Gastroenterology 2023;165:218–227

# Gallstones, Cholecystectomy, and Kidney Cancer: Observational and Mendelian Randomization Results Based on Large Cohorts

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**BACKGROUND & AIMS:** Gallstones (cholelithiasis) constitute a major health burden with high costs related to surgical removal of the gallbladder (cholecystectomy), generally indicated for symptomatic gallstones. The association between gallstones and cholecystectomy and kidney cancer is controversial. We comprehensively investigated this association, considering age at cholecystectomy and time from cholecystectomy to kidney cancer diagnosis, and assessed the causal effect of gallstones on kidney cancer risk by Mendelian randomization (MR). **METHODS:** We compared the risk of kidney cancer in cholecystectomized and noncholecystectomized patients (16.6 million in total) from the Swedish nationwide cancer, census, patient, and death registries using hazard ratios (HRs). For 2-sample and multivariable MR, we used summary statistics based on 408,567 UK Biobank participants. **RESULTS:** During a median follow-up of 13 years, 2627 of 627,870 cholecystectomized Swedish patients developed kidney cancer (HR, 1.17; 95% CI, 1.12–1.22). Kidney cancer risk was particularly increased in the first 6 months after cholecystectomy (HR, 3.79; 95% CI, 3.18–4.52) and in patients cholecystectomized before age 40 years (HR, 1.55; 95% CI, 1.39–1.72). MR results based on 18,417 patients with gallstones and 1788 patients with kidney cancer from the United Kingdom revealed a causal effect of gallstones on kidney cancer risk (9.6% risk increase per doubling in gallstone prevalence; 95% CI, 1.2%–18.8%). **CONCLUSIONS:** Both observational and causal MR estimates based on large prospective cohorts support an increased risk of kidney cancer in patients with gallstones. Our findings provide solid evidence for the compelling need to diagnostically rule out kidney cancer before and during gallbladder removal, to prioritize kidney cancer screening in patients undergoing cholecystectomy in their 30s, and to investigate the underlying mechanisms linking gallstones and kidney cancer in future studies.

*Keywords:* Cancer Prevention; Risk Prediction; Large Nationwide Cohorts; Causal Inference.

HEPATOBILIARY

Gallstones (cholelithiasis) are lithic deposits of digestive fluid in the gallbladder. Most gallstones are made up of cholesterol predominantly and are referred to as cholesterol gallstones, whereas pigment gallstones contain mostly bilirubin.[1] Gallstones are present in up to 25% of adults living in the Western world.[2] Although most gallstones remain asymptomatic, gallstone disease, referring to complications such as abdominal pain, cholecystitis, cholangitis, and pancreatitis, occurs in 20% of patients with gallstones and often requires surgical removal of the gallbladder (cholecystectomy).[3] Gallstone disease is one of the most common disorders of the digestive system, and risk factors for gallstones and gallstone disease include increasing age, female sex, type 2 diabetes, obesity, physical inactivity and other lifestyle factors.[4]

Bile acids, the major constituents of bile, are synthesized in the liver from cholesterol and are responsible for solubilization, digestion, and absorption of lipids in the intestine.[5] Under normal physiological conditions, bile acids regulate numerous physiological processes through the activation of 2 receptors, TGR5 (G protein-coupled bile acid receptor-1) and FXR (nuclear farnesoid X receptor), and are excreted only minimally in the urine.[6] However, certain pathological conditions, such as cholestasis and cholecystectomy can lead to increased bile acid excretion and cause oxidative damage.[7,8] The associated increased urinary excretion of bile acids can lead to oxidative stress and kidney damage, and may eventually lead to tumorigenesis.[6] In addition, excess bile acids lead to increased synthesis of secondary bile acids, the major components of which are lithocholic acid and deoxycholic acid.[9] Elevated levels of deoxycholic acid have been found to alter the gut microbiome, promote intestinal carcinogenesis, and suppress FXR, which acts as an antagonist of Wnt/$\beta$-catenin signaling.[9,10]

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***Abbreviations used in this paper:*** **BMI, body mass index; HR, hazard ratio; IVW, inverse variance weighted; MR, Mendelian randomization; MVMR, multivariate Mendelian randomization; OR, odds ratio; SNP, single nucleotide polymorphism.**

Most current article



0016-5085
https://doi.org/10.1053/j.gastro.2023.03.227

**WHAT YOU NEED TO KNOW**

BACKGROUND AND CONTEXT

Gallstone disease is a major health burden and symptomatic gallstones are often treated by surgical removal of the gallbladder (cholecystectomy). The risk of kidney cancer in cholecystectomized patients is poorly understood.

NEW FINDINGS

The risk of kidney cancer is particularly high in the first 6 months after cholecystectomy, and in patients who underwent a cholecystectomy before the age of 40 years. Mendelian randomization results provide robust evidence for a causal effect of gallstones on kidney cancer risk, partly mediated by smoking and diabetes.

LIMITATIONS

Information available in Swedish registries is limited and laterality of kidney cancer is not available in the UK Biobank.

CLINICAL RESEARCH RELEVANCE

The steady increase in the number of cholecystectomies may lead to an increased incidence of kidney cancer.

BASIC RESEARCH RELEVANCE

Further research is needed into the mechanisms linking gallstones, cholecystectomy, and kidney cancer.

In addition to these processes, gallstones may lead to local and systemic inflammation, as well as disruption of metabolic processes.[11,12] Surgical removal of the gallbladder may cause direct leakage of bile into the peritoneum in up to 2.7% of patients.[13] Furthermore, cholecystectomy leads to alteration of bile flux, with possible changes in bile salts, metabolic hormone levels, and bacterial microbiota, which can also lead to inflammation.[14,15] Consequently, both gallstones and cholecystectomy can promote tumor development by triggering several hallmarks of cancer.[16]

Several studies have found an association between gallstones and increased risks of digestive and kidney cancers.[17–20] However, the few studies conducted on the relationship between gallstones, cholecystectomy and kidney cancer have provided conflicting results. Two studies reported an association between gallstones and kidney cancer risk, although 1 study found no such association.[17,21,22] All studies conducted were based on a small number of kidney cancer cases, and the statistical power to perform stratified analyses by age at cholecystectomy was limited. In addition, it has been difficult to distinguish between gallstones and cholecystectomy as exposures that potentially increase the risk of kidney cancer, as none of the studies to date take into account the time between cholecystectomy and kidney cancer diagnosis, an important consideration, given that most kidney tumors develop over a period of many years.[23] To our knowledge, no study has yet investigated the causal effect of gallstones on kidney cancer risk using Mendelian randomization (MR).

The present investigation overcomes important shortcomings of previous studies and examines the relationship between gallstones and cholecystectomy and kidney cancer using 2 complementary approaches. In a large prospective observational study of 627,870 cholecystectomized patients, we estimated the relative risk of kidney cancer by taking into account patients' age at gallbladder removal and time after cholecystectomy. We also exploited gallstone risk variants as instrumental variables for 2-sample MR and used summary statistics from the UK Biobank to assess the causal effect of gallstones on kidney cancer risk, simultaneously accounting for established kidney cancer risk factors in multivariable MR.

With the increasing incidence of gallstones and related cholecystectomies, the burden of associated chronic diseases is also on the rise. Therefore, it is important to characterize the associations between gallstones and kidney cancer, and between cholecystectomy and kidney cancer, for better personalized prevention of this neoplasm.

## Materials and Methods

### *Prospective Data From the Swedish Registries*

Data from the Swedish National Patient Register, Swedish Cancer Registry, National Population Registry, national censuses, and the Death Registry, were linked using individually unique pseudonymized national registration numbers. These nationwide registered sources are updated every 1–3 years. The combined data sets include information on more than 16 million people. The Swedish National Patient Register contain nationwide data on surgical procedures from all private and public hospitals, as well as visits to specialized physicians in Sweden; hospital (inpatient) records from 1964 to 2018; and day clinic (outpatient) records from 2001 to 2018. Information on the date and performance of cholecystectomy was extracted from the inpatient and outpatient registers using the International Classification of Diseases, Ninth Revision, Clinical Modification[24] code 51.2 and its subcategories.

The Swedish Cancer Registry provided information on the date of cancer diagnosis, tumor topography and morphology, and diagnostic reports from physicians for the period 1958–2018. All cancer data were recorded according to the International Classification of Diseases, Seventh Revision.[25] Information on patients with kidney cancer was extracted using International Classification of Diseases, Seventh Revision code 180 (malignant neoplasm of kidney). There were no missing data on cancer status of individuals. The overall completeness of the Cancer Registry is estimated at 96% (it is probably even higher for nonhematologic organs).[26] Demographic information, such as sex and dates of birth, migration, and death were obtained from the National Population Registry, national censuses, and the Death Registry.

We used a population-based matched cohort design for the observational part of our study. For each cholecystectomized patient in Sweden, we selected 5 noncholecystectomized controls who were alive and free of kidney cancer, matched for sex, baseline age (year and month), and a propensity score. In total, 3,139,328 controls were included in the study and only 8 patients had fewer than 5 controls. The propensity score was calculated with logistic regression adjusted for sex, birth year, socioeconomic status (ie, farmer, manual workers, low- to middle-income office worker, high-income office worker or professional, company owner [other than farmer], or other or unspecified), and residential area (ie, large cities, southern Sweden, northern Sweden, or unspecified).

Follow-up of cholecystectomized patients started from the date of surgical gallbladder removal (month and year). Follow-up of noncholecystectomized individuals started at the same age (year and month) as their matched cholecystectomized counterparts. For all individuals, follow-up ended with cancer diagnosis, emigration, death, or the end of the study period (end of 2018), whichever came first. Median follow-up for cholecystectomized patients was 13.2 years (interquartile range, 5.3–23.6 years; mean, 15.8 years; and maximum, 54.8 years), and 94,606 cholecystectomized patients were followed up for at least 30 years. Median follow-up of noncholecystectomized controls was 13.6 years (interquartile range, 6.1–24.0 years; mean, 16.3 years; and maximum, 54.9 years), and 489,392 controls were followed for at least 30 years.

Cox proportional hazards regression was used to estimate the relative risk (hazard ratio [HR]) of first primary kidney cancer in cholecystectomized patients compared with their matched controls without a history of cholecystectomy. Stratified analyses were performed by sex, patient's age at cholecystectomy (ie, younger than 40 years, 40–49 years, 50–59 years, or 60 years or older), time since cholecystectomy (ie, 1–6 months, 7–36 months, or more than 3 years), and side of kidney cancer (ie, left or right). In addition to relative risks, absolute incidence rates of kidney cancer were calculated for cholecystectomized patients according to the time in years after gallbladder surgery and the patient's age at cholecystectomy (ie, younger than 40 years or 40 years or older). For comparison, kidney cancer incidence rates were also calculated for the control group (noncholecystectomized individuals) with an age at baseline equal to that of the matched cholecystectomized patients (Figure 1; 3-year moving average smoothing from the second year after cholecystectomy to reduce random variation). The main statistical analyses were planned in advance of their execution, and there were no data-driven changes to the planned analyses. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC), and absolute incidence rates of kidney cancer were represented using the R software environment for statistical computing and graphics.

## Prospective Data From the UK Biobank

We used the UK Biobank resource for subsequent MR analyses. The UK Biobank recruited 500,000 people from the United Kingdom aged between 40 and 69 years in 2006–2010.[27] Genotype calling was performed by Affymetrix (Santa Clara, CA) on the UK BiLEVE and the UK Biobank Axiom arrays. Genotype imputation was performed using the Haplotype Reference Consortium and UK10K haplotype resources.

## Assessment of the Causal Effect of Gallstones and Cholecystectomy on Kidney Cancer Risk by Mendelian Randomization

MR is an analytical method used to assess the causal effect of specific risk factors (exposures) on specific phenotypes (outcome) using genetic variants as instrumental variables.[28] A brief introduction to MR is provided in the Supplementary Methods, including the typical graph of MR studies adapted to the present investigation and a flowchart (Supplementary Figures 1 and 2). We applied 2-sample MR to investigate the causal effect of gallstones on the risk of kidney cancer. Summary statistics on the association between genetic variants and gallstones (sample I) were retrieved from the UK Biobank data set (18,417 cases of gallstone disease and 390,150 controls) in the study by Ferkingstad et al[29] (Supplementary Table 1), who identified 32 single nucleotide polymorphisms (SNPs) robustly associated with gallstone disease risk ($P < 5 \times 10^{-8}$ in the meta-analysis of UK Biobank and Icelandic data). Summary statistics on the association between these 32 SNPs and the risk of kidney cancer risk (sample II) were estimated using logistic regression adjusted for age, gender, and the first 20 genetic principal components based on unrelated participants of European descent from the UK Biobank (1788 cases of kidney cancer and 224,187 controls without any cancer diagnosis). We excluded 2 SNPs for subsequent analyses that were not imputed in the UK Biobank (rs756082276 and rs756935975), 2 SNPs in linkage disequilibrium ($r^2 > 0.01$) with other variants that explained a larger proportion of variance (rs45575636, rs34851490) and the palindromic SNP (rs1935), leaving 27 SNPs used as instrumental variables.

The variance in liability to gallstone disease explained by each SNP was calculated based on a multifactorial threshold model that postulates latent continuous liability under a normal distribution with mean 0 and variance 1.[30] This model assumes that the mean liability depends on the individual genotype; only those individuals whose liability exceeds a fixed threshold develop gallstone disease. Calculations relied on the allele frequencies and the per-allele odds ratios (ORs) reported by Ferkingstad et al,[29] and on the prevalence of gallstone disease, which was set at 15%. We visually inspected the funnel and scatter plots of the SNP–gallstone vs SNP–kidney cancer risk summary association statistics to detect weak instrument bias; calculated Cochran's $Q$ statistic using first-order inverse variance weights to detect heterogeneity, which indicates a possible violation of instrumental variable or modeling assumptions, of which pleiotropy is a likely major cause; and used the $P$ value for a nonzero MR–Egger intercept to assess horizontal pleiotropy.

Our primary objective was to test the evidence for a causal effect of gallstones on kidney cancer risk, which requires weaker MR assumptions than estimating its magnitude. As a secondary objective, we estimated the size of the causal effect and assessed its robustness by comparing inverse variance weighted (IVW), weighted median, and MR–Egger estimates of the OR. As additional sensitivity analyses, we also calculated the Wald ratio for the single SNP rs11887534, a missense variant in the ATP binding cassette subfamily G member 8 (*ABCG8*) gene implicated in the hepatic transport of cholesterol into the bile, and repeated MR analyses using summary statistics from nonoverlapping UK Biobank sample I and sample II considering the following 3 different exposures: self-reported and diagnosed gallstones, as in the original report by Ferkingstad et al[29]; diagnosed gallstones only; and cholecystectomy in patients with previously diagnosed gallstones. MR analyses stratified by sex were also performed. MR analyses were conducted using the R version of MR-Base, which provides convenient tools for harmonizing summary statistics, including standardization of effect alleles and removal of palindromic SNPs, and implements by default a random-effects model for the IVW method.

## Simultaneous Consideration of Established Kidney Cancer Risk Factors by Multivariable Mendelian Randomization

Type 2 diabetes, smoking, body mass index (BMI) and hypertension have been associated with the risk of kidney cancer

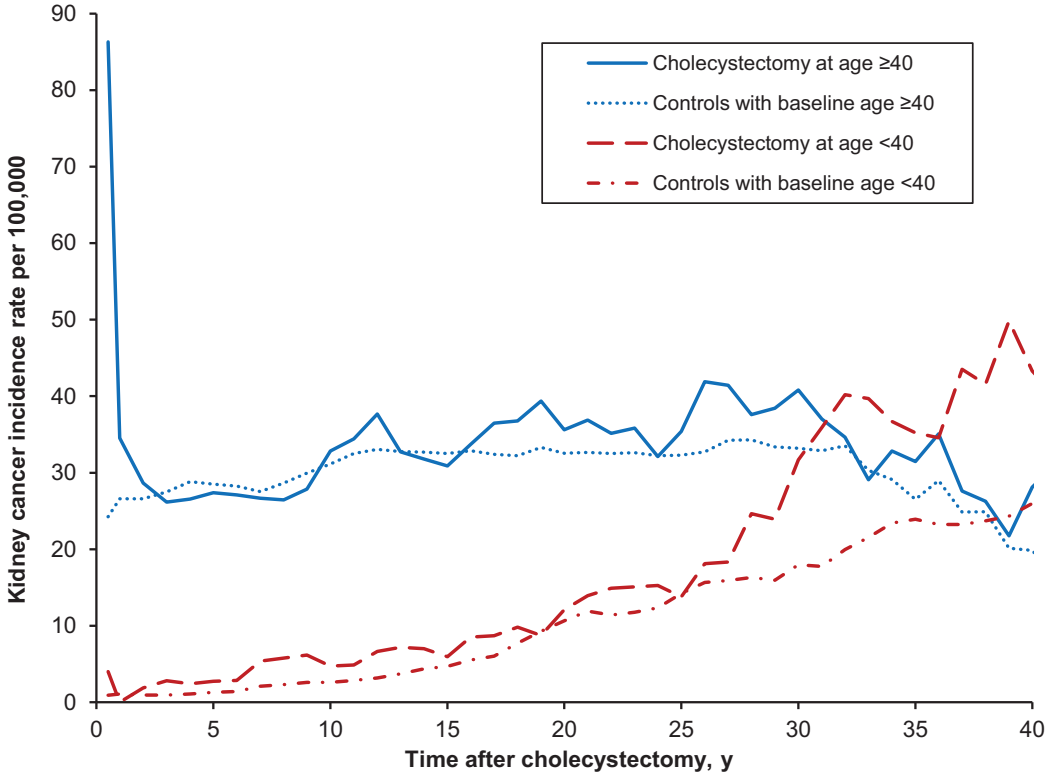


**Figure 1.** Incidence rate of kidney cancer after cholecystectomy by time after surgery and patient's age at the operation (younger than 40 years or 40 years or older). *Dotted lines* represent the corresponding rates for noncholecystectomized individuals with an age at baseline equal to the median age in the group of cholecystectomized patients.

(Supplementary Table 2).[31] Furthermore, a strong association between kidney stones and gallstones has been reported.[32] These risk factors may mediate the effect of gallstones on kidney cancer, and we used multivariable MR (MVMR) to assess the direct and mediated effects of gallstones on kidney cancer risk.[33] First, we used 2-sample MR to separately assess the causal effect of type 2 diabetes, smoking, BMI, kidney stones, and hypertension on kidney cancer risk. The investigated factors with a causal effect on kidney cancer risk were then considered together with gallstones in 2-way MVMR analyses to assess potential mediation. Finally, gallstones and all risk factors with a causal effect on kidney cancer risk were simultaneously considered in a multiway MVMR. All MVMR analyses were performed using the R package MVMR.

Summary statistics for diabetes, smoking, BMI, kidney stones, and hypertension were obtained from the original publications or the University of Bristol Integrative Epidemiology Unit OpenGWAS project database using the R package ieugwasr. Variants with minor allele frequencies $< 0.01$, palindromic SNPs with intermediate allele frequencies, and instruments without available summary statistics were removed. Supplementary Table 2 provides an overview of the studies and the number of available instruments for each kidney cancer risk factor.[34] Pairwise covariances between an instrument and pairs of exposures were estimated using the phenotypic correlation between exposures. Overlap between studies was limited and the summary statistics were assumed to be uncorrelated.

Summary statistics to reproduce all MR results are available at www.biometrie.uni-heidelberg.de/StatisticalGenetics/Software_ and_Data.

## Ethics Statements

This study was conducted in accordance with the Declaration of Helsinki. Swedish data access was approved by the Institutional Review Board (Ethics Committee) of Lund University (Dnr 2012/795 and Dnr 2016/679). To minimize the risk of identification of study participants, only access to pseudonymized secondary data was provided. Patient consent was waived because written informed consent is not needed for registry-based studies in Sweden. The North West Multicentre Research Ethics Committee approved the UK Biobank as a research tissue bank, for which researchers do not require separate ethics clearance. The UK data analyzed in this study were accessed under UK Biobank application 58030.

## Results

### *Observational Associations*

During the entire follow-up period, 2627 of 627,870 cholecystectomized Swedish patients were diagnosed with primary kidney cancer. Overall, a 17% increased relative risk of kidney cancer (HR,

**Table 1.** Relative Risk of Kidney Cancer After Cholecystectomy by Time After Surgery, Patient's Age at Surgery, and Sex

| Time since surgery, *mo* | Age at surgery, *y* | All | | | Women | | | Men | | | HR difference (women minus men) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n[a] | HR[b] | 95% CI | n[a] | HR[b] | 95% CI | n[a] | HR[b] | 95% CI | |
| Any | Any | 2627 | **1.17** | 1.12–1.22 | 1481 | **1.22** | 1.15–1.29 | 1146 | **1.12** | 1.05–1.19 | 0.10 |
| | <40 | 456 | **1.55** | 1.39–1.72 | 318 | **1.56** | 1.37–1.77 | 138 | **1.52** | 1.26–1.84 | 0.04 |
| | 40–49 | 467 | **1.11** | 1.01–1.23 | 268 | **1.16** | 1.02–1.33 | 199 | 1.05 | 0.91–1.23 | 0.11 |
| | 50–59 | 661 | 1.06 | 0.97–1.15 | 361 | 1.05 | 0.94–1.18 | 300 | 1.06 | 0.94–1.20 | –0.01 |
| | ≥60 | 1043 | **1.15** | 1.07–1.23 | 534 | **1.21** | 1.10–1.33 | 509 | 1.09 | 0.99–1.20 | 0.12 |
| 1–6 | Any | 202 | **3.79** | 3.18–4.52 | 98 | **4.27** | 3.30–5.53 | 104 | **3.41** | 2.68–4.34 | 0.86 |
| | <40 | 3 | **5.06** | 1.12–22.85 | 1 | 1.77 | 0.20–16.02 | 2 | NC | NC | NC |
| | 40–49 | 14 | **3.75** | 1.93–7.32 | 6 | **3.42** | 1.23–9.46 | 8 | **3.94** | 1.62–9.57 | –0.52 |
| | 50–59 | 36 | **4.58** | 2.97–7.08 | 16 | **5.65** | 2.86–11.13 | 20 | **4.00** | 2.27–7.07 | 1.65 |
| | ≥60 | 149 | **3.58** | 2.93–4.39 | 75 | **4.15** | 3.10–5.57 | 74 | **3.13** | 2.37–4.15 | 1.02 |
| 7–36 | Any | 294 | 1.08 | 0.95–1.22 | 150 | **1.20** | 1.00–1.43 | 144 | 0.98 | 0.82–1.17 | 0.22 |
| | <40 | 8 | **2.65** | 1.13–6.20 | 4 | 2.14 | 0.67–6.84 | 4 | 3.40 | 0.95–12.10 | –1.26 |
| | 40–49 | 27 | **1.57** | 1.02–2.42 | 15 | 1.52 | 0.85–2.70 | 12 | 1.64 | 0.85–3.15 | –0.12 |
| | 50–59 | 59 | 1.07 | 0.81–1.42 | 37 | 1.38 | 0.96–1.98 | 22 | 0.77 | 0.49–1.21 | 0.61 |
| | ≥60 | 200 | 1.01 | 0.87–1.17 | 94 | 1.07 | 0.86–1.33 | 106 | 0.96 | 0.78–1.18 | 0.11 |
| >36 | Any | 2131 | **1.11** | 1.06–1.17 | 1233 | **1.16** | 1.09–1.23 | 898 | 1.06 | 0.99–1.14 | 0.10 |
| | <40 | 445 | **1.53** | 1.38–1.70 | 313 | **1.55** | 1.37–1.76 | 132 | **1.47** | 1.21–1.79 | 0.08 |
| | 40–49 | 426 | 1.07 | 0.96–1.19 | 247 | 1.13 | 0.98–1.29 | 179 | 1.00 | 0.85–1.17 | 0.13 |
| | 50–59 | 566 | 1.00 | 0.92–1.10 | 308 | 0.98 | 0.87–1.10 | 258 | 1.04 | 0.91–1.18 | –0.06 |
| | ≥60 | 694 | 1.04 | 0.96–1.13 | 365 | 1.09 | 0.97–1.22 | 329 | 0.99 | 0.88–1.11 | 0.10 |

NC, not calculable.
[a]Number of patients with kidney cancer after cholecystectomy.
[b]Hazard ratio adjusted for sex, birth year, baseline age, socioeconomic status, and residential area. Bold values indicate that the 95% CI does not include 1.00.



1.17; 95% CI, 1.12–1.22) (Table 1) was found in cholecystectomized patients compared with non-cholecystectomized individuals.

The sample size of previous studies did not allow stratification by time after cholecystectomy, but our large prospective cohort permitted examination of the change in relative risk with time since surgical removal of the gallbladder. Most patients (n = 2131) developed kidney cancer more than 3 years after cholecystectomy, resulting in a relative risk of 1.11 (95% CI, 1.06–1.17) (Table 1). However, a particularly high relative risk of kidney cancer was observed in the first 6 months after cholecystectomy (n = 202; HR, 3.79; 95% CI, 3.18–4.52). Further stratification by sex showed a higher relative risk of kidney cancer in cholecystectomized women (HR, 1.22; 95% CI, 1.15–1.29) than in cholecystectomized men (HR, 1.12; 95% CI, 1.05–1.19; overlapping 95% CI) (Table 1). The proportion of cases of kidney cancer attributable to cholecystectomy (population attributable fraction) was 1.1% overall, 1.8% in women, and 0.6% in men.

When patient's age at cholecystectomy was taken into account, the relative risk of kidney cancer generally decreased with increasing age at surgery (Table 1). Patients who underwent cholecystectomy before the age of 40 years had a 55% higher risk of kidney cancer than non-cholecystectomized individuals of similar age (HR, 1.55; 95% CI, 1.39–1.72), whereas a 15% excess risk was observed for cholecystectomy after the age of 60 years (HR, 1.15; 95% CI, 1.07–1.23).

As the gallbladder is closer to the right kidney, we also examined possible differences in relative risks according to the side of the renal neoplasm (Table 2). The number of primary tumors in the right kidney (n = 872) was virtually identical to that in the left kidney (n = 873). The largest differences in relative risks were found in the first 6 months after surgery (right: HR, 4.91 vs left: HR, 3.64) and in patients cholecystectomized before the age of 40 years (right: HR, 1.51 vs left: HR, 1.71), but these differences did not reach the 5% level of statistical significance.

**Table 2.** Relative Risk of Kidney Cancer After Cholecystectomy by Time After Surgery, Patient's Age at Surgery, and Kidney Side

| Time since surgery, *mo* | Age at surgery, *y* | Any side | | | Right kidney | | | Left kidney | | | HR difference (right minus left) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n[a] | HR[b] | 95% CI | n[a] | HR[b] | 95% CI | n[a] | HR[b] | 95% CI | |
| Any | Any | 2627 | **1.17** | 1.12–1.22 | 872 | **1.22** | 1.13–1.31 | 873 | **1.22** | 1.13–1.32 | 0.00 |
| | <40 | 456 | **1.55** | 1.39–1.72 | 190 | **1.51** | 1.28–1.77 | 194 | **1.71** | 1.45–2.01 | –0.20 |
| | ≥40 | 2171 | **1.11** | 1.06–1.17 | 682 | **1.16** | 1.06–1.26 | 679 | **1.13** | 1.04–1.22 | 0.03 |
| 1–6 | Any | 202 | **3.79** | 3.18–4.52 | 56 | **4.91** | 3.45–6.99 | 46 | **3.64** | 2.52–5.25 | 1.27 |
| | <40 | 3 | **5.06** | 1.12–22.85 | 1 | NC | NC | 2 | NC | NC | NC |
| | ≥40 | 199 | **3.76** | 3.16–4.49 | 1 | **4.83** | 3.39–6.89 | 44 | **3.48** | 2.40–5.04 | 1.35 |
| >6 | Any | 2425 | **1.11** | 1.06–1.16 | 816 | **1.16** | 1.08–1.25 | 827 | **1.18** | 1.09–1.27 | –0.02 |
| | <40 | 453 | **1.54** | 1.39–1.71 | 189 | **1.50** | 1.27–1.76 | 192 | **1.69** | 1.44–1.99 | –0.19 |
| | ≥40 | 1972 | 1.04 | 0.99–1.09 | 627 | 1.08 | 0.99–1.18 | 635 | 1.07 | 0.99–1.17 | 0.01 |

NC, not calculable.
[a]Number of patients with kidney cancer after cholecystectomy.
[b]Hazard ratio adjusted for sex, birth year, baseline age, socioeconomic status, and residential area. Bold values indicate that the 95% CI does not include 1.00.

### *Causal Inference by Mendelian Randomization*

The variance in liability to gallstone disease explained jointly by the 27 SNPs used as instrumental variables in 2-sample MR was 7% and the variance explained by the missense variant rs11887534 at the *ABCG8* locus was 3%. MR analysis of the association between gallstones and kidney cancer risk revealed no heterogeneity among instrumental variables as a proxy for pleiotropy (IVW Cochran's $Q$ statistic $P = .43$) and no horizontal pleiotropy (MR–Egger intercept $P = .42$). Neither outliers nor weak instrument biases were evident in the scatter and funnel plots (Figure 2), where SNP rs11887534 clearly appeared as a high-leverage instrumental variable.

We found evidence of a causal effect of gallstones on kidney cancer risk, with a 9.6% increased risk of kidney cancer per doubling in gallstone prevalence (IVW OR, 1.096; 95% CI, 1.012–1.188; $P = .025$). The causal effect size estimated by weighted median (OR, 1.067; 95% CI, 0.949–1.200) and MR–Egger regression methods (OR, 1.048; 95% CI, 0.916–1.198), and the Wald ratio for rs11887534 (OR, 1.062; 95% CI, 0.930–1.212) were all consistent with the primary IVW estimate (overlapping 95% CIs, Supplementary Table 3), as were causal effect estimates based on nonoverlapping UK Biobank samples: IVW OR, 1.144 (95% CI, 1.042–1.257; $P = .005$) for the exposure "self-reported or diagnosed gallstones"; IVW OR, 1.153 (95% CI, 1.046–1.271; $P = .004$) for the exposure "diagnosed gallstones"; and IVW OR, 1.138 (95% CI, 1.041–1.244; $P = .004$) for the exposure "cholecystectomy with a prior gallstone diagnosis." In accordance with the observational results based on Swedish patients, MR analyses stratified by sex showed a slightly stronger causal effect of gallstones on the risk of kidney cancer in women (OR, 1.162; 95% CI, 1.004–1.346; $P = .045$) than in men (OR, 1.140; 95% CI, 0.996–1.304; $P = .057$) (Supplementary Table 4).

To investigate the mediating effects of type 2 diabetes, smoking initiation, BMI, kidney stones, and hypertension on the causal effect of gallstones on kidney cancer risk, we applied MVMR. Kidney stones and hypertension did not show a causal effect on kidney cancer risk according to univariate MR and were not considered in the subsequent MVMR analyses (Supplementary Table 5). Two-way MVMR indicated a direct effect of gallstones on kidney cancer risk, independent of type 2 diabetes ($P = .03$), smoking initiation ($P = .02$), and BMI ($P < .001$), as well as mediation of type 2 diabetes and smoking on the effect of gallstones on kidney cancer. For example, the original OR for gallstones (1.144) decreased to 1.116 when type 2 diabetes was included (Supplementary Table 5), suggesting a (144–116)/144 = 19% mediation through diabetes and 81% direct effect of gallstones on kidney cancer risk on the doubling scale of gallstone prevalence. The 4-way MVMR results also indicated a direct causal effect of gallstones on kidney cancer risk when simultaneously adjusting for type 2 diabetes, smoking initiation, and BMI ($P = .03$), and robust MVMR with Q-minimization resulted in slightly larger effect size estimates (OR, 1.13 vs OR, 1.09).

## Discussion

In this study, we investigated the association of gallstones and cholecystectomy with kidney cancer risk using 2 approaches: an observational matched study based on clinical and demographic data from a large cohort with up to 60 years of follow-up (Swedish cancer and inpatient/outpatient registries) and an MR study based on clinical and genotype data from a large prospective database (UK

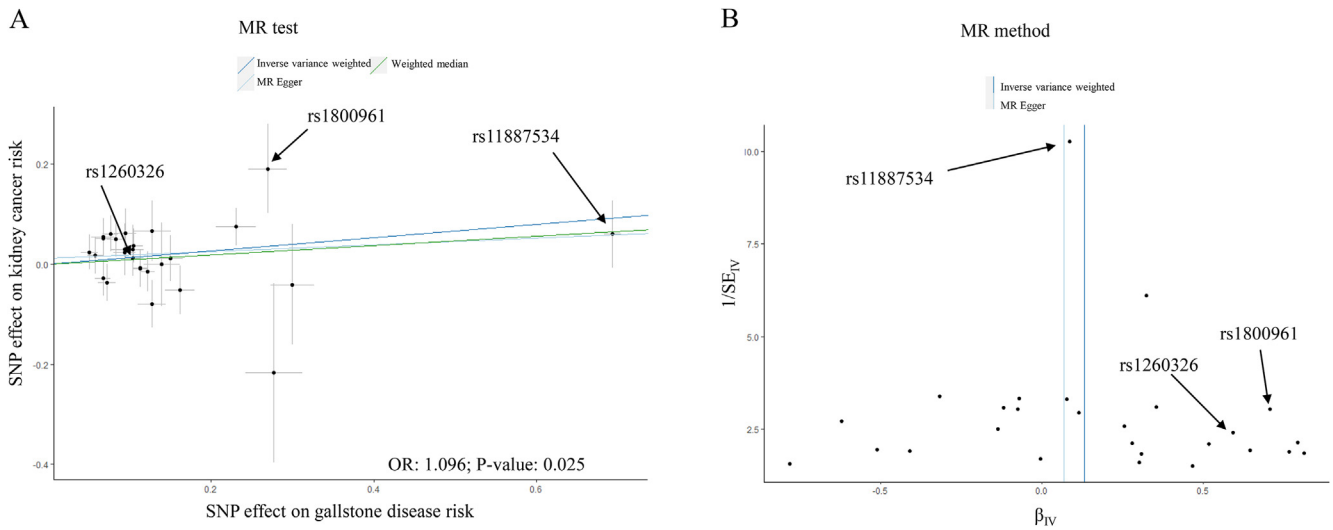


**Figure 2.** *Scatterplot* (*A*) and *funnel plot* (*B*) from the MR analysis on the causal effect of gallstones on kidney cancer risk. The SNPs rs11887534 (*ABCG8*), rs1800961 (*HNF4A*), and rs1260326 (*GCKR*) are marked with *arrows*.

Biobank). In the observational part of our study, we performed stratified analyses according to the time after surgical gallbladder resection, patient age at cholecystectomy, sex, and side of kidney cancer, with unprecedented precision. Taking into account the time after cholecystectomy allowed us to distinguish to some extent between gallstones and cholecystectomy as separate risk factors for kidney cancer. Cholecystectomized patients presented an overall 17% increased risk of developing kidney cancer. The relative risk was particularly high in the first 6 months after cholecystectomy (279% risk increase), but a 11% risk excess was observed even with follow-up starting 3 years after surgery. The relative risk of kidney cancer decreased with increasing age at cholecystectomy. MR results consistently supported a causal effect of gallstones on the risk of kidney cancer.

Our results provide solid evidence for an association between gallstones and kidney cancer risk. The relative risk of kidney cancer was particularly pronounced within the first 6 months after cholecystectomy. Because kidney cancer generally takes several years to develop, it is unlikely that cholecystectomy itself triggered tumor development in patients diagnosed with kidney cancer shortly after gallbladder removal.[23] Possible reasons for the increased risk of kidney cancer in the first 6 months after cholecystectomy are surveillance bias, a causal effect of gallstones on kidney cancer risk, and potential confounders.[17,21]

In addition to the increased risk shortly after gallbladder surgery, cholecystectomized patients showed an increased risk of kidney cancer more than 3 years after gallbladder removal. The risk increase was striking in patients cholecystectomized before the age of 40 years when they reached the age of 60 years (Figure 1), corresponding to a time lag of 25–30 years. Neither heterogeneity among instrumental variables nor horizontal pleiotropy or weak instrument biases were evident in MR analyses, favoring IVW over weighted median and MR–Egger causal effect estimates. The consistent results of sensitivity analyses based on

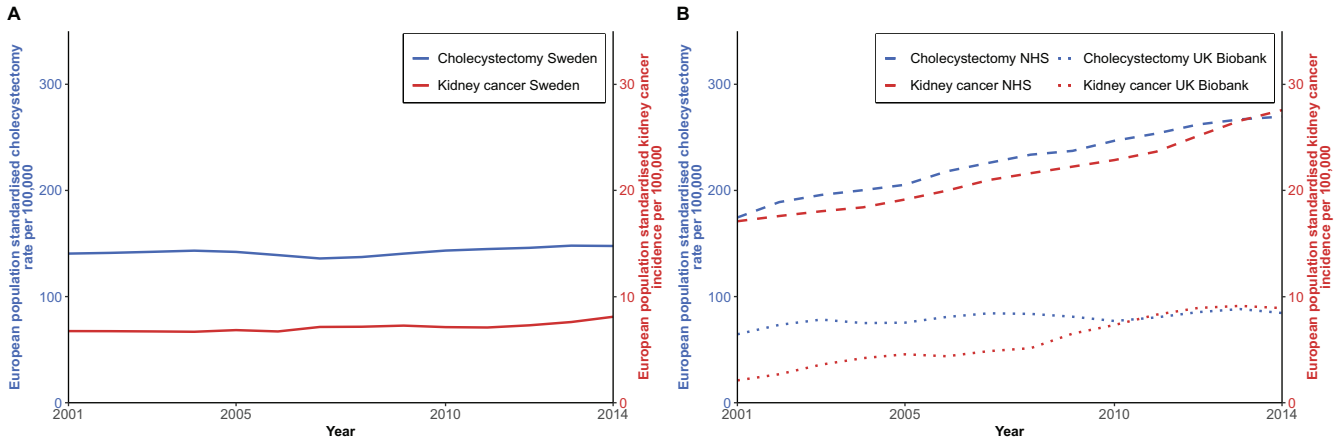


**Figure 3.** Incidence of cholecystectomy and kidney cancer per 100,000 person-years in Sweden (*A*) und United Kingdom (*B*). *Blue lines* depict cholecystectomies rates and *red lines* show kidney cancer incidences per 100,000 person-years, standardized to the European population. NHS, National Health Service.

nonoverlapping samples from the UK Biobank added further plausibility to a causal effect of both gallstones and gallbladder surgery on kidney cancer risk: OR of 1.144 per 2-fold increase in the prevalence of "self-reported or diagnosed gallstones," OR of 1.153 for "diagnosed gallstones," and OR of 1.138 for "cholecystectomy after gallstone diagnosis."

Using MR, we found that genetic susceptibility to gallstones was associated with kidney cancer risk, suggesting that gallstones are a causal risk factor for developing kidney cancer. To better understand the possible mechanisms behind this association, we included established risk factors for kidney cancer in the MVMR analyses.[22] Accounting for BMI did not reduce the estimated effect of gallstones on kidney cancer risk, but we observed partial mediation of this effect by type 2 diabetes (19% mediation) and smoking initiation (24% mediation). Age, diabetic nephropathy, and end-stage renal disease are common risk factors for type 2 diabetes and kidney cancer, and cigarette smoke releases harmful chemicals that spread to the kidneys, damaging DNA and making it harder for kidney cells to repair DNA damage (35668219, 33944952, and 34980891). Two SNPs (rs1260326 and rs1800961) used as instrumental variables for gallstone disease in the present study have also been associated with type 2 diabetes and metabolic traits, such as cholesterol and C-reactive protein levels, which may reflect systemic inflammation.[34] The 2 SNPs are missense variants; rs1260326 is located in the glucokinase regulatory protein (*GCKR*) gene and rs1800961 is located near the hepatocyte nuclear factor 4$\alpha$ (*HNF4A*) gene. SNP rs1800961 showed a strong positive influence on the causal effect of gallstones on kidney cancer risk in MR analyses (see Figure 2, Supplementary Tables 1 and 3) and has been found to control the expression of *HNF1A*, a transcription factor expressed mainly in the liver, gut, pancreas, and kidney, that regulates insulin secretion, lipid metabolism, glucose reabsorption, and water absorption.[35] Interestingly, in vivo studies of hnf1a-null mice have found hyperbileacidemia and hypercholesterolemia in these mice.[35,36]

However, when gallstones, diabetes, smoking, and BMI were considered together, MVMR showed an independent, direct effect of gallstones on kidney cancer risk. The high-leverage SNP rs11887534 in the MR analysis (see Figure 2) is a missense variant located in *ABCG8*, which encodes for a transporter that promotes excretion of cholesterol and sterols into bile, and facilitates the transport of sterols back into the intestinal lumen.[37] The genetic variant leads to enhanced cholesterol secretion, resulting in cholesterol-supersaturated bile, which itself provokes gallstone formation.[38]

In addition, physiological changes associated with surgical removal of the gallbladder may lead to the development of kidney cancer; cholecystectomy alters the composition of bile with increased levels of secondary bile acids, enhances the enterohepatic circulation of bile, and exposes the gastrointestinal tract to increased concentrations of secondary bile acids. This may lead to carcinogenic and pro-inflammatory processes, oxidative stress, inhibition of FXR as a suppressor of Wnt/$\beta$-catenin signaling, disruption of metabolic hormone levels and bacterial microbiota, and potentially initiate or promote the development of kidney cancer.[9,10,39] With regard to hormone regulation, both observational and MR analyses indicated that cholecystectomized women were at higher risk of developing kidney cancer than cholecystectomized men, whereas in the general population, the risk of kidney cancer is higher in men than in women.[40] Although the relative risk differences between women and men did not reach statistical significance and should therefore be interpreted with caution, female sex is an important risk factor for gallstones, probably because estrogens stimulate increased cholesterol storage in the bile, which ultimately leads to gallstone formation.[15] The possible mechanisms driving the development of kidney cancer after cholecystectomy in women, but not in men, remain to be investigated.

From a clinical perspective, the identified association between gallstones and cholecystectomy and kidney cancer is particularly alarming because the number of cholecystectomies performed each year is steadily increasing and we found strong evidence that this may translate into an increased incidence of kidney cancer. Figure 3 provides data from large national databases on cholecystectomy rates and kidney cancer incidence in Sweden and the United Kingdom (*panel A*: data from the Swedish registries, *panel B*: data from the National Health Service in England and the UK Biobank). Although in Sweden, the number of cholecystectomies and kidney cancer cases increased only slightly between 2001 and 2014, the UK plots showed an important increase in both surgical removal of the gallbladder and kidney cancer diagnoses. The likely contribution of common external (ie, environmental) factors modulating epigenetic mechanisms and individual genetic susceptibility to gallstones, and the time lag between gallstone formation, cholecystectomy, changes in bile acid metabolism, local and chronic inflammation, and the development of kidney cancer, add complexity to the interdependency between the risk factors investigated and kidney cancer as the final outcome, which we have tried to disentangle in the present study.

Our study benefitted from long-standing and large-scale nationwide data on diseases and surgical procedures in Sweden compiled since 1964, with the possibility of record linkage to a high-quality nationwide cancer registry that has been in operation since 1958, with >96% completeness.[26] The large number of cholecystectomized patients (n = 627,870) allowed stratified analyses by time since cholecystectomy, age at gallbladder removal, sex, and tumor side. Surveillance bias was explicitly addressed by both considering and excluding cancer cases diagnosed in the first 6 months after surgery. A limitation common to registry-based studies was the lack of detailed clinical information, such as the exact indication for cholecystectomy, although most patients undergo surgery due to symptomatic cholelithiasis and related complications. Potential confounding factors, such as obesity, diet, ethnicity, cigarette smoking, education, and physical activity, may also exist. We adjusted

all HRs for residential area and socioeconomic status, which may remove the effect of lifestyle differences to some extent. Furthermore, we were able to strengthen observational findings by providing novel evidence for a causal link between gallstones and kidney cancer risk using MR. Unfortunately, information on the laterality of kidney cancer was not available in the UK Biobank and MR analyses stratified by tumor side were not possible.

In conclusion, this is the largest study to date investigating the association of gallstones and cholecystectomy with kidney cancer risk, which complements traditional epidemiological research with causal inference using MR. Our results suggest that gallstones and cholecystectomy increase the risk of kidney cancer. Although elucidation of the underlying mechanisms requires further research, our study provides robust evidence for the compelling need to diagnostically rule out kidney cancer before and during gallbladder removal, and to prioritize kidney cancer screening in patients cholecystectomized at an early age.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://doi.org/10.1053/j.gastro.2023.03.227.

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**Received June 21, 2022. Accepted March 25, 2023.**

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**Acknowledgments**
The authors would like to thank Rajiv Kumar and Tamara Perez Jeldres for constructive discussions. The manuscript was edited by an English native speaker.

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Dominique Scherer, PhD (Conceptualization: Supporting; Formal analysis: Equal; Funding acquisition: Supporting; Investigation: Equal; Methodology: Equal; Project administration: Equal; Validation: Equal; Visualization: Equal; Writing – original draft: Equal).

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Justo Lorenzo Bermejo, PhD (Conceptualization: Lead; Funding acquisition: Lead; Investigation: Supporting; Methodology: Lead; Project administration: Lead; Resources: Equal; Supervision: Lead; Visualization: Supporting; Writing – original draft: Equal).

**Conflicts of interest**
The authors disclose no conflicts.

**Funding**
This study was supported by the European Union's Horizon 2020 research and innovation program under grant agreement 825741; the European Union within the initiative "Biobanking and Biomolecular Research Infrastructure—Large Prospective Cohorts" (collaborative study "Identification of Biomarkers for Gallbladder Cancer Risk Prediction -Towards Personalized Prevention of an Orphan Disease"; grant 313010); the high performance computing initiative in Baden-Württemberg (bwHPC) and the German Research Foundation (DFG) through grant INST 35/1134-1 FUGG and INST 35/1503-1 FUGG; the UK Biobank Resource (application 58030); grants from the Swedish Research Council to Jan Sundquist (2020-01175) and to Kristina Sundquist (2018-02400), as well as Medical Training and Research Agreement funding from Region Skåne awarded to Kristina Sundquist. The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Data Availability**
This study leveraged the Swedish Cancer Registry, the Swedish National Patient Register, and the UK Biobank. Raw data from these registries and studies cannot be shared by the study authors. However, additional information and relevant contact details are available at: https://www.socialstyrelsen.se/en/statistics-and-data/registers/; https://www.socialstyrelsen.se/en/statistics-and-data/registers/national-cancer-register/; https://www.socialstyrelsen.se/en/statistics-and-data/registers/; and https://www.ukbiobank.ac.uk/.

# Supplementary Methods

## *A Brief Introduction to Mendelian Randomization*

MR is an analytical method used to assess the causal effect of a given risk factor (exposure) on a phenotype of interest (outcome).[e1] The rationale for MR studies lies in the use of genetic variants as instrumental variables for the exposure under investigation. Genetic variants are randomly assigned at conception, mimicking the design of a randomized controlled trial, and the use of genetic variants strongly associated with the exposure of interest ensures that causal effect estimates from MR studies are less likely to be affected by confounding, measurement error, and reverse causation than results from traditional observational studies.[e2]

The selection of valid instrumental variables is essential for conducting an MR study (Supplementary Figure 1). In order to be able to test the causal effect of a particular exposure (eg, gallstones in this study) on a particular outcome (eg, kidney cancer in this study), the genetic variants must fulfill the following 3 criteria: be strongly associated with the exposure; not be associated with a confounder of the exposure–outcome association; and be associated with the outcome exclusively through the exposure of interest.[e3]

The first criterion can be met by selecting instrumental variables that are robustly associated with the investigated exposure (statistical association at the genome-wide level of statistical significance). MR studies that do not meet this criterion often result in low statistical power and potentially biased results. As the second and third criteria are often more difficult to address, alternative methods that are relatively robust against validity violations have been developed, such as MR–Egger regression and weighted median MR estimates. MVMR allows the causal effect of multiple exposures on an outcome of interest to be investigated simultaneously.

## *Flowchart Describing the Mendelian Randomization Analyses*

The flowchart in Supplementary Figure 2 represents the main and sensitivity MR analyses, from the selection of instrumental variables for gallstone disease to 2-sample MR based on 27 selected genetics variants.

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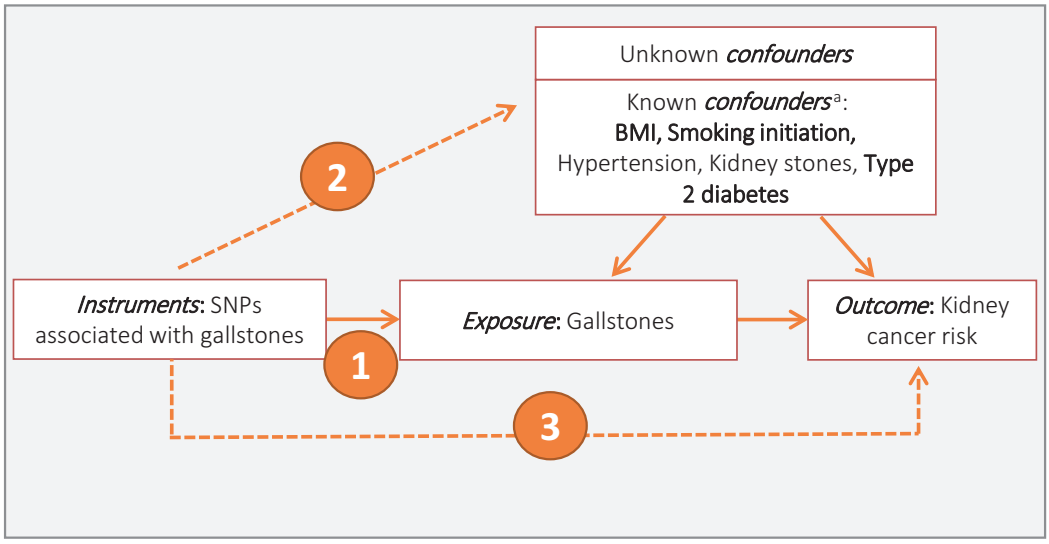
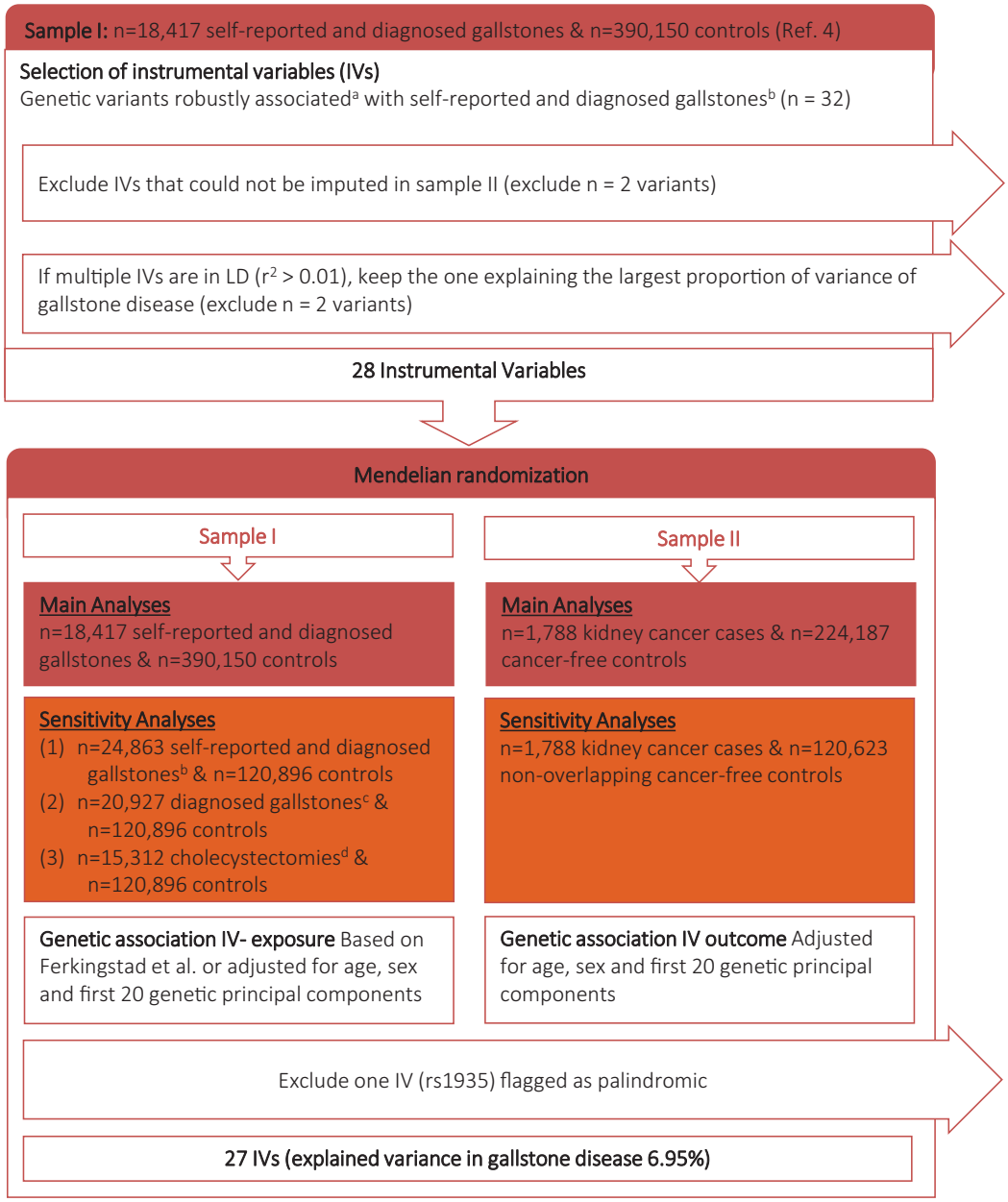



**Supplementary Figure 1.** Core assumptions for a valid genetic variant used as an instrumental variable in MR studies. In addition to the main exposure investigated (gallstones), type 2 diabetes, smoking initiation, and BMI also showed a causal effect on kidney cancer risk in the present study, and their causal effects on kidney cancer risk were assessed simultaneously with gallstones in MVMR.




**Supplementary Figure 2.** Flowchart representing the main and sensitivity MR analyses. [a]Ferkingstad et al[29] used the significance thresholds $2.0 \times 10^{-7}$ for high-impact variants (including stop-gained, frameshift, splice acceptor, or donor), $3.9 \times 10^{-8}$ for moderate-impact variants (including missense, splice-region variants, and in-frame indels), $3.6 \times 10^{-9}$ for low-impact variants (including upstream and downstream variants), and $5.9 \times 10^{-10}$ for lowest-impact variants (including intron and intergenic variants). [b]Self-reported and diagnosed gallstones: The exposure was ascertained in UK Biobank based on self-reported noncancer illness (data field 20002) and International Classification of Diseases, Ninth Revision (ICD-9-CM) code 574 or International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM)[e4] code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital. [d]Diagnosed gallstones: The exposure was ascertained based on ICD-9-CM code 574 or ICD-10-CM code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital. [e]Cholecystectomy: The exposure was ascertained using code J18 from the Office of Population Censuses and Surveys Classification of Interventions and Procedures, Fourth Revision,[e5] obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones. LD, linkage disequilibrium.

**Supplementary Table 1.** Summary Statistics on the Genetic Association Between 27 Single Nucleotide Polymorphisms and Risk of Gallstone Disease (Exposure) and Kidney Cancer (Outcome)

| SNP | Effect allele | Alternate allele | Explained variance[a] | Exposure | | | | Outcome | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\beta$ | EAF | SE | *P* value | $\beta$ | EAF | SE | *P* value |
| rs11012737 | A | G | 0.0010 | 0.077 | 0.31 | 0.009 | $4.4 \times 10^{-16}$ | 0.061 | 0.31 | 0.036 | .093 |
| rs11641445 | T | C | 0.0006 | 0.058 | 0.31 | 0.010 | $1.4 \times 10^{-9}$ | 0.018 | 0.31 | 0.037 | .628 |
| rs1169288 | C | A | 0.0015 | –0.094 | 0.31 | 0.014 | $1.3 \times 10^{-11}$ | –0.024 | 0.31 | 0.037 | .513 |
| **rs11887534** | **C** | **G** | **0.0288** | **0.693** | **0.07** | **0.010** | $\mathbf{1.0 \times 10^{-300}}$ | **0.060** | **0.07** | **0.068** | **.376** |
| rs12004 | G | T | 0.0008 | 0.068 | 0.30 | 0.014 | $2.3 \times 10^{-6}$ | 0.055 | 0.30 | 0.037 | .134 |
| **rs1260326** | **T** | **C** | **0.0012** | **–0.083** | **0.61** | **0.011** | $\mathbf{5.6 \times 10^{-14}}$ | **–0.049** | **0.39** | **0.035** | **.156** |
| rs12633863 | G | A | 0.0023 | 0.113 | 0.55 | 0.009 | $1.5 \times 10^{-25}$ | –0.008 | 0.45 | 0.034 | .816 |
| rs12968116 | T | C | 0.0008 | –0.094 | 0.13 | 0.017 | $2.1 \times 10^{-8}$ | –0.029 | 0.12 | 0.052 | .576 |
| rs13280055 | A | G | 0.0010 | 0.104 | 0.13 | 0.014 | $3.8 \times 10^{-14}$ | 0.029 | 0.13 | 0.050 | .554 |
| rs17138478 | A | C | 0.0008 | 0.095 | 0.13 | 0.014 | $7.5 \times 10^{-12}$ | 0.062 | 0.13 | 0.050 | .216 |
| rs17240268 | A | G | 0.0011 | –0.128 | 0.09 | 0.023 | $3.6 \times 10^{-8}$ | –0.066 | 0.09 | 0.061 | .278 |
| rs174567 | G | A | 0.0008 | 0.068 | 0.35 | 0.010 | $1.3 \times 10^{-12}$ | –0.028 | 0.35 | 0.036 | .436 |
| **rs1800961** | **T** | **C** | **0.0019** | **0.270** | **0.03** | **0.023** | $\mathbf{9.2 \times 10^{-21}}$ | **0.191** | **0.03** | **0.089** | **.032** |
| rs2070959 | G | A | 0.0008 | 0.068 | 0.32 | 0.010 | $1.3 \times 10^{-12}$ | 0.052 | 0.32 | 0.036 | .152 |
| rs212100 | T | C | 0.0019 | –0.151 | 0.84 | 0.015 | $5.4 \times 10^{-26}$ | –0.012 | 0.16 | 0.046 | .799 |
| rs2290846 | A | G | 0.0021 | 0.113 | 0.29 | 0.009 | $4.4 \times 10^{-23}$ | –0.008 | 0.29 | 0.037 | .821 |
| rs2291428 | C | G | 0.0022 | 0.122 | 0.24 | 0.009 | $2.9 \times 10^{-22}$ | –0.015 | 0.24 | 0.040 | .712 |
| rs2292553 | G | A | 0.0005 | –0.051 | 0.56 | 0.011 | $1.8 \times 10^{-6}$ | –0.024 | 0.44 | 0.034 | .484 |
| rs2469991 | T | A | 0.0009 | –0.073 | 0.29 | 0.011 | $3.8 \times 10^{-11}$ | 0.037 | 0.29 | 0.037 | .321 |
| rs28929474 | T | C | 0.0016 | 0.300 | 0.02 | 0.026 | $5.9 \times 10^{-17}$ | –0.041 | 0.02 | 0.120 | .735 |
| rs4148808 | C | T | 0.0026 | –0.163 | 0.14 | 0.018 | $2.6 \times 10^{-24}$ | 0.052 | 0.14 | 0.048 | .285 |
| rs55971546 | T | C | 0.0006 | 0.140 | 0.04 | 0.022 | $3.0 \times 10^{-10}$ | –0.001 | 0.04 | 0.083 | .994 |
| rs56398830 | A | G | 0.0007 | 0.278 | 0.01 | 0.035 | $1.6 \times 10^{-15}$ | –0.217 | 0.01 | 0.179 | .226 |
| rs601338 | G | A | 0.0021 | –0.105 | 0.51 | 0.011 | $3.6 \times 10^{-21}$ | –0.037 | 0.49 | 0.034 | .272 |
| rs6471717 | G | A | 0.0017 | 0.104 | 0.66 | 0.009 | $1.3 \times 10^{-20}$ | 0.012 | 0.34 | 0.036 | .737 |
| rs686030 | C | A | 0.0013 | –0.128 | 0.86 | 0.017 | $2.0 \times 10^{-13}$ | 0.080 | 0.14 | 0.047 | .092 |
| rs708686 | T | C | 0.0079 | 0.231 | 0.27 | 0.024 | $2.6 \times 10^{-16}$ | 0.075 | 0.27 | 0.038 | .049 |

NOTE. Bold type indicates statistics for the SNPs rs11887534 in the hepatic cholesterol transporter ATP-binding cassette subfamily G member 8 (*ABCG8)* gene, rs1260326 in the glucokinase regulatory protein (*GCKR*) gene and also associated with BMI and diabetes, and rs1800961 near the hepatocyte nuclear factor 4$\alpha$ (*HNF4A*) gene and also associated with diabetes. EAF, effect allele frequency.

[a]Explained variance in gallstone liability.

**Supplementary Table 2.** Overview of the Studies Used to Obtain the Summary Association Statistics for the Investigated Exposures

| First author | Year | Exposure | Sample origin | Study size | | No. of instrumental variables |
|---|---|---|---|---|---|---|
| | | | | Cases, n | Controls, n | |
| Ferkingstad[29] | 2018 | Gallstones | UK Biobank | 18,417 | 390,150 | 27 |
| Mahajan[e6] | 2018 | Type 2 diabetes | 31 studies (do not include the UK Biobank) | 55,005 | 400,308 | 113 |
| Liu[e7] | 2019 | Smoking initiation | 26 studies (include the UK Biobank) | 557,321 | 680,770 | 359 |
| Hoffmann[e8] | 2018 | BMI | GERA and GIANT | — | 334,487[a] | 272 |
| Howles[e9] | 2019 | Kidney stones | UK Biobank | 6536 | 388,508 | 8 |
| Evangelou[e10] | 2018 | Hypertension (SBP, DBP, PP) | International Consortium of Blood Pressure | — | 299,024[a] | 233 (SBP)<br>282 (DBP)<br>213 (PP) |

DBP, diastolic blood pressure; GERA, Genetic Epidemiology Research on Adult Health and Aging cohort; GIANT, Genetic Investigation of Anthropomorphic Traits; PP, pulse pressure; SBP, systolic blood pressure.
[a]Participants.

**Supplementary Table 3.** Results of Mendelian Randomization Analyses Based on Summary Statistics From Partially Overlapping and Nonoverlapping UK Biobank Samples (Unrelated and of European Ancestry)

| | Partially overlapping UK Biobank samples: Self-reported and diagnosed gallstones[a] (n = 18,417) | | | Nonoverlapping UK Biobank samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Self-reported and diagnosed gallstones[a] (n = 24,863) | | | Diagnosed gallstones[b] (n = 20,927) | | | Cholecystectomies[c] (n = 15,312) | | |
| Method | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value |
| IVW | 1.096 | 1.012–1.188 | .025 | 1.144 | 1.042–1.257 | .005 | 1.153 | 1.046–1.271 | .004 | 1.138 | 1.041–1.244 | .004 |
| Weighted median | 1.067 | 0.949–1.200 | .280 | 1.123 | 0.988–1.277 | .076 | 1.132 | 0.991–1.292 | .068 | 1.123 | 0.989–1.274 | .074 |
| MR–Egger | 1.048 | 0.916–1.198 | .503 | 1.067 | 0.921–1.236 | .396 | 1.072 | 0.917–1.254 | .390 | 1.063 | 0.919–1.230 | .419 |
| Wald ratio | | | | | | | | | | | | |
| For rs11887534 | 1.062 | 0.930–1.212 | .376 | 1.126 | 0.980–1.294 | .094 | 1.133 | 0.979–1.312 | .095 | 1.123 | 0.091–1.290 | .093 |
| For rs1260326 | 1.508 | 0.856–2.657 | .156 | 1.704 | 0.930–3.122 | .085 | 1.756 | 0.926–3.329 | .085 | 1.576 | 0.940–2.643 | .085 |
| For rs1800961 | 1.632 | 1.044–2.551 | .032 | 1.799 | 1.086–2.981 | .023 | 1.814 | 1.087–3.028 | .023 | 1.748 | 1.082–2.826 | .023 |

NOTE. SNP rs11887534 is located in the hepatic cholesterol transporter *ABCG8* gene, SNP rs1260326 is located in the *GCKR* gene and is also associated with BMI and diabetes, and SNP rs1800961 is located near the *HNF4A* gene and is also associated with diabetes.

[a]Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported noncancer illness (data field 20002) and ICD-9-CM[24] code 574 or ICD-10-CM[e4] code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.

[b]Diagnosed gallstones: The exposure was ascertained based on ICD-9-CM code 574 or ICD-10-CM code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.

[c]Cholecystectomy: The exposure was ascertained using code J18 from the Office of Population Censuses and Surveys Classification of Interventions and Procedures, Fourth Version,[e5] obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.

[d]Per doubling in gallstone prevalence.

**Supplementary Table 4.** Results of Sex-Stratified Mendelian Randomization Analyses Based on Summary Statistics From Nonoverlapping UK Biobank Samples (Unrelated and of European Ancestry)

| | Women | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nonoverlapping UK Biobank samples | | | | | | | | |
| | Self-reported and diagnosed gallstones[a] (n = 17,345) | | | Diagnosed gallstones[b] (n = 14,223) | | | Cholecystectomies[c] (n = 11,036) | | |
| Method | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value |
| IVW | 1.162 | 1.004–1.346 | .045 | 1.172 | 1.005–1.366 | .043 | 1.168 | 1.011–1.350 | .035 |
| Weighted median | 1.139 | 0.939–1.382 | .186 | 1.145 | 0.925–1.418 | .214 | 1.141 | 0.932–1.396 | .280 |
| MR–Egger | 1.003 | 0.802–1.255 | .979 | 1.005 | 0.794–1.271 | .971 | 1.014 | 0.808–1.272 | .908 |

| | Men | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nonoverlapping UK Biobank samples | | | | | | | | |
| | Self-reported and diagnosed gallstones[a] (n = 7622) | | | Diagnosed gallstones[b] (n = 6795) | | | Cholecystectomies[c] (n = 4314) | | |
| Method | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value | OR[d] | 95% CI | *P* value |
| IVW | 1.140 | 0.996–1.304 | .057 | 1.140 | 0.994–1.316 | .068 | 1.116 | 0.989–1.263 | .081 |
| Weighted median | 1.156 | 0.974–1.373 | .098 | 1.159 | 0.966–1.392 | .113 | 1.135 | 0.959–1.343 | .142 |
| MR–Egger | 1.127 | 0.901–1.410 | .304 | 1.120 | 0.881–1.425 | .364 | 1.093 | 0.876–1.364 | .438 |

[a]Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported noncancer illness (data field 20002) and ICD-9-CM[24] code 574 or ICD-10-CM[e4] code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.
[b]Diagnosed gallstones: The exposure was ascertained based on ICD-9-CM code 574 or ICD-10-CM code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.
[c]Cholecystectomy: The exposure was ascertained using code J18 from the Office of Population Censuses and Surveys Classification of Interventions and Procedures, Version 4,[e5] obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.
[d]Per doubling in gallstone prevalence.

**Supplementary Table 5.** Results of Univariate, 2-Way (Gallstones and Type 2 Diabetes, Gallstones and Smoking Initiation, and Gallstones and BMI) and 4-Way (Gallstones, Type 2 Diabetes, Smoking Initiation and BMI) Multivariable Mendelian Randomization Analyses With Kidney Cancer Risk as the Outcome of Interest Based on Nonoverlapping UK Biobank Samples

| Variable | OR[a] IVW | 95% CI | *P* value | *Q P* value | OR[a] robust MVMR with Q-minimization |
|---|---|---|---|---|---|
| Univariate | | | | | |
| Gallstones[b] | 1.144 | 1.042–1.257 | .005 | .39 | — |
| Type 2 diabetes | 1.098 | 1.010–1.193 | .030 | .03 | — |
| Smoking initiation | 1.256 | 1.079–1.463 | .003 | .09 | — |
| BMI | 1.282 | 1.088–1.509 | .003 | .32 | — |
| Kidney stones | 1.009 | 0.839–1.213 | .926 | .13 | — |
| SBP | 1.010 | 0.993–1.023 | .237 | .90 | — |
| DBP | 1.007 | 0.980–1.035 | .620 | .08 | — |
| PP | 0.977 | 0.950–1.004 | .096 | .01 | — |
| 2-way | | | | | |
| Gallstones–type 2 diabetes | | | | | |
| Gallstones[b] | 1.116 | 1.015–1.227 | .03 | .05 | 1.075 |
| Diabetes | 1.092 | 1.008–1.183 | .03 | | 1.060 |
| Gallstones–smoking initiation | | | | | |
| Gallstones[b] | 1.109 | 1.016–1.210 | .02 | .08 | 1.088 |
| Smoking initiation | 1.225 | 1.050–1.428 | .01 | | 1.225 |
| Gallstones–BMI | | | | | |
| Gallstones[b] | 1.157 | 1.063–1.259 | <.001 | .38 | 1.112 |
| BMI | 1.179 | 0.997–1.394 | .06 | | 1.126 |
| 4-way | | | | | |
| Gallstones–type 2 diabetes–smoking initiation–BMI | | | | | |
| Gallstones[b] | 1.091 | 1.008-1.181 | .03 | .04 | 1.133 |
| Type 2 diabetes | 1.079 | 1.015-1.148 | .02 | | 1.044 |
| Smoking initiation | 1.216 | 1.052-1.405 | .008 | | 1.227 |
| BMI | 1.076 | 0.912-1.268 | .39 | | 1.185 |

DBP, diastolic blood pressure; PP, pulse pressure; SBP: systolic blood pressure.
[a]Per doubling in exposure prevalence or per SBP, DBP, and PP unit increase.
[b]Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported noncancer illness (data field 20002) and ICD-9-CM[24] code 574 or ICD-10-CM[e4] code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.