$ individuals of European ancestry (18). Sex-specific estimates for BMI were obtained from a previous

GWAS within the GIANT consortium including 171,977 women and 152,893 men (19). For alcohol consumption, GWAS data on drinks per week for 537,349 participants were used (8). Further multivariable MR analyses were performed including GWAS data for educational attainment to control for socioeconomic status (20). For breast cancer, we also examined whether the effect of smoking was independent of age at menarche in a multivariable MR setting using GWAS data in up to ~370,000 women (21). For multivariable MR analyses, genome-wide significant signals of the exposures assessed (i.e. BMI, alcohol consumption, educational attainment, and age at menarche) independent of smoking-related variants (R^2 LD <0.001) were also included in the models (Supplementary Tables 2–3). As a positive control analysis, we examined the association between ever having smoked regularly and lung cancer using data from the International Lung Cancer Consortium (ILCCO) GWAS, which comprises 11,348 cases and 15,861 controls (22). The genetic instrument for lifetime amount of smoking has previously been shown to be strongly and robustly associated with lung cancer risk [odds ratio [OR] per 1-standard deviation [SD] increment: 4.21 (95% confidence interval [CI]: 2.98-5.96); P: <0.01] (Supplementary Table 4) (7). Missing variants in any of the exposures used in the multivariable MR analyses and in the outcomes assessed were replaced by a suitable proxy (minimum LD $R^2 = 0.8$) where available.

The statistical analyses were implemented in the Mendelian randomization R package (23) and MR-PRESSO version 1.0 in the R environment (17).

Results

Mendelian randomization estimates for lifetime amount of smoking

In the IVW model, a genetic predisposition to higher lifetime amount of smoking was positively associated with risk of breast cancer [OR per 1-SD increment: 1.13 (95% CI: 1.00-1.26); P: 0.04], with similar effect estimates found for ER+ [OR: 1.11 (95% CI: 0.97-1.26); P: 0.12] and ER- breast cancer [OR: 1.15 (95% CI: 0.96-1.37); P: 0.14] (Table 1). Genetic predisposition to lifetime amount of smoking was also positively associated with risks of colorectal cancer [OR: 1.21 (95% CI: 1.04-1.40); P: 0.01], colon cancer [OR: 1.31 (95% CI: 1.11-1.55); P: <0.01], and rectal cancer [OR: 1.36 (95% CI: 1.07-1.73); P: 0.01] (Table 1). Similar magnitude effect estimates were found for distal colon cancer [OR: 1.50 (95% CI: 1.21-1.87); P: <0.01] and proximal colon cancer [OR: 1.19 (95% CI: 0.98-1.46); P: 0.09] (P-heterogeneity: 0.13). A positive effect of lifetime amount of smoking on colorectal cancer risk was found for men [OR: 1.32 (95% CI: 1.11-1.59); P: <0.01], but not women [OR: 1.11 (95% CI: 0.90-1.37); P: 0.34], although the evidence for a difference by sex was weak (P-heterogeneity: 0.21).

Evidence of effect heterogeneity (Cochran's Q P-values <0.01) was found for most models. Little evidence of directional pleiotropy was found for all models (nearly zero MR-Egger intercept values), with similar magnitude effect estimates generally found for the weighted median method (Table 1). The MR-Egger analyses also yielded similar effect estimates to the IVW models for all endpoints, with the exception of ER- breast cancer, for which an inverse effect estimate was found (Table 1). The MR-PRESSO and the analysis removing

one variant at a time, yielded similar results in all analyses performed (Table 1 and Supplementary Tables 5A–6A).

Multivariable MR analyses, adjusting for BMI and alcohol consumption, resulted in raised effect estimates for breast cancer [OR: 1.28 (95% CI: 1.09-1.49); P: <0.01], ER+ breast cancer [OR: 1.25 (95% CI: 1.05-1.49); P: 0.01], and ER- breast cancer [OR: 1.45 (95% CI: 1.14-1.85); P: <0.01], with this strengthening of association driven mostly by adjustment for BMI (Table 1). Conversely, the positive associations between genetic predisposition to lifetime amount of smoking and colorectal cancer were slightly attenuated in the multivariable model [OR: 1.18 (95% CI: 0.98-1.43); P: 0.09] (Table 1), with adjustment for BMI being most influential [OR: 1.15 (95% CI: 0.97-1.36); P: 0.12]. However, the positive associations for colon cancer [OR: 1.25 (95% CI: 1.02-1.53); P: 0.03], distal colon cancer [OR: 1.39 (95% CI: 1.07-1.80); P: 0.01] and rectal cancer [OR: 1.33 (95% CI: 0.99-1.79); P: 0.05] remained after BMI and alcohol consumption multivariable adjustments (Figure 1A). In the multivariable MR models accounting for educational attainment, a genetic predisposition to higher lifetime amount of smoking was positively associated with risk of breast cancer [OR: 1.18 (95% CI: 1.00-1.38); P: 0.05] but attenuated towards the null for colorectal cancer [OR: 1.11 (95% CI: 0.93-1.32); P: 0.24]. A positive association remained between lifetime smoking and distal colon cancer after accounting for educational attainment in multivariable MR models [OR: 1.45 (95% CI: 1.10-1.91); P: 0.01]. Multivariable MR analyses, adjusting for age at menarche, resulted in similar effect estimates to the IVW models for breast cancer [OR: 1.12 (95% CI: 0.99-1.26); P: 0.06], ER+ breast cancer [OR: 1.11 (95% CI: 0.97-1.26); P: 0.13], and ER- breast cancer [OR: 1.13 (95% CI: 0.94-1.36); P: 0.18] (Supplementary Table 7).

Mendelian randomization estimates for ever having smoked regularly

In the IVW model, no association was found between genetic predisposition to ever having smoked regularly and risks of breast cancer [OR: 1.01 (95% CI: 0.90-1.14); P: 0.85], ER+ breast cancer [OR: 1.01 (95% CI: 0.88-1.15); P: 0.88], and ER- breast cancer [OR: 1.03 (95% CI: 0.87-1.21); P: 0.74] (Table 2). For colorectal cancer, we found no association between genetic predisposition to ever having smoked regularly and risks of colorectal cancer [OR: 0.97 (95% CI: 0.86-1.10); P: 0.68], colon cancer [OR: 0.99 (95% CI: 0.86-1.13); P: 0.84] and rectal cancer [OR: 1.00 (95% CI: 0.83-1.20); P: 0.99] (Table 2). Similar null results were found for distal colon cancer [OR: 1.00 (95% CI: 0.85-1.18); P: 0.99], proximal colon cancer [OR: 0.97 (95% CI: 0.82-1.15); P: 0.73], and for colorectal cancer in women [OR: 0.94 (95% CI: 0.80-1.09); P: 0.40] and men [OR: 1.02 (95% CI: 0.88-1.18); P: 0.80].

For all colorectal and breast cancer models, estimates from MR-Egger and the weighed median approach did not provide any evidence of a causal effect. In all analyses, there was no evidence of aggregated directional pleiotropy using MR-Egger (P-pleiotropy>0.05). We detected heterogeneity among the causal estimates of the 112 index variants (all P-heterogeneity<0.01 for colorectal and breast cancer). In the sensitivity analyses using MR-PRESSO or removing one variant at a time, the results for all analyses were near identical (Table 2 and Supplementary Tables 5B–6B). The multivariable analyses, adjusting for BMI

and alcohol consumption, showed no association for ever having smoked regularly with breast cancer [OR: 1.12 (95% CI: 0.94-1.33); P: 0.20], ER+ breast cancer [OR: 1.08 (95% CI: 0.89-1.31); P: 0.43], and ER- breast cancer [OR: 1.16 (95% CI: 0.92-1.46); P: 0.21] (Figure 1B). No association was observed for ever having smoked regularly and risk of breast cancer after multivariable adjustment for educational attainment and age at menarche (Supplementary Table 8). Similarly, null findings were observed for ever having smoked regularly and risk of colorectal cancer after multivariable adjustment for BMI and alcohol, with no apparent differential effects of these two exposures. Ever having smoked regularly was not related to risk of colorectal cancer after multivariable adjustment for educational attainment (Supplementary Table 8).

In the positive control analysis, assessing the association between genetic predisposition to ever having smoked regularly and lung cancer, we found a strong positive effect estimate [OR: 1.90 (95% CI: 1.56-2.31); P: <0.01], demonstrating the validity of the genetic instrument (Supplementary Table 4).

Discussion

In this MR analysis, genetic predisposition to higher lifetime amount of smoking was associated with elevated risks of breast cancer and colorectal cancer. In contrast, no association was found between genetic predisposition to ever having smoked regularly and risks of breast and colorectal cancer.

The association between smoking and breast cancer risk is unclear. Experimental evidence has shown that chemical compounds found in tobacco smoke can induce mammary cancers in rodents (24–26), and smoking related DNA adducts and p53 mutation have been identified in breast tissue (27,28). Results from recent pooled analyses of cohort studies supported these experimental studies, with positive associations found between smoking and breast cancer risk, especially for women who initiated smoking before first childbirth (4,29-31). However, causal inference of the smoking and breast cancer relationship is hampered by the epidemiological association being relatively weak (e.g. the relative risk for current versus never smokers was estimated to range from 1.07 to 1.12 in recent pooled analyses (4,30)) and the possibility that confounding by other breast cancer risk factors, such as alcohol consumption and BMI, may induce bias (4). MR analyses are less susceptible to conventional confounding (6) and our use of novel multivariable MR methods meant we were able to estimate the direct effect of genetic predisposition to ever having smoked regularly and lifetime amount of smoking on breast cancer risk conditional on alcohol consumption and BMI (as it is likely that genetic variants associated with smoking exert pleiotropic effects with other addictive behaviors) (32). Importantly, for breast cancer, the positive associations for lifetime amount of smoking were strongest in our multivariable MR models where we adjusted for BMI and alcohol intake. In addition, the positive associations for lifetime amount of smoking and risk of breast cancer were robust in the multivariable models accounting for possible pleiotropic effects of educational attainment, an indicator of socioeconomic status, and age at menarche.

We found similar magnitude positive effect estimates between being genetically predisposed to lifetime amount of smoking and both ER+ and ER- breast cancer. Recent prospective studies have generally found that smoking-related exposures were more consistently and strongly associated with ER+ than ER- breast cancer risk (4).

Tobacco smoke contains many carcinogens, that can damage DNA and the colorectal mucosa (33,34). Findings from an experimental study of mice found that a tobacco-specific nitrosamine derived from nicotine acts as an initiator of colorectal cancer development (35). The strongest positive effect estimates for genetic predisposition to lifetime amount of smoking we found were for distal colon cancer and rectal cancer. For both anatomical subsites, the positive associations remained stable in multivariable MR models that conditioned for BMI and alcohol consumption, two established colorectal cancer risk factors (36). There is conflicting observational evidence for the association between smoking and risk of colorectal cancer by anatomical subsite. An earlier meta-analysis of case-control and cohort studies reported stronger positive relationships for rectal cancer and proximal colon cancer (37). However, a recent joint Nurses' Health Study and Health Professionals Follow-up Study, found no heterogeneity for the positive association between pack-years of smoking and risks of distal colon, proximal colon, and rectal cancer (38).

The positive effect estimate we found between lifetime amount of smoking and colorectal cancer risk was most apparent for men. Evidence for heterogeneity by sex for the association between smoking and colorectal cancer is mixed. It has previously been suggested that the effect of smoking is either limited or stronger in men than for women (39), partly attributed to interactions with endogenous sex steroid hormone levels and/or adiposity (5). Two older meta-analyses of prospective cohort studies have shown that risks for current smoking are higher in men than women (5,40). However, subsequent studies have reported positive associations between smoking and colorectal cancer among women (41–45).

We found no association between ever having smoked regularly and risks of both breast and colorectal cancer. The genetic instrument we used for ever smoking was a binary phenotype indicating if an individual ever smoked regularly. Similar null associations for ever having smoked regularly and risks of breast and colorectal cancers were reported in a recent MR study that used UK Biobank and BCAC data (46). Our positive control analysis showed the validity of the genetic instrument for ever having smoking regularly with an expected strong and robust positive association found for lung cancer risk. However, compared to lung cancer, smoking is more weakly associated with risks of breast and colorectal cancer (4,5,30), and it is possible that the null results we found for genetic disposition to ever smoking regularly with these cancers are a consequence of this phenotype crudely capturing exposure to cigarette smoke, with intensity and duration not accounted for. In particular, observational evidence suggests that ever smokers who started to smoke more than 1 year after the first childbirth had no increased risk for breast cancer, while those who initiated smoking more than 10 years before their first childbirth had a 60% increased risk of breast cancer, compared with never smokers (29). For colorectal cancer, it has been found that pack-years or duration among ever smokers are the smoking variables most consistently associated with higher disease risk (47). In contrast, the genetic instrument for lifetime amount of smoking, which was positively associated with both breast and colorectal cancer

risks in our analyses, combined information in a score on smoking duration, smoking initiation, and number of cigarettes per day. Since this index captures smoking duration (age at initiation and age at cessation in current and former smokers) and heaviness (number of cigarettes smoked per day), we did not perform separate MR analyses for age of initiation of regular smoking, smoking cessation, and number of cigarettes per day.

To our knowledge, this is the first MR study to report causal associations for a smoking phenotype with breast and colorectal cancers. We conducted multiple sensitivity analyses to assess, and adjust for, the influence of horizontal pleiotropy on our results and, overall, our results were robust. However, MR-Egger results for breast cancer are not consistent with those in the IVW models and Weighted median approach and are the least reliable due to low I_{GX}^2 statistic (50% for lifetime amount of smoking and 71% for of ever having smoked regularly) which tests the suitability of this method (48). Further, the agnostic use of all smoking-related variants irrespective of their biological function could explain the heterogeneity observed in MR results; however multivariable MR methodology was employed to account for possible pleiotropic effects. A limitation of our study was that our use of summary-level data meant we were unable to assess the associations by menopausal status (although ~85% of breast cancer cases were post-menopausal), subgroups of other risk factors (e.g. BMI, exogenous hormone use), and conduct analyses for localized and advanced breast and colorectal cancers.

In summary, using a comprehensive MR analytical framework, we estimated potential causal associations between smoking and risks of breast cancer and colorectal cancer. Our findings are consistent with prior observational evidence and provide support for higher lifetime amount of smoking being a causal risk factor for these common malignancies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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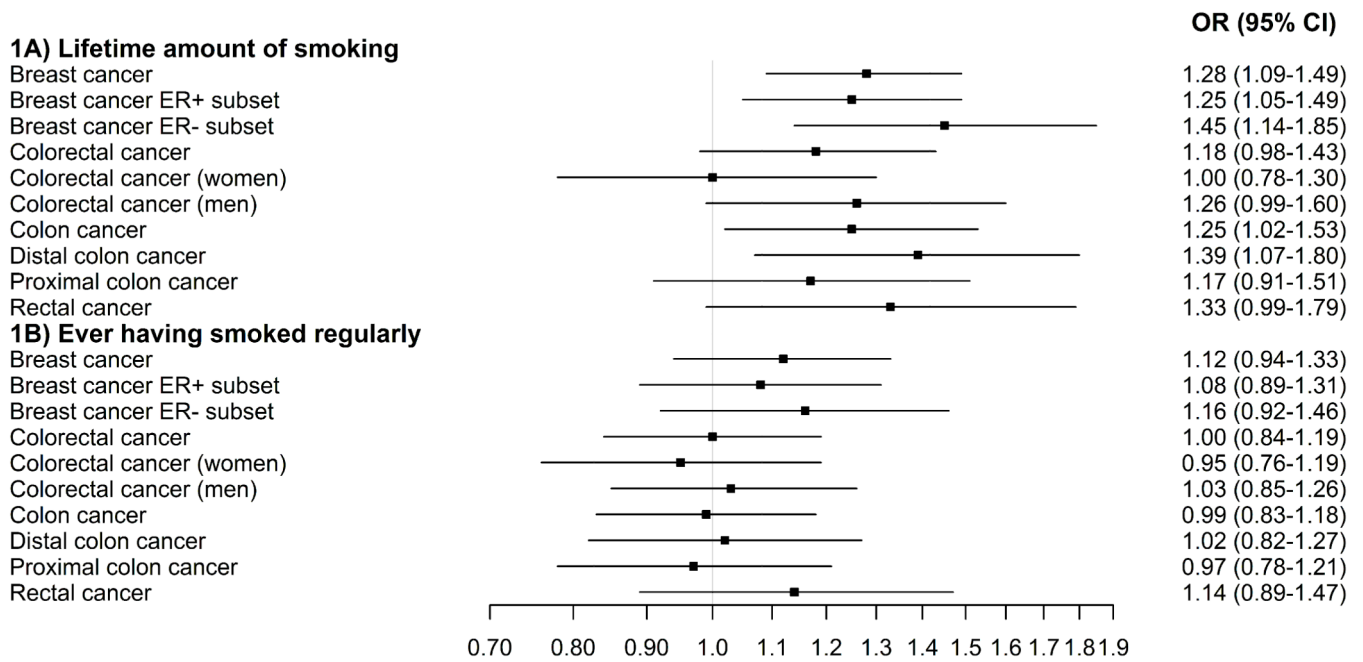


Figure 1. Multivariable Mendelian randomization estimates, adjusted for body mass index and alcohol consumption, for the causal effect of (A) lifetime amount of smoking, and (B) ever having smoked regularly on cancer risk.
 OR: odds ratio; CI: confidence intervals; BMI: body mass index; ER: estrogen receptor

Table 1
Mendelian randomization estimates for the causal effect of lifetime amount of smoking on cancer risk.

Methods	OR	95% CI	P-value	P-value for pleiotropy or heterogeneity
Breast cancer				
IVW	1.13	(1.00-1.26)	0.04	< 0.01
MR-Egger	1.42	(0.90-2.24)	0.13	0.30
MR-Egger intercept	-0.004 (-0.010-0.003)			
Weighted median	1.16	(1.02-1.34)	0.03	NA
MR PRESSO (rs2867112)	1.15	(1.03-1.28)	0.01	< 0.01
Multivariable IVW (BMI)	1.25	(1.08-1.45)	< 0.01	< 0.01
Multivariable IVW (alcohol consumption)	1.12	(0.97-1.29)	0.13	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.28	(1.09-1.49)	< 0.01	< 0.01
Breast cancer ER+ subset				
IVW	1.11	(0.97-1.26)	0.12	< 0.01
MR-Egger	1.67	(1.00-2.80)	0.05	0.11
MR-Egger intercept	-0.006 (-0.014-0.001)			
Weighted median	1.12	(0.95-1.31)	0.17	NA
MR PRESSO (rs11783093)	1.08	(0.96-1.23)	0.21	< 0.01
Multivariable IVW (BMI)	1.23	(1.05-1.44)	0.01	< 0.01
Multivariable IVW (alcohol consumption)	1.11	(0.95-1.31)	0.20	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.25	(1.05-1.49)	0.01	< 0.01
Breast cancer ER- subset				
IVW	1.15	(0.96-1.37)	0.14	< 0.01
MR-Egger	0.70	(0.34-1.45)	0.33	0.17
MR-Egger intercept	0.008 (-0.003-0.018)			
Weighted median	1.21	(0.95-1.54)	0.12	NA
MR PRESSO (rs2867112)	1.20	(1.01-1.42)	0.04	0.05
Multivariable IVW (BMI)	1.33	(1.06-1.68)	0.01	< 0.01
Multivariable IVW (alcohol consumption)	1.17	(0.96-1.43)	0.13	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.45	(1.14-1.85)	< 0.01	< 0.01
Colorectal cancer				
IVW	1.21	(1.04-1.40)	0.01	0.01
MR-Egger	1.58	(0.87-2.89)	0.13	0.36
MR-Egger intercept	-0.004 (-0.013-0.005)			
Weighted median	1.23	(1.01-1.50)	0.04	NA
MR PRESSO	1.21	(1.04-1.40)	0.01	0.01
Multivariable IVW (BMI)	1.15	(0.97-1.36)	0.12	< 0.01
Multivariable IVW (alcohol consumption)	1.23	(1.04-1.45)	0.02	0.01
Multivariable IVW (BMI, alcohol consumption)	1.18	(0.98-1.43)	0.09	< 0.01
Colorectal cancer (women)				

Methods	OR	95% CI	P-value	P-value for pleiotropy or heterogeneity
IVW	1.11	(0.90-1.37)	0.34	< 0.01
MR-Egger	1.96	(0.83-4.66)	0.12	0.18
MR-Egger intercept	-0.009	(-0.022-0.004)		
Weighted median	1.08	(0.82-1.44)	0.59	NA
MR PRESSO (rs62175972)	1.08	(0.88-1.32)	0.47	0.02
Multivariable IVW (BMI)	1.00	(0.79-1.27)	0.99	0.01
Multivariable IVW (alcohol consumption)	1.11	(0.87-1.41)	0.39	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.00	(0.78-1.30)	0.98	0.01
Colorectal cancer (men)				
IVW	1.32	(1.11-1.59)	< 0.01	0.52
MR-Egger	1.17	(0.56-2.44)	0.67	0.74
MR-Egger intercept	0.002	(-0.009-0.013)		
Weighted median	1.36	(1.03-1.79)	0.03	NA
MR PRESSO	1.32	(1.11-1.59)	< 0.01	0.52
Multivariable IVW (BMI)	1.27	(1.02-1.58)	0.03	0.18
Multivariable IVW (alcohol consumption)	1.35	(1.10-1.65)	< 0.01	0.50
Multivariable IVW (BMI, alcohol consumption)	1.26	(0.99-1.60)	0.06	0.15
Colon cancer				
IVW	1.31	(1.11-1.55)	< 0.01	0.08
MR-Egger	1.57	(0.80-3.09)	0.19	0.59
MR-Egger intercept	-0.003	(-0.013-0.007)		
Weighted median	1.37	(1.09-1.72)	0.01	??
MR PRESSO	1.31	(1.11-1.55)	< 0.01	0.08
Multivariable IVW (BMI)	1.23	(1.02-1.47)	0.03	0.09
Multivariable IVW (alcohol consumption)	1.30	(1.07-1.57)	0.01	0.02
Multivariable IVW (BMI, alcohol consumption)	1.25	(1.02-1.53)	0.03	0.04
Distal colon cancer				
IVW	1.50	(1.21-1.87)	< 0.01	0.05
MR-Egger	1.92	(0.79-4.70)	0.15	0.57
MR-Egger intercept	-0.004	(-0.017-0.010)		
Weighted median	1.42	(1.04-1.93)	0.03	NA
MR PRESSO	1.50	(1.21-1.87)	< 0.01	0.05
Multivariable IVW (BMI)	1.39	(1.10-1.75)	< 0.01	0.17
Multivariable IVW (alcohol consumption)	1.44	(1.12-1.85)	< 0.01	0.01
Multivariable IVW (BMI, alcohol consumption)	1.39	(1.07-1.80)	0.01	0.12
Proximal colon cancer				
IVW	1.19	(0.98-1.46)	0.09	0.25
MR-Egger	1.06	(0.47-2.41)	0.88	0.77
MR-Egger intercept	0.002	(-0.010-0.014)		
Weighted median	1.23	(0.92-1.65)	0.17	NA
MR PRESSO	1.19	(0.98-1.46)	0.09	0.25

Methods	OR	95% CI	P-value	P-value for pleiotropy or heterogeneity
Multivariable IVW (BMI)	1.11	(0.89-1.40)	0.35	0.11
Multivariable IVW (alcohol consumption)	1.24	(0.98-1.56)	0.07	0.13
Multivariable IVW (BMI, alcohol consumption)	1.17	(0.91-1.51)	0.22	0.07
Rectal cancer				
IVW	1.36	(1.07-1.73)	0.01	< 0.01
MR-Egger	1.11	(0.42-2.93)	0.84	0.67
MR-Egger intercept	0.003 (-0.011-0.018)			
Weighted median	1.31	(0.95-1.81)	0.10	NA
MR PRESSO	1.36	(1.07-1.73)	0.01	< 0.01
Multivariable IVW (BMI)	1.38	(1.06-1.79)	0.02	< 0.01
Multivariable IVW (alcohol consumption)	1.40	(1.07-1.82)	0.01	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.33	(0.99-1.79)	0.05	< 0.01

OR: odds ratio; CI: confidence intervals; IVW: Inverse-variance weighted; BMI: body mass index; ER: estrogen receptor; P-value for pleiotropy in MR-Egger regression; P-value for heterogeneity in inverse-variance weighted analysis; Variants in the parenthesis of the MR PRESSO method were identified as outlying and excluded

Table 2
Mendelian randomization estimates for the causal effect of ever having smoked regularly on cancer risk.

Methods	OR	95% CI	P-value	P-value for pleiotropy or heterogeneity
Breast cancer				
IVW	1.01	(0.90-1.14)	0.85	< 0.01
MR-Egger	1.31	(0.82-2.10)	0.25	0.26
MR-Egger intercept	-0.006 (-0.016-0.004)			
Weighted median	1.04	(0.94-1.15)	0.50	NA
MR PRESSO (rs6731872, rs62246017, rs7072776, and rs8103660)	1.02	(0.94-1.10)	0.64	< 0.01
Multivariable IVW (BMI)	1.05	(0.90-1.22)	0.52	< 0.01
Multivariable IVW (alcohol consumption)	1.05	(0.91-1.21)	0.48	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.12	(0.94-1.33)	0.20	< 0.01
Breast cancer ER+ subset				
IVW	1.01	(0.88-1.15)	0.88	< 0.01
MR-Egger	1.58	(0.94-2.66)	0.08	0.08
MR-Egger intercept	-0.010 (-0.021-0.001)			
Weighted median	1.00	(0.89-1.13)	0.95	NA
MR PRESSO (rs62246017, rs11783093, rs7072776, and rs8103660)	0.96	(0.88-1.05)	0.40	0.01
Multivariable IVW (BMI)	1.04	(0.88-1.23)	0.66	< 0.01
Multivariable IVW (alcohol consumption)	1.05	(0.89-1.23)	0.56	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.08	(0.89-1.31)	0.43	< 0.01
Breast cancer ER- subset				
IVW	1.03	(0.87-1.21)	0.74	< 0.01
MR-Egger	0.76	(0.39-1.47)	0.41	0.35
MR-Egger intercept	0.007 (-0.007-0.021)			
Weighted median	1.05	(0.88-1.26)	0.59	NA
MR PRESSO (rs12739243, rs6731872, rs1549979, rs7696257, and rs8103660)	1.08	(0.94-1.24)	0.27	< 0.01
Multivariable IVW (BMI)	1.07	(0.88-1.30)	0.51	< 0.01
Multivariable IVW (alcohol consumption)	1.06	(0.87-1.29)	0.59	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.16	(0.92-1.46)	0.21	< 0.01
Colorectal cancer				
IVW	0.97	(0.86-1.10)	0.68	< 0.01
MR-Egger	1.10	(0.67-1.79)	0.71	0.63
MR-Egger intercept	-0.003 (-0.013-0.008)			
Weighted median	0.97	(0.83-1.13)	0.67	NA
MR PRESSO (rs77215829)	0.99	(0.88-1.12)	0.87	< 0.01
Multivariable IVW (BMI)	1.00	(0.86-1.15)	0.96	< 0.01
Multivariable IVW (alcohol consumption)	0.98	(0.85-1.14)	0.82	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.00	(0.84-1.19)	0.98	< 0.01
Colorectal cancer (women)				

Methods	OR	95% CI	P-value	P-value for pleiotropy or heterogeneity
IVW	0.94	(0.80-1.09)	0.40	< 0.01
MR-Egger	1.30	(0.70-2.43)	0.40	0.28
MR-Egger intercept	-0.007 (-0.021-0.006)			
Weighted median	0.94	(0.77-1.15)	0.56	NA
MR PRESSO	0.94	(0.80-1.09)	0.40	< 0.01
Multivariable IVW (BMI)	0.92	(0.76-1.12)	0.42	< 0.01
Multivariable IVW (alcohol consumption)	0.94	(0.78-1.14)	0.53	< 0.01
Multivariable IVW (BMI, alcohol consumption)	0.95	(0.76-1.19)	0.64	< 0.01
Colorectal cancer (men)				
IVW	1.02	(0.88-1.18)	0.80	0.05
MR-Egger	0.93	(0.51-1.67)	0.79	0.74
MR-Egger intercept	0.002 (-0.010-0.015)			
Weighted median	1.04	(0.84-1.28)	0.71	NA
MR PRESSO	1.02	(0.88-1.18)	0.80	0.05
Multivariable IVW (BMI)	1.05	(0.89-1.24)	0.56	0.06
Multivariable IVW (alcohol consumption)	1.03	(0.86-1.23)	0.76	0.05
Multivariable IVW (BMI, alcohol consumption)	1.03	(0.85-1.26)	0.74	0.05
Colon cancer				
IVW	0.99	(0.86-1.13)	0.84	< 0.01
MR-Egger	1.03	(0.60-1.75)	0.92	0.88
MR-Egger intercept	-0.001 (-0.012-0.011)			
Weighted median	1.02	(0.85-1.22)	0.86	NA
MR PRESSO (rs77215829)	1.00	(0.88-1.14)	0.95	0.02
Multivariable IVW (BMI)	1.00	(0.86-1.16)	0.99	< 0.01
Multivariable IVW (alcohol consumption)	1.00	(0.85-1.18)	0.99	< 0.01
Multivariable IVW (BMI, alcohol consumption)	0.99	(0.83-1.18)	0.90	< 0.01
Distal colon cancer				
IVW	1.00	(0.85-1.18)	0.99	0.03
MR-Egger	0.83	(0.43-1.60)	0.58	0.57
MR-Egger intercept	0.004 (-0.010-0.018)			
Weighted median	0.97	(0.78-1.22)	0.82	NA
MR PRESSO (rs9627272)	0.98	(0.84-1.15)	0.81	0.13
Multivariable IVW (BMI)	1.02	(0.85-1.22)	0.87	0.04
Multivariable IVW (alcohol consumption)	1.01	(0.83-1.23)	0.94	0.03
Multivariable IVW (BMI, alcohol consumption)	1.02	(0.82-1.27)	0.84	0.04
Proximal colon cancer				
IVW	0.97	(0.82-1.15)	0.73	< 0.01
MR-Egger	1.22	(0.63-2.38)	0.55	0.48
MR-Egger intercept	-0.005 (-0.019-0.009)			
Weighted median	0.98	(0.78-1.23)	0.88	NA
MR PRESSO	0.97	(0.82-1.15)	0.73	< 0.01

Methods	OR	95% CI	P-value	P-value for pleiotropy or heterogeneity
Multivariable IVW (BMI)	0.97	(0.81-1.17)	0.77	< 0.01
Multivariable IVW (alcohol consumption)	1.00	(0.82-1.22)	0.99	< 0.01
Multivariable IVW (BMI, alcohol consumption)	0.97	(0.78-1.21)	0.79	< 0.01
Rectal cancer				
IVW	1.00	(0.83-1.20)	0.99	< 0.01
MR-Egger	1.00	(0.48-2.11)	0.99	0.99
MR-Egger intercept	0.000 (-0.016-0.016)			
Weighted median	1.03	(0.81-1.30)	0.82	NA
MR PRESSO	1.00	(0.83-1.20)	0.99	< 0.01
Multivariable IVW (BMI)	1.04	(0.84-1.29)	0.70	< 0.01
Multivariable IVW (alcohol consumption)	1.08	(0.86-1.34)	0.51	< 0.01
Multivariable IVW (BMI, alcohol consumption)	1.14	(0.89-1.47)	0.29	< 0.01

OR: odds ratio; CI: confidence intervals; IVW: Inverse-variance weighted; BMI: body mass index; ER: estrogen receptor; P-value for pleiotropy in MR-Egger regression; P-value for heterogeneity in inverse-variance weighted analysis; Variants in the parenthesis of the MR PRESSO method were identified as outlying and excluded