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Obesity, Type 2 Diabetes, Lifestyle Factors, and Risk of Gallstone Disease: A Mendelian Randomization Investigation

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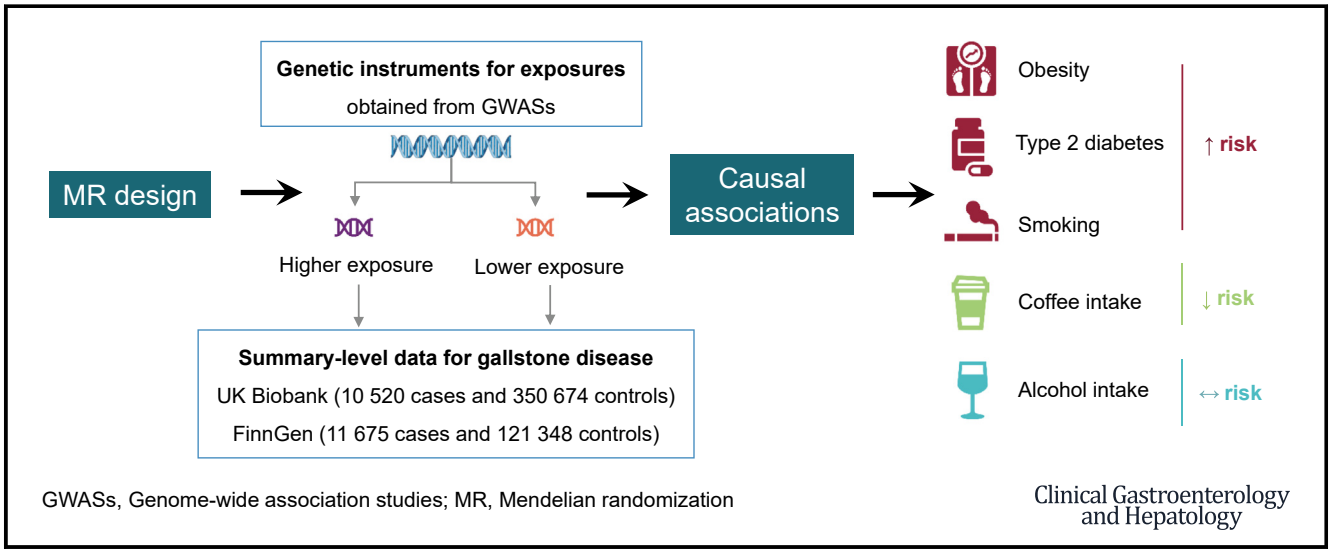

BACKGROUND & AIMS: **Obesity, type 2 diabetes, and lifestyle factors (cigarette smoking, alcohol drinking, and coffee consumption) have been associated with the risk of developing gallstone disease in observational studies, but whether these associations are causal is undetermined. We conducted a Mendelian randomization study to assess these associations.**

METHODS: **Genetic instruments associated with the exposures at the genome-wide significance ($p < 5\times10^{-8}$) level were selected from corresponding genome-wide association studies (n = 224 459 to 1 232 091 individuals). Summary-level data for gallstone disease were obtained from the UK Biobank (10 520 cases and 350 674 non-cases) and FinnGen consortium (11 675 cases and 121 348 non-cases). Univariable and multivariable Mendelian randomization analyses were conducted. Results from UK Biobank and FinnGen were combined using fixed-effects meta-analysis.**

Abbreviations used in this paper: BMI, body mass index; CI, confidence interval; LD, linkage disequilibrium; MR, Mendelian randomization; OR, odds ratio; SNP, single nucleotide polymorphism.

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RESULTS: **The odds ratios were 1.63 (95% confidence interval (CI), 1.49, 1.79) for one standard deviation (SD) increase in body mass index, 1.81 (95% CI, 1.60, 2.05) for one SD increase in waist circumference, 1.13 (95% CI, 1.09, 1.17) for one unit increase in the log-odds ratio of type 2 diabetes and 1.25 (95% CI, 1.16, 1.34) for one SD increase in prevalence of smoking initiation. The associations for body mass index and type 2 diabetes persisted after mutual adjustment. Genetically predicted coffee consumption was inversely associated with gallstone disease after adjustment for body mass index and smoking (odds ratio per 50% increase 0.44, 95% CI, 0.21, 0.91). There was no association with alcohol consumption.**

CONCLUSIONS: **This study supports independent causal roles of obesity, type 2 diabetes, and smoking in gallstone disease.**

Keywords: Gallstones; Lifestyle Factors; Type 2 Diabetes.

Gallstone disease affects around 10%–20% of the global adult population and is associated with the highest socioeconomic costs of all gastrointestinal conditions.[1–3] Serious complications, such as cholecystitis, cholangitis, and pancreatitis, as well as gallstone recurrence, influence health status and quality of life of gallstone patients after cholecystectomy.[4] Obesity,[1,2,4] type 2 diabetes,[5–7] and cigarette smoking[8] have been proposed as risk factors, with alcohol[4,9,10] and coffee[4,11,12] consumption as protective factors for gallstones in traditional observational studies. However, the causality of these observational findings cannot be determined, as residual confounding may have biased the results. For example, obesity and type 2 diabetes are closely interrelated, and their independent association with cholelithiasis is uncertain. Likewise, cigarette smoking, alcohol drinking, and coffee consumption are overlapping behaviors,[13] and this may introduce residual confounding in observational studies.

Mendelian randomization (MR) leverages the random allocation of genetic variants during conception as instrumental variables to estimate the causal association between an exposure (eg, body mass index [BMI]) and health outcome.[14] Here, the MR design was used to assess the potential causal associations of overall obesity (measured as BMI), abdominal obesity (measured as waist circumference), type 2 diabetes, and lifestyle factors (cigarette smoking, alcohol drinking, and coffee consumption) with risk of gallstone disease.

Materials and Methods

Study Design and Data Sources

Study design overview and the assumptions of a MR study are shown in Figure 1. Genetic instruments for the exposures were obtained from published genome-wide association studies.[15–19] Data for gallstone disease were obtained from UK Biobank[20] and the FinnGen consortium.[21] Detailed information on used data sources is displayed in Table 1. All studies had been approved by a relevant ethical review board and participants had given informed consent.

Genetic Instrument Selection

Genetic instruments for BMI,[15] waist circumference (with and without adjustment for BMI),[16] cigarette smoking,[17] alcohol drinking,[17] coffee consumption,[18] and type 2 diabetes[19] were selected at genome-wide significance threshold ($P < 5\times10^{-8}$) from corresponding genome-wide association studies. A set of genetic instruments associated with smoking index (taking into account smoking status as well as smoking duration, heaviness, and cessation in ever smokers)[22] were used in a supplementary analysis. Linkage disequilibrium (LD) among single nucleotide polymorphisms (SNPs) for each risk factor was calculated based on 1000 Genomes LD reference panel (European population) using the PLINK clumping method. SNPs in LD ($r^2 > 0.01$ and clump window <10 kb) were excluded and the SNP with the lowest *P* value was retained. In the analysis of coffee consumption, 5 SNPs were excluded due to pleiotropic associations with other potential risk factors (in particular BMI) at genome-wide significance (rs1260326 in the *GCKR* gene, rs574367 in *SEC16B*, rs10865548 in *TMEM18*, rs66723169 in *MC4R*, and rs34060476 in *MLXIPL*).[23]

Data Sources for Gallstone Disease

Summary-level genetic data for gallstone disease (cholelithiasis, defined by International Classification of Diseases-Tenth Revision code K80) were obtained from the UK Biobank cohort[20] and the FinnGen consortium.[21] The second wave of analyses of UK Biobank data from the Neale lab was used in the present study. Data from the Neale lab included 361,194 UK Biobank participants after exclusion of individuals of non-European ancestry, closely related individuals (or at least 1 of a related pair of individuals), individuals with sex chromosome aneuploidies, and individuals who had withdrawn consent from the UK Biobank study. Association tests had been adjusted for age, sex, and up to 20 principal components. The R3 release of the FinnGen consortium data was used. In that dataset, individuals with ambiguous gender, high genotype missingness (>5%), excess heterozygosity (±4

SDs), and non-Finnish ancestry had been excluded. Association tests had been adjusted for age, sex, 10 genetic principal components, and genotyping batch.

Statistical Analysis

A multiplicative random-effects inverse-variance weighted model was used to obtain the MR estimates. Derived estimates based on UK Biobank and the FinnGen consortium were combined using fixed-effects meta-analysis. A multivariable random-effects inverse-variance weighted model was used to adjust for BMI in the analysis of type 2 diabetes and vice versa. We also conducted a 2-step multivariable MR analysis in the analysis of coffee consumption to adjust for BMI and smoking initiation. Likewise, multivariable MR analysis was performed to adjust for smoking initiation in the analysis of alcohol drinking as these 2 traits are genetically correlated ($r_g = 0.34$).[17] Three other methods, including the weighted median, MR-Egger regression, and MR-PRESSO (Pleiotropy Residual Sum and Outlier) methods, were used as sensitivity analyses. The weighted median model provides consistent estimates on the condition that $\geq$50% of the weight in the analysis comes from valid instrumental variables.[24] MR-Egger regression analysis can detect and correct for directional pleiotropy whereas it compromises power.[25] The *P* value for the MR-Egger intercept was used to indicate directional pleiotropy. The MR-PRESSO approach can detect outliers and generate estimates after outliers removing.[26] The MR-PRESSO distortion test aims at examining the differences between the estimates before and after outlier correction and a $P < .05$ of distortion test indicates a significant difference in estimates before and after outlier correction.[26] Cochrane's Q value was estimated to assess the heterogeneity among used SNPs for each risk factor. Odds ratios (ORs) and corresponding confidence intervals (CIs) of gallstone disease were scaled to a 1-SD increase in BMI and waist circumference, a 1-unit increase in log OR of type 2 diabetes, a 1-SD increase in prevalence of smoking initiation, a 1-SD increase of lifetime smoking index, a 1-SD increase of log-transformed alcoholic drinks/wk, and a 50% increase in coffee consumption. All analyses were performed using the TwoSampleMR,[27] Mendelianrandomization,[28] and MR-PRESSO[26] packages in R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Genetically predicted higher BMI, waist circumference, and liability to type 2 diabetes were associated with elevated risk of gallstone disease in UK Biobank data, FinnGen consortium data, and meta-analysis ($P < .001$) (Figures 2 and 3). The combined ORs of gallstones were 1.63 (95% CI, 1.49–1.79) per a 1-SD increase in BMI, 1.81 (95% CI, 1.60–2.05) per a 1-SD increase in waist circumference, and 1.13 (95% CI, 1.09–1.17) for a 1-unit increase in log OR of type 2 diabetes. The associations were consistent in all sensitivity analyses, though pleiotropy was detected in the analysis of type 2 diabetes in UK Biobank data by the MR-Egger regression. After removing outliers in the MR-PRESSO analysis, the association between type 2 diabetes and cholelithiasis persisted and the *P* value for the distortion test were above .05 (Table 2). Associations for BMI and type 2 diabetes persisted and attenuated slightly after mutual adjustment (Figure 2). The association between genetically predicted waist circumference and gallstone disease did not remain in the UK Biobank data and attenuated greatly in the FinnGen consortium data after adjusting for BMI (Figure 3).

Among lifestyle factors, genetic predisposition to smoking initiation was associated with gallstones in the meta-analysis of data from the UK Biobank and FinnGen consortium (for a 1-SD increase in smoking prevalence: OR, 1.25; 95% CI, 1.16–1.34) (Figure 3 and Table 2). This positive association was replicated in the sensitivity analysis of lifetime smoking index. There were no associations of alcohol or coffee consumption with gallstones in the primary analysis (Figure 3) or the sensitivity analyses of each data source (Table 2). After adjustment for BMI, coffee consumption showed an inverse association with gallstones (per a 50% increase in coffee consumption: OR, 0.50; 95% CI, 0.29–0.88). This association remained after additional adjustment for smoking initiation (OR, 0.44; 95% CI, 0.21–0.91). In a meta-analysis of estimates from the UK Biobank and FinnGen consortium, coffee consumption showed an association with gallstones in analysis based on the weighted median method with an OR of 0.78 (95% CI, 0.63–0.97). Genetically predicted alcohol drinking (combined OR, 1.06; 95% CI, 0.77–1.47) was not associated with gallstones in the

What You Need to Know

Background

Obesity, type 2 diabetes, and lifestyle factors have been associated with the risk of developing gallstone disease in observational studies, but whether these associations are causal is undetermined.

Findings

Genetic predisposition to obesity, type 2 diabetes, and smoking initiation was associated with an increased risk of gallstone disease. Genetically predicted coffee consumption but not alcohol consumption was associated with a reduced risk of gallstone disease.

Implications for patient care

Preventing obesity and diabetes and modifying lifestyle factors may act as prevention strategies for gallstone disease.

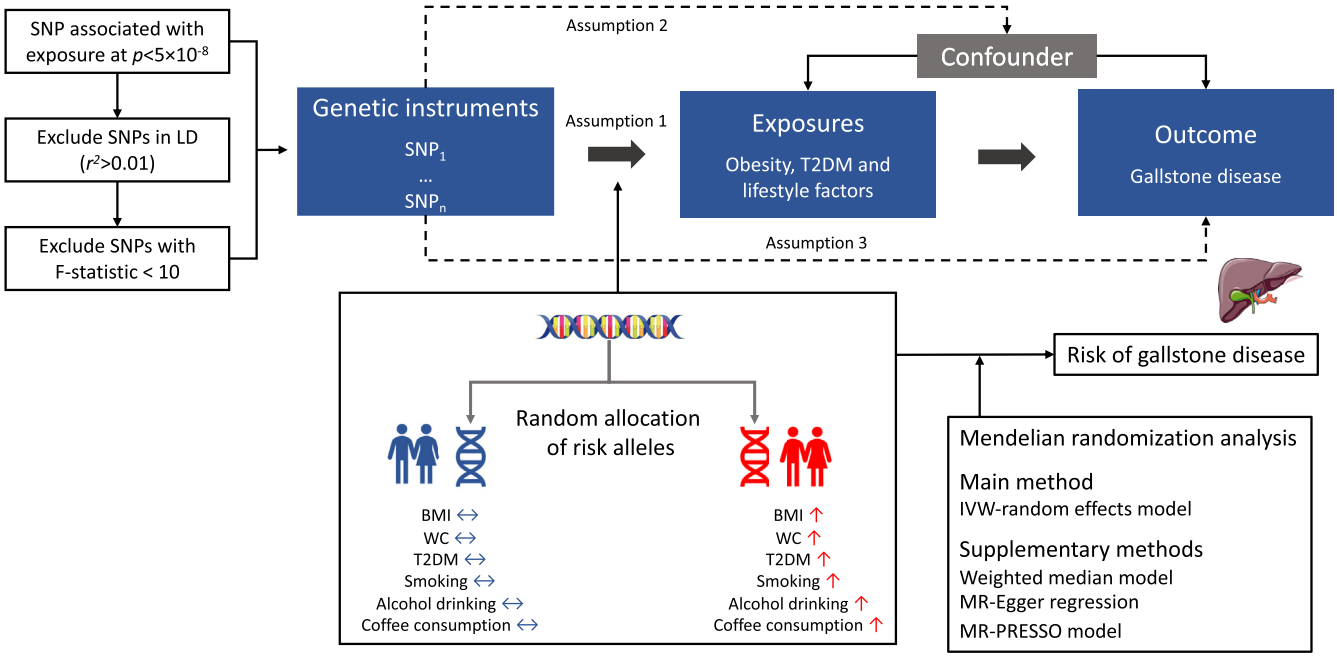

Figure 1. Study design overview and assumptions of the MR design. Assumption 1 indicates that the genetic variants proposed as instrumental variables should be robustly associated with the risk factor of interest; assumption 2 indicates that the used genetic variants should not be associated with potential confounders, and assumption 3 indicates that the selected genetic variants should affect the risk of the outcome merely through the risk factor, not via alternative pathways. The MR design can reduce residual confounding and reverse causality, thereby reinforcing the causal inference of an exposure-outcome association. The basis of this is that genetic variants, selected as instrumental variable for studying the effect of modifying the exposure, are randomly allocated at conception and are therefore less vulnerable to confounding from environmental factors and reverse causation. IVW, inverse-variance weighted; T2DM, type 2 diabetes mellitus; WC, waist circumference.

multivariable MR analysis adjusting for smoking initiation.

Discussion

The present MR study supports that both obesity and type 2 diabetes are independently and causally associated with the risk of gallstone disease. Moreover, our results provide support for a causal association between smoking and risk of gallstone disease. A possible causal association is observed between high coffee consumption and decreased risk of gallstone disease. There is no evidence that alcohol drinking is causally associated with gallstones.

BMI, representing overall obesity, has been associated with gallstones in observational studies[4,29] and in a 1-sample MR study in 77,679 Danish adults,[30] which was corroborated by the present 2-sample MR analysis in British and Finnish populations. The association between waist circumference and gallstones did not remain after adjustment for BMI, which indicated that central obesity is not a more important risk factor than overall fat mass for gallstone disease. The association between obesity and gallstones was slightly attenuated in the multivariable MR analysis with adjustment for genetically predicted type 2 diabetes liability, which may suggest that type 2 diabetes partly mediates this association. Several mechanisms, including alterations of metabolic factors, hepatic secretion of supersaturated bile, dyslipidemia, intestinal and gallbladder hypomotility, gallbladder stasis, decreased secretion of bile acids, cholesterol crystallization and precipitation, and supersaturated gallbladder bile, may underlie the increased risk of gallstones among obese individuals.[31] Even though obesity plays a causal role in the development of gallstones, both bariatric surgery and a low-calorie diet have been shown to increase the risk of asymptomatic and symptomatic gallstones.[32,33] Such increased risk might be related to a rapid weight loss.[34]

Studies of the association of type 2 diabetes with risk of gallstones have not been entirely consistent. A meta-analysis of 10 cohort studies showed an overall 56% increased risk of gallstones among diabetes patients compared with individuals without diabetes.[7] An increased risk of gallstones in type 2 diabetes patients was also found in a cross-sectional study with more than 4 million diabetes patients[6] but not in several other case-control studies.[5,35] In 1 of those case-control studies, type 2 diabetes was associated with gallstones in the univariable (crude) analysis, but the association was attenuated after adjustment for obesity and other risk factors.[35] The present MR study based on a large number of gallstone disease cases revealed a possibly causal association between type 2 diabetes and gallstones, and the

Table 1. Detailed Information on Used Studies and Consortia

Exposure or Outcome	Unit	Participants Included in Analysis	Adjustments	Identified SNPs	PubMed ID or URL
BMI	SD	339,224 individuals of multiancestries	Age and any necessary study-specific covariates	97	25673413
Waist circumference	SD	224,459 individuals of European ancestry	Age and study-specific covariates	47	25673412
Waist circumference adjusted for BMI	SD	224,459 individuals of European ancestry	Age, BMI, and study-specific covariates	70	25673412
Type 2 diabetes	1-unit in log odds ratio of type 2 diabetes	228,499 type 2 diabetes cases and 1,178,783 noncases of multiancestries	Age, sex, and the first 10 genetic principal components	558	32541925
Smoking initiation	SD in prevalence of smoking initiation	1,232,091 European-descent individuals	Age, sex, and the first 10 genetic principal components	378	30643251
Lifetime smoking index	SD change of lifetime smoking index	462,690 European-descent individuals	Genotyping chip and sex	126	31689377
Alcohol drinking	SD increase of log-transformed alcoholic drinks/wk	941,280 European-descent individuals	Age, sex, and the first 10 genetic principal components	99	30643251
Coffee consumption	50% change	375,833 European-descent individuals	Age, sex, BMI, total energy, proportion of typical food intake, and 20 genetic principal components	14	31046077
Gallstone disease[a]	—	10,520 gallstone disease cases and 350,674 noncases	Age, sex, and up to 20 genetic principal components	—	UK Biobank (http://www.nealelab.is/uk-biobank)
Gallstone disease[a]	—	11,675 gallstone disease cases and 121,348 noncases	Age, sex, 10 genetic principal components, and genotyping batch	—	FinnGen consortium (https://www.finngen.fi/fi)

BMI, body mass index; SD, standard deviation; SNPs, single nucleotide polymorphism.
[a]Defined by the International Classification of Diseases 10th Revision code K80. Unit for coffee consumption was rescaled to 50% increase based on 1% increase reported in genome-wide association study.

association was likely independent of BMI. Several possible mechanisms may explain the association between type 2 diabetes and gallstone disease. For example, hepatic insulin resistance has been shown to directly promote the formation of cholesterol gallstones.[36] Gallbladder hypomotility in diabetes might be an additional predisposing factor for cholesterol gallstone formation.[3]

Epidemiological data on the association between cigarette smoking and gallstones are inconsistent, with positive[8,37] and null findings[38] reported. Our study provided evidence to support a causal role of cigarette smoking in the development of gallstone, and further, that the association is likely independent of BMI. The different effect sizes of smoking on gallstones in UK Biobank and FinnGen consortium data might be related to differences in smoking patterns. Meta-analyses on alcohol consumption in relation to gallstones revealed a dose-response inverse relationship[9,10] and a J-shaped association (the risk of gallstones decreased with increasing alcohol consumption up to 30 g/d and thereafter plateaued).[10] However, our study did not confirm the inverse association[4,9] but could not rule out a possible weak nonlinear association of alcohol drinking with gallstones.

With regard to coffee consumption, an inverse association with risk of overall and symptomatic gallstone disease has been found in observational studies[4,11,12] and in a 1-sample MR study based on 114,220 Danish individuals (including 7294 cases).[39] The present MR study supported a possible association between high coffee consumption and lower risk of gallstones. Potential mechanisms underlying this inverse association might be that components in coffee stimulate

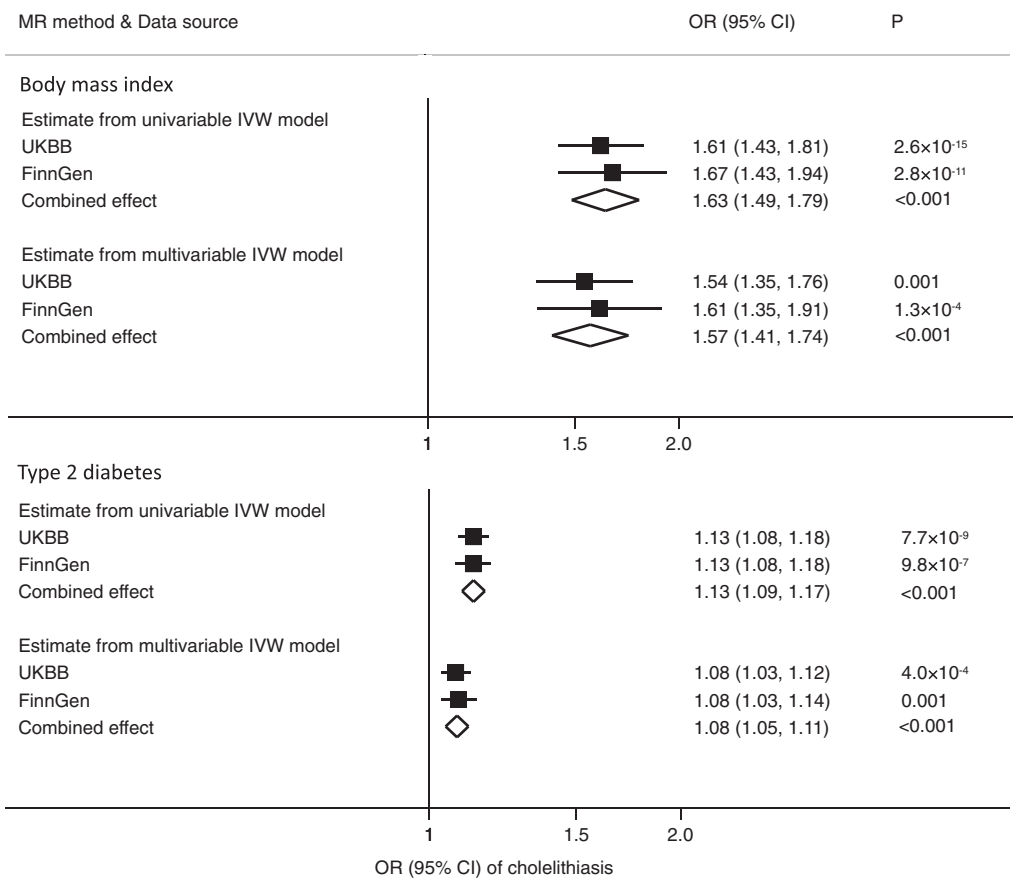

Figure 2. Associations of BMI and type 2 diabetes with gallstone disease in univariable and multivariable MR analyses. The multivariable MR analysis adjusted for type 2 diabetes in the analysis of BMI and vice versa. The ORs of gallstone disease were scaled to a 1-SD increase in BMI and a 1-unit increase in log OR of type 2 diabetes. IVW, inverse-variance weighted; UKBB, UK Biobank.

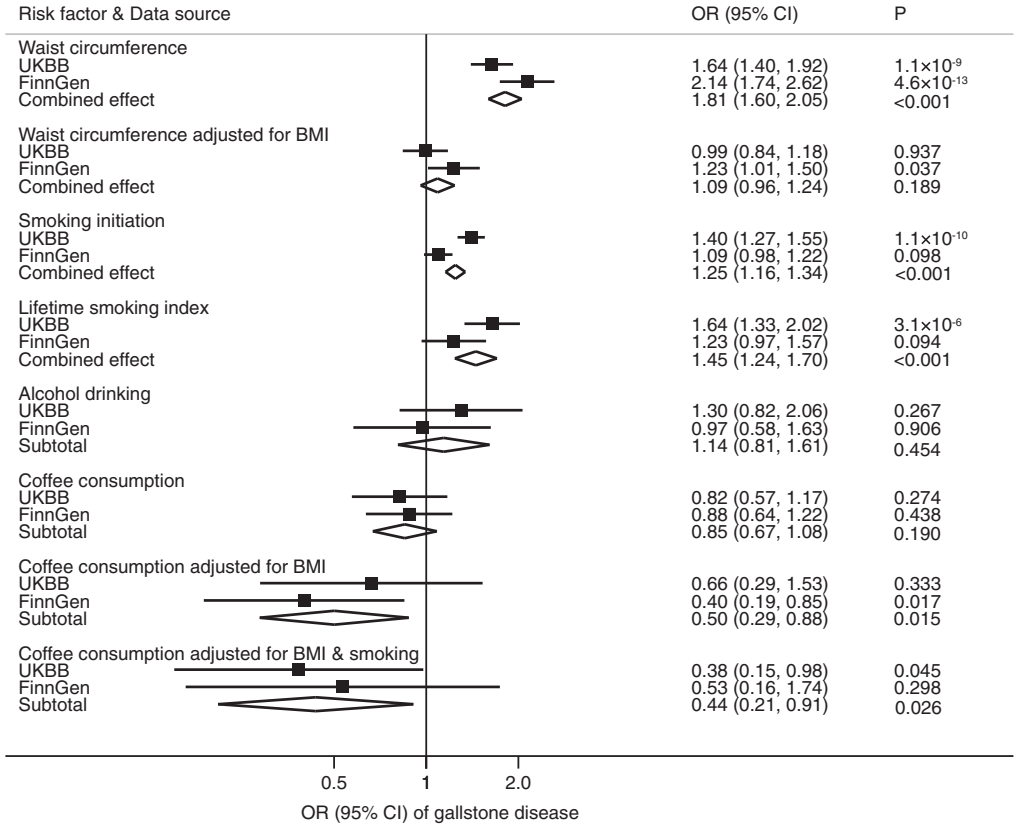

Figure 3. Associations of genetically predicted waist circumference and modifiable lifestyle factors with gallstone disease. Estimates for coffee consumption with adjustment for BMI were derived from the multivariable inverse-variance weighted model with random effects. The ORs of gallstone disease were scaled to a 1-SD increase in waist circumference, a 1-SD increase in prevalence of smoking initiation, a 1-SD increase of lifetime smoking index, a 1-SD increase of log-transformed alcoholic drinks/wk, and a 50% increase in coffee consumption. Estimates were derived using the multiplicative random-effects inverse-variance weighted MR method and combined using fixed-effects meta-analysis. UKBB, UK Biobank.

Table 2. Associations of Genetically Predicted Risk Factors With Gallstone Disease in MR Sensitivity Analyses

			Weighted Median			MR-Egger			MR-PRESSO				
	Used SNPs	Cochrane's Q	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	$P_{\text{pleiotropy}}$[a]	$P_{\text{distortion test}}$[b]
UK Biobank													
BMI	93	91.79	1.53	1.26–1.85	1.75×10^{-5}	1.50	1.12–2.00	.008	1.61	1.43–1.81	5.52×10^{-12}	.591	.554
Waist circumference	45	45.69	1.48	1.15–1.91	.003	1.74	1.14–2.68	.015	1.64	1.40–1.92	2.42×10^{-7}	.763	.433
Waist circumference adjusted for BMI	70	105.77	1.06	0.86–1.31	.579	1.16	0.56–2.39	.687	0.99	0.84–1.18	.937	.003	.665
Type 2 diabetes	488	707.89	1.10	1.04–1.17	.001	1.02	0.94–1.11	.592	1.12	1.08–1.17	2.47×10^{-8}	.009	.709
Smoking initiation	309	406.80	1.30	1.14–1.48	7.60×10^{-5}	1.10	0.72–1.69	.666	1.39	1.25–1.54	9.10×10^{-10}	.253	.841
Lifetime smoking index	126	164.77	1.58	1.20–2.07	.001	0.62	0.28–1.39	.251	1.56	1.28–1.89	1.85×10^{-5}	.016	.569
Alcohol drinking	82	240.89	1.00	0.60–1.65	.995	1.09	0.45–2.68	.848	1.07	0.79–1.46	.654	.662	.050
Coffee consumption	9	27.10	0.81	0.57–1.15	.248	0.76	0.37–1.58	.488	0.99	0.77–1.28	.953	.829	<.001
FinnGen consortium													
BMI	93	122.80	1.74	1.40–2.18	1.06×10^{-6}	2.09	1.45–3.01	1.63×10^{-4}	1.62	1.40–1.87	4.86×10^{-9}	.189	.730
Waist circumference	45	66.74	2.27	1.76–2.93	3.44×10^{-10}	3.08	1.79–5.30	2.13×10^{-4}	2.05	1.69–2.48	5.60×10^{-9}	.163	.662
Waist circumference adjusted for BMI	70	116.12	1.31	1.03–1.65	.027	0.70	0.30–1.63	.420	1.23	1.01–1.49	.037	<.001	.188
Type 2 diabetes	468	761.75	1.10	1.03–1.18	.008	1.11	1.00–1.22	.044	1.12	1.07–1.17	1.40×10^{-6}	.636	.620
Smoking initiation	295	333.40	1.11	0.96–1.29	.160	0.83	0.53–1.30	.414	1.09	0.98–1.22	.100	.214	NA
Lifetime smoking index	124	178.11	1.14	0.84–1.55	.411	0.29	0.11–0.77	.015	1.23	0.97–1.57	.096	.004	NA
Alcohol drinking	78	193.20	0.97	0.57–1.65	.918	0.60	0.17–2.13	.434	0.87	0.59–1.26	.459	.697	.754
Coffee consumption	9	16.86	0.77	0.59–1.02	.066	0.78	0.39–1.54	.495	0.82	0.66–1.02	.118	.697	.620

NOTE. The ORs of gallstone disease were scaled to 1-SD increase in BMI and waist circumference, 1-unit increase in log-odds ratio of type 2 diabetes, 1-SD increase in prevalence of smoking initiation, 1-SD increase of lifetime smoking index, 1-SD increase of log-transformed alcoholic drinks/week and 50% increase in coffee consumption.

BMI, body mass index; CI, confidence interval; MR, Mendelian randomization; NA, not available; OR, odds ratio; SNP, single nucleotide polymorphism.

[a]*P* values for pleiotropy were derived from MR-Egger test and a *P* value <.05 indicates a possible pleiotropic effect.

[b]*P* values for distortion were derived from MR-PRESSO test and a *P* value <.05 indicates a difference between estimates before and after outlier removal. *P* of distortion test was not available for the analysis of smoking initiation and lifetime smoking index based on FinnGen consortium due to no outlier detected.

cholecystokinin release, enhance gallbladder motility, inhibit gallbladder fluid absorption, decrease cholesterol crystallization in bile, downregulate the hepatic low-density lipoprotein receptor, and decrease 3-hydroxyl-3-methylglutaryl CoA reductase activity.[40]

The major strengths of the present study are the MR design and the large number of gallstone disease cases. The similar results in 2 independent populations supported the robustness and reliability of our results for the associations of obesity and type 2 diabetes with gallstones. Genetic associations with the International Classification of Diseases–defined gallstone endpoint in UK Biobank has been replicated in both Icelandic and Danish cohorts,[41,42] which supported a high validity of gallstone definition in our study. Population stratification bias is unlikely to have influenced our results, as the majority of recruited participants in the genome-wide association studies were of European origin and adjustment was made for population structure through genetic principal components. However, this population confinement might also limit the generalizability of our findings to other populations.

A limitation of the present study is that we could not distinguish between cholesterol and pigment gallstones, which have different etiologies. Nevertheless, the vast majority of gallstones in the Western world are cholesterol gallstones.

A potential concern in any MR study is pleiotropy. There are 2 types of pleiotropy in MR studies—vertical and horizontal pleiotropy.[43,44] In this study, genetic instruments for BMI influenced the risk of gallstone disease partly via type 2 diabetes, which would represent vertical pleiotropy (or mediated pleiotropy), as BMI was a causal risk factor for type 2 diabetes.[45] Vertical pleiotropy is not a barrier in causal inference of MR studies.[43,44] The genetic instruments for coffee consumption had moderate correlations with BMI and smoking.[18] Given that coffee consumption is less likely to cause obesity and smoking initiation, this phenomenon called horizontal pleiotropy would bias causal estimation. We found an inverse association between genetically predicted coffee consumption and gallstones after removal of pleiotropy by excluding pleiotropic genetic variants and after adjusting for BMI and smoking through multivariable MR analysis.[43,44] Considering the consistent associations of genetic predisposition to type 2 diabetes and smoking initiation with gallstone disease across the 2 data sources and different MR models, these results are unlikely to be driven by horizontal pleiotropy.

In conclusion, the present study provides MR evidence supporting causal roles of obesity, type 2 diabetes, and cigarette smoking in gallstones. The possible association between coffee consumption and gallstone disease needs verification.

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Conflicts of Interest
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