Journal Pre-proof

Gallstones, cholecystectomy and kidney cancer: observational and Mendelian randomisation results based on large cohorts

Elham Kharazmi, MD, PhD, Dominique Scherer, PhD, Felix Boekstegers, PhD, Qunfeng Liang, MSc, Kristina Sundquist, MD, PhD, Jan Sundquist, MD, PhD, Mahdi Fallah, MD, PhD, Justo Lorenzo Bermejo, PhD

Gallstones, cholecystectomy and kidney cancer: observational and Mendelian randomisation results based on large cohorts

Short Title: Gallstones, cholecystectomy and kidney cancer

Elham Kharazmi, MD, PhD[1,2,3§]; Dominique Scherer, PhD[1§]; Felix Boekstegers, PhD[1]; Qunfeng Liang, MSc[2]; Kristina Sundquist, MD, PhD[3,4,5]; Jan Sundquist, MD, PhD[3,4,5]; Mahdi Fallah, MD, PhD[2,3,6§]; Justo Lorenzo Bermejo, PhD[1,7,§,*]

[1] *Statistical Genetics Research Group, Institute of Medical Biometry, Heidelberg University, 69120 Heidelberg, Germany*
[2] *Risk Adapted Prevention Group, Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), 69120 Heidelberg, Germany*
[3] *Center for Primary Health Care Research, Lund University, 202 13 Malmö, Sweden*
[4] *Department of Family Medicine and Community Health, Department of Population Health Science and Policy, Icahn School of Medicine at Mount Sinai, New York, USA*
[5] *Center for Community-based Healthcare Research and Education (CoHRE), Department of Functional Pathology, School of Medicine, Shimane University, 693-8501 Izumo, Japan*
[6] *Institute of Primary Health Care (BIHAM), University of Bern, 3012 Bern, Switzerland*
[7] *Department of Biostatistics for Precision Oncology, Institut de Cancérologie Strasbourg Europe, 67033, Strasbourg, France*

§ *Contributed equally*

Abbreviations
CI – confidence interval; IVW – inverse variance weighted; MR – Mendelian randomization; OR – odds ratio; HR – hazard ratio; SNP – single nucleotide polymorphism

*Correspondence to:
    Prof. Dr. Justo Lorenzo Bermejo
    Statistical Genetics Research Group
    Institute of Medical Biometry
    Heidelberg University
    Im Neuenheimer Feld 130.3
    69120 Heidelberg, Germany
    E-mail address: lorenzo@imbi.uni-heidelberg.de
    Phone: +49 6221 56 4180
    Fax: +49 6221 56 4195

ABSTRACT

Background and 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, cholecystectomy and kidney cancer is controversial. We comprehensively investigated this association, considering age at cholecystectomy and time from cholecystectomy to kidney cancer diagnosis, and assessing the causal effect of gallstones on kidney cancer risk by Mendelian randomisation (MR).

Methods: We compared the risk of kidney cancer in cholecystectomised and non-cholecystectomised individuals (16.6 million in total) from the Swedish nationwide cancer, census, patient and death registries using hazard ratios (HRs). For two-sample and multivariable MR, we used summary statistics based on 408,567 UK Biobank participants.

Results: During a median follow-up of 13 years, 2,627 of 627,870 cholecystectomised 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 cholecystectomised before age 40 (HR=1.55, 95% CI 1.39-1.72). MR results based on 18.417 gallstone and 1,788 kidney cancer patients from the UK 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 gallstone patients. Our findings provide solid evidence for the compelling need to diagnostically rule out kidney cancer before and during gallbladder removal, to prioritise kidney cancer screening in patients undergoing cholecystectomy in their 30s, and to investigate the underlying mechanisms linking gallstones and kidney cancer in future studies.

Key Words: cancer prevention, risk prediction, large nation-wide cohorts, causal inference

INTRODUCTION

Gallstones (cholelithiasis) are lithic deposits of digestive fluid in the gallbladder. Most gallstones are predominantly made up of cholesterol and referred to as cholesterol gallstones, whereas pigment gallstones mostly contain bilirubin.[1] Gallstones are present in up to 25% of adults living in the Western world.[2] While most gallstones remain asymptomatic, gallstone disease, referring to complications such as abdominal pain, cholecystitis, cholangitis and pancreatitis, occurs in 20% of gallstone patients and often requires the surgical removal of the gallbladder (cholecystectomy).[3] Gallstone disease is one of the most common disorders of the digestive system, and the 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 synthesised in the liver from cholesterol and are responsible for solubilisation, digestion, and absorption of lipids in the intestine.[5] Under normal physiological conditions, bile acids regulate numerous physiological processes through the activation of two receptors, TGR5 (G protein-coupled bile acid receptor-1) and FXR (nuclear farnesoid X receptor), and are only minimally excreted 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 kidneys 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 shown to alter the gut microbiome, promote intestinal carcinogenesis and suppress FXR, which acts as an antagonist of Wnt/β-catenin signalling.[9][10]

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 tumour development by triggering several hallmarks of cancer.[16]

Several studies have found an association between gallstones and increased risks of digestive and kidney cancer.[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, while one 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 tumours 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 randomisation (MR).

The present investigation overcomes important shortcomings of previous studies and examines the relationship between gallstones, cholecystectomy and kidney cancer using two complementary approaches: (1) In a large prospective observational study of 627,870 cholecystectomised patients, we estimate the relative risk of kidney cancer by taking into account patients' age at gallbladder removal and time after cholecystectomy. (2) We exploit gallstone risk variants as instrumental variables for two-sample MR, and use 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 characterise the associations between gallstones and kidney cancer, and between cholecystectomy and kidney cancer, for better personalised prevention of this neoplasm.

MATERIALS AND METHODS

Prospective data from the Swedish registries

Data from the Swedish National Patient Registers, the Swedish Cancer Registry, the National Population Registry, national censuses and the Death Registry have been linked using individually unique pseudonymised national registration numbers. These nationwide registered sources are updated every 1-3 years. The combined datasets include information on more than

16 million individuals. The Swedish National Patient Registers contain nationwide data on surgical procedures from all private and public hospitals as well as visits to specialised 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 ICD-9 code 51.2 and its subcategories.

The Swedish Cancer Registry provided information on the date of cancer diagnosis, tumour topography and morphology, and diagnostic reports from physicians for the period 1958-2018. All cancer data were recorded according to the 7th revision of the International Classification of Diseases (ICD-7). Information on patients with kidney cancer was extracted using ICD-7 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 non-haematological organs).[24] 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 cholecystectomised patient in the whole of Sweden, we selected five non-cholecystectomised 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 five controls. The propensity score was calculated with logistic regression adjusted for sex, birth year, socioeconomic status [farmer, manual workers, low to middle-income office worker, high-income office worker/professional, company owner (other than farmer) or other/unspecified], and residential area (large cities, southern Sweden, northern Sweden, or unspecified).

Follow-up of cholecystectomised patients started from the date of surgical gallbladder removal (month and year). Follow-up of non-cholecystectomised individuals started at the same age (year and month) as their matched cholecystectomised 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. The median follow-up of cholecystectomised patients was 13.2 years (interquartile range 5.3-23.6, mean 15.8, maximum 54.8 years), and 94,606 cholecystectomised patients were followed up for at least 30 years. The median follow-up of non-

cholecystectomised controls was 13.6 years (interquartile range 6.1-24.0, mean 16.3, 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 cholecystectomised patients compared with their matched controls without a history of cholecystectomy. Stratified analyses were performed by sex, patient's age at cholecystectomy (<40, 40-49, 50-59 or ≥60 years), time since cholecystectomy (1-6 months, 7-36 months or >3 years), and side of kidney cancer (left or right). In addition to relative risks, absolute incidence rates of kidney cancer were calculated for cholecystectomised patients according to the time in years after gallbladder surgery and the patient's age at cholecystectomy (<40 or ≥40 years). For comparison, kidney cancer incidence rates were also calculated for the control group (non-cholecystectomised individuals) with an age at baseline equal to that of the matched cholecystectomised 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 the SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA), 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 the subsequent MR analyses. The UK Biobank recruited 500,000 people from the UK aged between 40 and 69 years in 2006–2010.[25] Genotype calling was performed by Affymetrix (Santa Clara, CA, USA) 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 MR

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.[26] 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 flow chart. We applied two-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 dataset (18,417 cases of gallstone disease and 390,150 controls) in the study by Ferkingstad et al. (**Supplementary Table S1**), who identified 32 single nucleotide variants (SNPs) robustly associated with gallstone disease risk (P-value $<5\times10^{-8}$ in the meta-analysis of UK Biobank and Icelandic data).[27] 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 (1,788 cases of kidney cancer and 224,187 controls without any cancer diagnosis). We excluded for subsequent analyses two SNPs that were not imputed in the UK Biobank (rs756082276 and rs756935975), two 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.[28] 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., and on the prevalence of gallstone disease, which was set at 15%.[27] We (1) visually inspected the funnel and scatter plots of the SNP–gallstone versus SNP–kidney cancer risk summary association statistics to detect weak instrument bias; (2) calculated Cochran's Q statistic using first-order inverse variance weights to detect heterogeneity, which indicates a possible violation of instrumental variable or modelling assumptions, of which pleiotropy is a likely major cause; and (3) used the P-value for a non-zero 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 non-overlapping UK Biobank Sample I and Sample II considering three different exposures: (1) self-reported and diagnosed gallstones, as in the original report by Ferkingstad et al., (2) diagnosed gallstones only, and (3) 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 harmonising summary statistics, including standardisation 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 MR

Type 2 diabetes, smoking, body-mass index (BMI) and hypertension have been associated with the risk of kidney cancer (**Supplementary Table S2**).[29] Furthermore, a strong association between kidney stones and gallstones has been reported.[30] 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.[31] First, we used two-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 two-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 multi-way 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 (IEU) 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 S2** provides an overview of the studies and the number of available instruments for each kidney cancer risk factor.[32] 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 minimise the risk of identification of study participants, only access to pseudonymised 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 analysed in this study were accessed under UK Biobank application number 58030.

RESULTS

Observational associations

During the entire follow-up period, 2,627 out of 627,870 cholecystectomised Swedish patients were diagnosed with primary kidney cancer. Overall, a 17% increased relative risk of kidney cancer (HR=1.17, 95% CI 1.12-1.22, **Table 1**) was found in cholecystectomised patients compared with non-cholecystectomised 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=2,131) 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 cholecystectomised women (HR=1.22, 95% CI 1.15-1.29) than in cholecystectomised men (HR=1.12, 95% CI 1.05-1.19; overlapping 95% confidence intervals; **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 showed a 55% higher risk of kidney cancer than non-cholecystectomised 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 (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 tumours 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 (HR right=4.91 versus HR left=3.64) and in patients cholecystectomised before the age of 40 (HR right=1.51 versus SIR left=1.71), but these differences did not reach the 5% level of statistical significance.

Causal inference by MR

The variance in liability to gallstone disease explained jointly by the 27 SNPs used as instrumental variables in two-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-value*=.43) and no horizontal pleiotropy (MR–Egger intercept *P-value*=.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-value*=.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% confidence intervals, **Supplementary Table S3**), as were causal effect estimates based on non-overlapping UK Biobank samples: IVW OR=1.144 (95% CI 1.042-1.257, *P-value*=.005) for the exposure "self-reported or diagnosed gallstones", IVW OR=1.153 (95% CI 1.046-1.271, *P-value*=.004) for the exposure "diagnosed gallstones" and IVW OR=1.138 (95% CI 1.041-1.244, *P-value*=.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-*

value=.045) than in men (OR=1.140, 95% CI 0.996-1.304, *P-value*=.057, **Supplementary Table S4**).

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 S5**). Two-way MVMR indicated a direct effect of gallstones on kidney cancer risk, independent of type 2 diabetes (*P-value*=.03), smoking initiation (*P-value*=.02) and BMI (*P-value* <.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 OR=1.116 when type 2 diabetes was included (**Supplementary Table S5**), 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-value*=.03), and robust MVMR with Q-minimisation resulted in slightly larger effect size estimates (OR=1.13 versus OR=1.09).

DISCUSSION

In this study, we investigated the association of gallstones and cholecystectomy with kidney cancer risk using two approaches: (1) 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 (2) an MR study based on clinical and genotype data from a large prospective database (UK 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. Cholecystectomised 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. Since kidney cancer generally takes several years to develop, it is unlikely that cholecystectomy itself triggered tumour 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, cholecystectomised patients showed an increased risk of kidney cancer more than 3 years after gallbladder removal. The risk increase was striking in patients cholecystectomised before the age of 40 when they reached the age of 60 (**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, favouring IVW over weighted median and MR–Egger causal effect estimates. The consistent results of sensitivity analyses based on non-overlapping samples from the UK Biobank added further plausibility to a causal effect of both gallstones and gallbladder surgery on kidney cancer risk: OR=1.144 per 2-fold increase in the prevalence of "self-reported or diagnosed gallstones", OR=1.153 for "diagnosed gallstones" and OR=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, 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.[32] The two SNPs are missense variants; rs1260326 is a located in the glucokinase regulatory protein (*GCKR*) gene and rs1800961 is located near the hepatocyte nuclear factor 4 alpha (*HNF4A*) gene. SNP rs1800961 shows a strong positive influence on the causal effect of gallstones on kidney cancer risk in MR analyses (see **Figure 2**, **Table S1** and **Table S3**) and has been shown to control the expression of *HNF1A*, a transcription factor mainly expressed in the liver, gut, pancreas and kidney that regulates insulin secretion, lipid metabolism, glucose reabsorption and water absorption.[33] Interestingly, *in vivo* studies of hnf1a-null mice have shown hyperbileacidemia and hypercholesterolemia in these mice.[33 34]

On the other hand, 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 the excretion of cholesterol and sterols into bile, and facilitates the transport of sterols back into the intestinal lumen.[35] The genetic variant leads to an enhanced cholesterol secretion resulting in cholesterol-supersaturated bile, which itself provokes gallstone formation.[36]

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/β-catenin signalling, disruption of metabolic hormone levels and bacterial microbiota, and potentially initiate or promote the development of kidney cancer.[37 9 10] With regard to hormone regulation, both observational and MR analyses indicated that cholecystectomised women were at higher risk of developing kidney cancer than cholecystectomised men, whereas in the general population the risk of kidney cancer is higher in men than in women.[38] 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 oestrogens 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, cholecystectomy and kidney cancer is particularly alarming because the number of cholecystectomies performed each year is steadily increasing and we provide strong evidence that this may translate into an increased incidence of kidney cancer. **Figure 3** shows data from large national databases on cholecystectomy rates and kidney cancer incidence in Sweden and the UK (Panel A: data from the Swedish registries, Panel B: data from the National Health Service in England and the UK Biobank). While in Sweden the number of cholecystectomies and kidney cancer cases increased only slightly between 2001 and 2014, the UK plots show an important increase in both surgical removal of the gallbladder and kidney cancer diagnoses. The likely contribution of common external (i.e. 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 completeness of over 96%.[24] The large number of cholecystectomised patients (627,870) allowed stratified analyses by time since cholecystectomy, age at gallbladder removal, sex and tumour 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 the majority of 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 tumour 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. While 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 prioritise kidney cancer screening in patients cholecystectomised at an early age.

REFERENCES

1. Lammert F, Gurusamy K, Ko CW, et al. Gallstones. *Nat Rev Dis Primers* 2016;2:16024. doi: 10.1038/nrdp.2016.24 [published Online First: 2016/04/29]
2. Gurusamy KS, Davidson BR. Gallstones. *BMJ* 2014;348:g2669. doi: 10.1136/bmj.g2669 [published Online First: 2014/04/24]
3. Marschall HU, Einarsson C. Gallstone disease. *J Intern Med* 2007;261(6):529-42. doi: 10.1111/j.1365-2796.2007.01783.x [published Online First: 2007/06/06]
4. Stokes CS, Krawczyk M, Lammert F. Gallstones: environment, lifestyle and genes. *Dig Dis* 2011;29(2):191-201. doi: 10.1159/000323885 [published Online First: 2011/07/08]
5. Di Ciaula A, Garruti G, Lunardi Baccetto R, et al. Bile Acid Physiology. *Ann Hepatol* 2017;16(Suppl. 1: s3-105.):s4-s14. doi: 10.5604/01.3001.0010.5493 [published Online First: 2017/10/29]
6. Li S, Li C, Wang W. Bile acid signaling in renal water regulation. *Am J Physiol Renal Physiol* 2019;317(1):F73-F76. doi: 10.1152/ajprenal.00563.2018 [published Online First: 2019/05/16]
7. Barrera F, Azocar L, Molina H, et al. Effect of cholecystectomy on bile acid synthesis and circulating levels of fibroblast growth factor 19. *Ann Hepatol* 2015;14(5):710-21. [published Online First: 2015/08/11]
8. Ahmed M. Functional, Diagnostic and Therapeutic Aspects of Bile. *Clin Exp Gastroenterol* 2022;15:105-20. doi: 10.2147/CEG.S360563 [published Online First: 2022/07/29]
9. Cao H, Xu M, Dong W, et al. Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis. *Int J Cancer* 2017;140(11):2545-56. doi: 10.1002/ijc.30643 [published Online First: 2017/02/12]
10. Yao Y, Li X, Xu B, et al. Cholecystectomy promotes colon carcinogenesis by activating the Wnt signaling pathway by increasing the deoxycholic acid level. *Cell Commun Signal* 2022;20(1):71. doi: 10.1186/s12964-022-00890-8 [published Online First: 2022/05/26]
11. Knab LM, Boller AM, Mahvi DM. Cholecystitis. *Surg Clin North Am* 2014;94(2):455-70. doi: 10.1016/j.suc.2014.01.005 [published Online First: 2014/04/01]
12. Grigor'eva IN, Romanova TI. Gallstone Disease and Microbiome. *Microorganisms* 2020;8(6) doi: 10.3390/microorganisms8060835 [published Online First: 2020/06/06]
13. Rio-Tinto R, Canena J. Endoscopic Treatment of Post-Cholecystectomy Biliary Leaks. *GE Port J Gastroenterol* 2021;28(4):265-73. doi: 10.1159/000511527 [published Online First: 2021/08/14]
14. Chen Y, Wu S, Tian Y. Cholecystectomy as a risk factor of metabolic syndrome: from epidemiologic clues to biochemical mechanisms. *Lab Invest* 2018;98(1):7-14. doi: 10.1038/labinvest.2017.95 [published Online First: 2017/09/12]
15. Perez-Moreno P, Riquelme I, Garcia P, et al. Environmental and Lifestyle Risk Factors in the Carcinogenesis of Gallbladder Cancer. *J Pers Med* 2022;12(2) doi: 10.3390/jpm12020234 [published Online First: 2022/02/26]
16. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646-74. doi: 10.1016/j.cell.2011.02.013

17. Tavani A, Rosato V, Di Palma F, et al. History of cholelithiasis and cancer risk in a network of case-control studies. *Ann Oncol* 2012;23(8):2173-78. doi: 10.1093/annonc/mdr581 [published Online First: 2012/01/11]

18. Ward HA, Murphy N, Weiderpass E, et al. Gallstones and incident colorectal cancer in a large pan-European cohort study. *Int J Cancer* 2019;145(6):1510-16. doi: 10.1002/ijc.32090 [published Online First: 2018/12/27]

19. Pang Y, Lv J, Kartsonaki C, et al. Causal effects of gallstone disease on risk of gastrointestinal cancer in Chinese. *Br J Cancer* 2021;124(11):1864-72. doi: 10.1038/s41416-021-01325-w [published Online First: 2021/03/28]

20. Kharazmi E, Sundquist K, Sundquist J, et al. Risk of Gynecological Cancers in Cholecystectomized Women: A Large Nationwide Cohort Study. *Cancers (Basel)* 2022;14(6) doi: 10.3390/cancers14061484 [published Online First: 2022/03/26]

21. Chen YK, Yeh JH, Lin CL, et al. Cancer risk in patients with cholelithiasis and after cholecystectomy: a nationwide cohort study. *J Gastroenterol* 2014;49(5):923-31. doi: 10.1007/s00535-013-0846-6 [published Online First: 2013/06/29]

22. Johansen C, Chow WH, Jorgensen T, et al. Risk of colorectal cancer and other cancers in patients with gall stones. *Gut* 1996;39(3):439-43. doi: 10.1136/gut.39.3.439 [published Online First: 1996/09/01]

23. Gofrit ON, Yutkin V, Zorn KC, et al. The growth rate of "clinically significant" renal cancer. *Springerplus* 2015;4:580. doi: 10.1186/s40064-015-1385-9 [published Online First: 2015/11/07]

24. Barlow L, Westergren K, Holmberg L, et al. The completeness of the Swedish Cancer Register: a sample survey for year 1998. *Acta oncologica (Stockholm, Sweden)* 2009;48(1):27-33. doi: 10.1080/02841860802247664 [published Online First: 2008/09/04]

25. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015;12(3):e1001779. doi: 10.1371/journal.pmed.1001779 [published Online First: 2015/04/01]

26. Burgess S, Davey Smith G, Davies NM, et al. Guidelines for performing Mendelian randomization investigations. *Wellcome Open Res* 2019;4:186. doi: 10.12688/wellcomeopenres.15555.2 [published Online First: 2020/08/11]

27. Ferkingstad E, Oddsson A, Gretarsdottir S, et al. Genome-wide association meta-analysis yields 20 loci associated with gallstone disease. *Nature communications* 2018;9(1):5101. doi: 10.1038/s41467-018-07460-y [published Online First: 2018/12/07]

28. So HC, Gui AH, Cherny SS, et al. Evaluating the heritability explained by known susceptibility variants: a survey of ten complex diseases. *Genet Epidemiol* 2011;35(5):310-7. doi: 10.1002/gepi.20579

29. Padala SA, Barsouk A, Thandra KC, et al. Epidemiology of Renal Cell Carcinoma. *World J Oncol* 2020;11(3):79-87. doi: 10.14740/wjon1279 [published Online First: 2020/06/05]

30. Taylor EN, Chan AT, Giovannucci EL, et al. Cholelithiasis and the risk of nephrolithiasis. *J Urol* 2011;186(5):1882-7. doi: 10.1016/j.juro.2011.06.067 [published Online First: 2011/09/29]

31. Sanderson E, Spiller W, Bowden J. Testing and correcting for weak and pleiotropic instruments in two-sample multivariable Mendelian randomization. *Stat Med* 2021;40(25):5434-52. doi: 10.1002/sim.9133 [published Online First: 2021/08/03]

32. Sollis E, Mosaku A, Abid A, et al. The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. *Nucleic Acids Res* 2023;51(D1):D977-D85. doi: 10.1093/nar/gkac1010 [published Online First: 2022/11/10]

33. Miyachi Y, Miyazawa T, Ogawa Y. HNF1A Mutations and Beta Cell Dysfunction in Diabetes. *International journal of molecular sciences* 2022;23(6) doi: 10.3390/ijms23063222 [published Online First: 2022/03/26]

34. Shih DQ, Bussen M, Sehayek E, et al. Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. *Nature genetics* 2001;27(4):375-82. doi: 10.1038/86871 [published Online First: 2001/03/30]

35. Yu L, Li-Hawkins J, Hammer RE, et al. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest* 2002;110(5):671-80. doi: 10.1172/JCI16001 [published Online First: 2002/09/05]
36. Buch S, Schafmayer C, Volzke H, et al. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. *Nature genetics* 2007;39(8):995-9. doi: 10.1038/ng2101 [published Online First: 2007/07/17]
37. Tiderington E, Lee SP, Ko CW. Gallstones: new insights into an old story. *F1000Res* 2016;5 doi: 10.12688/f1000research.8874.1 [published Online First: 2016/08/11]
38. Scelo G, Li P, Chanudet E, et al. Variability of Sex Disparities in Cancer Incidence over 30 Years: The Striking Case of Kidney Cancer. *Eur Urol Focus* 2018;4(4):586-90. doi: 10.1016/j.euf.2017.01.006 [published Online First: 2017/07/30]

Table 1. Relative risk of kidney cancer after cholecystectomy by time after surgery, patient's age at surgery and sex

Time since surgery (months)	Age at surgery (years)	All			Women			Men			HR difference (women's minus men's)
		N	HR	95% CI	N	HR	95% CI	N	HR	95% CI	
Any	Any	2,627	**1.17**	1.12 1.22	1,481	**1.22**	1.15 1.29	1,146	**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	1,043	**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		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	2,131	**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

N: Number of patients with kidney cancer after cholecystectomy; HR: hazard ratio adjusted for sex, birth year, baseline age, socioeconomic status and residential area; CI: confidence interval. Bold values indicate that the 95% confidence interval does not include 1.00. NC: Not calculable.

Table 2. Relative risk of kidney cancer after cholecystectomy by time after surgery, patient's age at surgery and kidney side

Time since surgery (months)	Age at surgery (years)	Any side			Right kidney			Left kidney			HR difference (right minus left)
		N	HR	95% CI	N	HR	95% CI	N	HR	95% CI	
Any	Any	2,627	**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	2,171	**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 NC	2	NC	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	2,425	**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	1,972	1.04	0.99 1.09	627	1.08	0.99 1.18	635	1.07	0.99 1.17	0.01

N: Number of patients with kidney cancer after cholecystectomy; HR: hazard ratio adjusted for sex, birth year, baseline age, socioeconomic status and residential area; CI: confidence interval. Bold values indicate that the 95% confidence interval does not include 1.00. NC: Not calculable.

Figure 1. Incidence rate of kidney cancer after cholecystectomy by time after surgery and patient's age at the operation (<40 years or ≥40 years or more. Dotted lines represent the corresponding rates for non-cholecystectomised individuals with an age at baseline equal to the median age in the group of cholecystectomised patients.

Figure 2: Scatterplot (A) and funnel plot (B) from the Mendelian randomisation analysis on the causal effect of gallstones on kidney cancer risk. The SNP rs11887534 (*ABCG8*), rs1800961 (*HNF4A*) and rs1260326 (*GCKR*) are marked with arrows. MR: mendelian randomisation; IVW OR: inverse variance weighted odds ratio; SNP: single nucleotide polymorphism

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

A.

MR test

Inverse variance weighted   Weighted median
MR Egger

rs1800961

rs1260326   rs11887534

SNP effect on kidney cancer risk

OR: 1.096; P-value: 0.025

SNP effect on gallstone disease risk

B.

MR method

Inverse variance weighted
MR Egger

rs11887534

$1/SE_{IV}$

rs1800961

rs1260326

β_{IV}

A

B
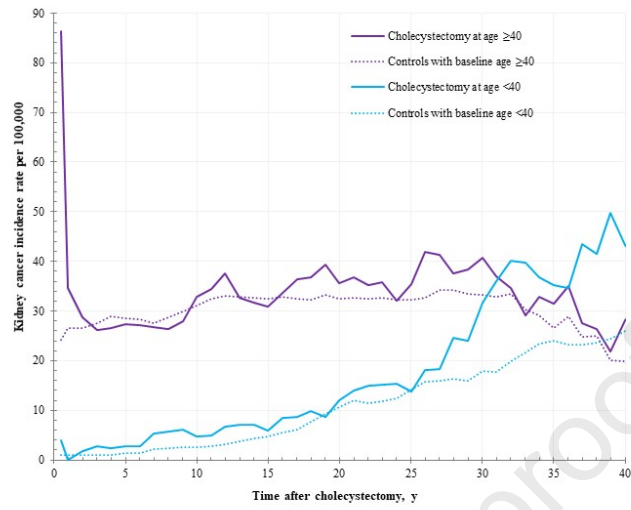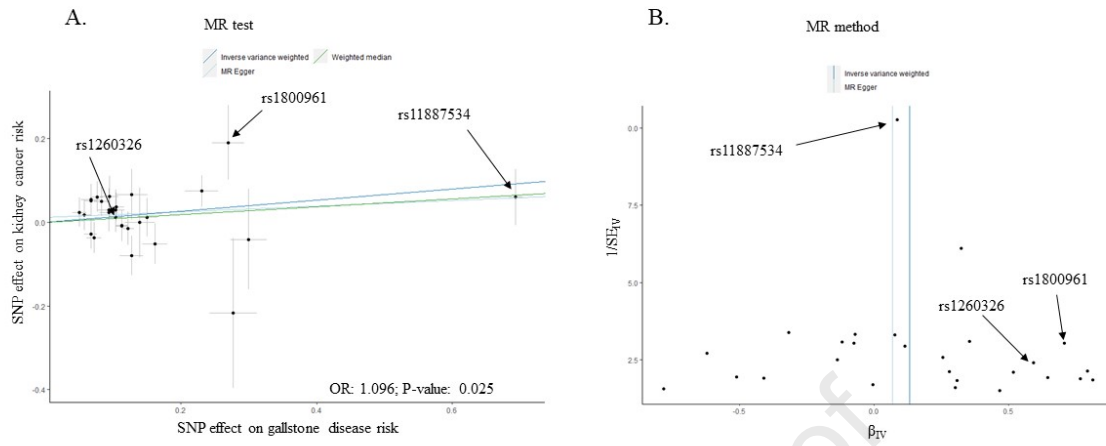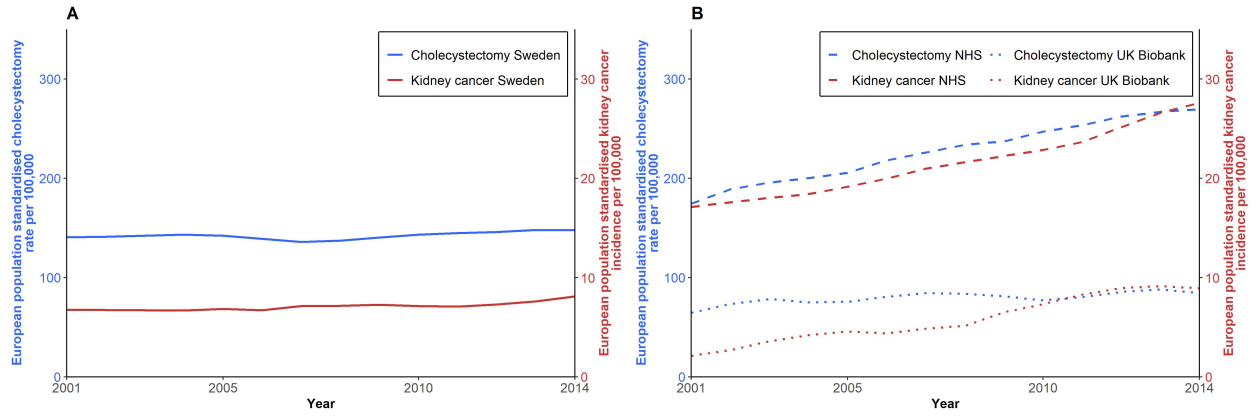
What You Need to Know (25-30 words for each section)

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 cholecystectomised 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. Mendelian randomisation 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 and Basic Research Relevance
The steady increase in the number of cholecystectomies may lead to an increased incidence of kidney cancer. Further research is needed into the mechanisms linking gallstones, cholecystectomy and kidney cancer.

Lay summary (25-30 words)

The number of gallbladder operations to treat gallstones continues to rise. Patients with gallstone disease have an increased risk of kidney cancer shortly after and even many years after gallbladder surgery.

Table S1. Summary statistics on the genetic association between 27 single nucleotide polymorphisms (SNPs) and the risk of gallstone disease (exposure) and kidney cancer (outcome). 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 body mass index and diabetes, and **rs1800961** near the hepatocyte nuclear factor 4alpha (*HNF4A*) gene and also associated with diabetes are highlighted.

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

SNP			EAF			SE					
rs686030	C	A	0.0013	-0.128	0.86	0.017	2.0×10^{-13}	0.080	0.14	0.047	.092
rs708686	T	C	0.0079	0.231	0.27	0.024	2.6×10^{-16}	0.075	0.27	0.038	.049

SNP: single nucleotide polymorphism; EAF: effect allele frequency; SE: standard error; Explained variance: Explained variance in gallstone liability

Table S2. Overview of the studies used to obtain the summary association statistics for the investigated exposures.

Exposure	Sample origin	Study size	Number of instrumental variables	Authors	Year	Ref
Gallstones	UK Biobank	n=18,417 cases n=390,150 controls	27	Ferkingstad *et al.*	2018	[1]
Type 2 diabetes	31 studies (do not include the UK Biobank)	n=55,005 cases n=400,308 controls	113	Mahajan *et al.*	2018	[2]
Smoking Initiation	26 studies (include the UK Biobank)	n=557,321 cases n=680,770 controls	359	Liu *et al.*	2019	[3]
BMI	GERA and GIANT	n=334,487 individuals	272	Hoffmann *et al.*	2018	[4]
Kidney stones	UK Biobank	n=6,536 cases n=388,508 controls	8	Howles *et al.*	2019	[5]
Hypertension (SBP, DBP, PP)	International Consortium of Blood Pressure	n=299,024 individuals	233 (SBP) 282 (DBP) 213 (PP)	Evangelou *et al.*	2018	[6]

BMI: body mass index; SBP: Systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure

Table S3. Results of Mendelian randomisation analyses based on summary statistics from partially overlapping (left column) and non-overlapping (three far right columns) UK Biobank samples (unrelated and of European ancestry). SNP **rs11887534** is located in the hepatic cholesterol transporter *ABCG8* gene, SNP **rs1260326** is located in the *GCKR* gene and is also associated with body mass index and diabetes, and SNP **rs1800961** is located near the *HNF4A* gene and is also associated with diabetes.

| Method | Partially overlapping UK Biobank samples | | | Non-overlapping UK Biobank samples | | | | | | | | |
| | Self-reported and diagnosed gallstones n=18,417 | | | Self-reported and diagnosed gallstones n=24,863 | | | Diagnosed gallstones n=20,927 | | | Cholecystectomies n=15,312 | | |
	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
Inverse variance weighted	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
Wald ratio 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
Wald ratio 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

MR: mendelian randomisation; OR: odds ratio per doubling in gallstone prevalence; CI: confidence interval

Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported non-cancer illness (Data-Field 20002) and ICD-9 code 574 or ICD-10 code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.

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

Cholecystectomy: The exposure was ascertained using the code J18 from the 4th version of the Office of Population Censuses and Surveys Classification of Interventions and Procedures, obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.

Table S4. Results of sex-stratified Mendelian randomisation analyses based on summary statistics from non-overlapping UK Biobank samples (unrelated and of European ancestry).

WOMEN	Non-overlapping UK Biobank samples								
	Self-reported and diagnosed gallstones n=17,345			Diagnosed gallstones n=14,223			Cholecystectomies n=11,036		
Method	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
Inverse variance weighted	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	Non-overlapping UK Biobank samples								
	Self-reported and diagnosed gallstones n=7,622			Diagnosed gallstones n=6,795			Cholecystectomies n=4,314		
Method	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
Inverse variance weighted	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

MR: mendelian randomisation; OR: odds ratio per doubling in gallstone prevalence; CI: confidence interval

Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported non-cancer illness (Data-Field 20002) and ICD-9 code 574 or ICD-10 code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.

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

Cholecystectomy: The exposure was ascertained using the code J18 from the 4th version of the Office of Population Censuses and Surveys Classification of Interventions and Procedures, obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.

Table S5. 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 randomisation analyses with kidney cancer risk as the outcome of interest based on non-overlapping UK Biobank samples.

	OR_{IVW}	95% CI	*P-value*	*Q P-value*	$OR_{\text{robust MVMR with Q-minimisation}}$
Univariate					
Gallstones[1]	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[1]	1.116	1.015-1.227	.03	.05	1.075
Diabetes	1.092	1.008-1.183	.03		1.060
Gallstones – Smoking initiation					
Gallstones[1]	1.109	1.016-1.210	.02	.08	1.088
Smoking initiation	1.225	1.050-1.428	.01		1.225
Gallstones – BMI					
Gallstones[1]	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[1]	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

OR: odds ratio per doubling in exposure prevalence or per SBP, DBP and PP unit increase; IVW: Inverse variance weighted; CI: confidence interval;
MVMR: Multivariable Mendelian randomisation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

[1]*Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported non-cancer illness (Data-Field 20002) and ICD-9 code 574 or ICD-10 code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.*

References

1. Ferkingstad E, Oddsson A, Gretarsdottir S, et al. Genome-wide association meta-analysis yields 20 loci associated with gallstone disease. *Nature communications.* 2018;9(1):5101.
2. Mahajan A, Taliun D, Thurner M, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nature genetics.* 2018;50(11):1505-1513.
3. Liu M, Jiang Y, Wedow R, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature genetics.* 2019;51(2):237-244.
4. Hoffmann TJ, Choquet H, Yin J, et al. A Large Multiethnic Genome-Wide Association Study of Adult Body Mass Index Identifies Novel Loci. *Genetics.* 2018;210(2):499-515.
5. Howles SA, Wiberg A, Goldsworthy M, et al. Genetic variants of calcium and vitamin D metabolism in kidney stone disease. *Nature communications.* 2019;10(1):5175.
6. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nature genetics.* 2018;50(10):1412-1425.

Table S1. Summary statistics on the genetic association between 27 single nucleotide polymorphisms (SNPs) and the risk of gallstone disease (exposure) and kidney cancer (outcome). 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 body mass index and diabetes, and **rs1800961** near the hepatocyte nuclear factor 4alpha (*HNF4A*) gene and also associated with diabetes are highlighted.

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

rs686030	C	A	0.0013	-0.128	0.86	0.017	2.0×10^{-13}	0.080	0.14	0.047	.092
rs708686	T	C	0.0079	0.231	0.27	0.024	2.6×10^{-16}	0.075	0.27	0.038	.049

SNP: single nucleotide polymorphism; EAF: effect allele frequency; SE: standard error; Explained variance: Explained variance in gallstone liability

Table S2. Overview of the studies used to obtain the summary association statistics for the investigated exposures.

Exposure	Sample origin	Study size	Number of instrumental variables	Authors	Year	Ref
Gallstones	UK Biobank	n=18,417 cases n=390,150 controls	27	Ferkingstad *et al.*	2018	[1]
Type 2 diabetes	31 studies (do not include the UK Biobank)	n=55,005 cases n=400,308 controls	113	Mahajan *et al.*	2018	[2]
Smoking Initiation	26 studies (include the UK Biobank)	n=557,321 cases n=680,770 controls	359	Liu *et al.*	2019	[3]
BMI	GERA and GIANT	n=334,487 individuals	272	Hoffmann *et al.*	2018	[4]
Kidney stones	UK Biobank	n=6,536 cases n=388,508 controls	8	Howles *et al.*	2019	[5]
Hypertension (SBP, DBP, PP)	International Consortium of Blood Pressure	n=299,024 individuals	233 (SBP) 282 (DBP) 213 (PP)	Evangelou *et al.*	2018	[6]

BMI: body mass index; SBP: Systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure

Table S3. Results of Mendelian randomisation analyses based on summary statistics from partially overlapping (left column) and non-overlapping (three far right columns) UK Biobank samples (unrelated and of European ancestry). SNP **rs11887534** is located in the hepatic cholesterol transporter *ABCG8* gene, SNP **rs1260326** is located in the *GCKR* gene and is also associated with body mass index and diabetes, and SNP **rs1800961** is located near the *HNF4A* gene and is also associated with diabetes.

| Method | Partially overlapping UK Biobank samples | | | Non-overlapping UK Biobank samples | | | | | | | | |
| | Self-reported and diagnosed gallstones n=18,417 | | | Self-reported and diagnosed gallstones n=24,863 | | | Diagnosed gallstones n=20,927 | | | Cholecystectomies n=15,312 | | |
	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
Inverse variance weighted	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
Wald ratio 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
Wald ratio 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

MR: mendelian randomisation; OR: odds ratio per doubling in gallstone prevalence; CI: confidence interval

Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported non-cancer illness (Data-Field 20002) and ICD-9 code 574 or ICD-10 code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.

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

Cholecystectomy: The exposure was ascertained using the code J18 from the 4th version of the Office of Population Censuses and Surveys Classification of Interventions and Procedures, obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.

Table S4. Results of sex-stratified Mendelian randomisation analyses based on summary statistics from non-overlapping UK Biobank samples (unrelated and of European ancestry).

WOMEN	Non-overlapping UK Biobank samples								
	Self-reported and diagnosed gallstones n=17,345			Diagnosed gallstones n=14,223			Cholecystectomies n=11,036		
Method	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
Inverse variance weighted	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	Non-overlapping UK Biobank samples								
	Self-reported and diagnosed gallstones n=7,622			Diagnosed gallstones n=6,795			Cholecystectomies n=4,314		
Method	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
Inverse variance weighted	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

MR: mendelian randomisation; OR: odds ratio per doubling in gallstone prevalence; CI: confidence interval

Self-reported and diagnosed gallstones: The exposure was ascertained based on self-reported non-cancer illness (Data-Field 20002) and ICD-9 code 574 or ICD-10 code K80 obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital.

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

Cholecystectomy: The exposure was ascertained using the code J18 from the 4th version of the Office of Population Censuses and Surveys Classification of Interventions and Procedures, obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.

Table S5. 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 randomisation analyses with kidney cancer risk as the outcome of interest based on non-overlapping UK Biobank samples.

	OR_{IVW}	95% CI	*P-value*	*Q P-value*	$OR_{robust\ MVMR\ with\ Q-minimisation}$
Univariate					
Gallstones[1]	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[1]	1.116	1.015-1.227	.03	.05	1.075
Diabetes	1.092	1.008-1.183	.03		1.060
Gallstones – Smoking initiation					
Gallstones[1]	1.109	1.016-1.210	.02	.08	1.088
Smoking initiation	1.225	1.050-1.428	.01		1.225
Gallstones – BMI					
Gallstones[1]	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[1]	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

OR: odds ratio per doubling in exposure prevalence or per SBP, DBP and PP unit increase; IVW: Inverse variance weighted; CI: confidence interval;
MVMR: Multivariable Mendelian randomisation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

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

References

1. Ferkingstad E, Oddsson A, Gretarsdottir S, et al. Genome-wide association meta-analysis yields 20 loci associated with gallstone disease. *Nature communications.* 2018;9(1):5101.
2. Mahajan A, Taliun D, Thurner M, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nature genetics.* 2018;50(11):1505-1513.
3. Liu M, Jiang Y, Wedow R, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature genetics.* 2019;51(2):237-244.
4. Hoffmann TJ, Choquet H, Yin J, et al. A Large Multiethnic Genome-Wide Association Study of Adult Body Mass Index Identifies Novel Loci. *Genetics.* 2018;210(2):499-515.
5. Howles SA, Wiberg A, Goldsworthy M, et al. Genetic variants of calcium and vitamin D metabolism in kidney stone disease. *Nature communications.* 2019;10(1):5175.
6. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nature genetics.* 2018;50(10):1412-1425.

Supplementary Methods

A brief introduction to Mendelian randomization (MR)

MR is an analytical method used to assess the causal effect of a given risk factor (exposure) on a phenotype of interest (outcome)[1]. 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 randomised 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[2].

The selection of valid instrumental variables is essential for conducting an MR study (Figure S1). In order to be able to test the causal effect of a particular exposure (e.g. gallstones in this study) on a particular outcome (e.g. kidney cancer in this study), the genetic variants must fulfil the following three criteria[3]:

(1) Be strongly associated with the exposure

(2) Not be associated with a confounder of the exposure-outcome association

(3) Be associated with the outcome exclusively through the exposure of interest
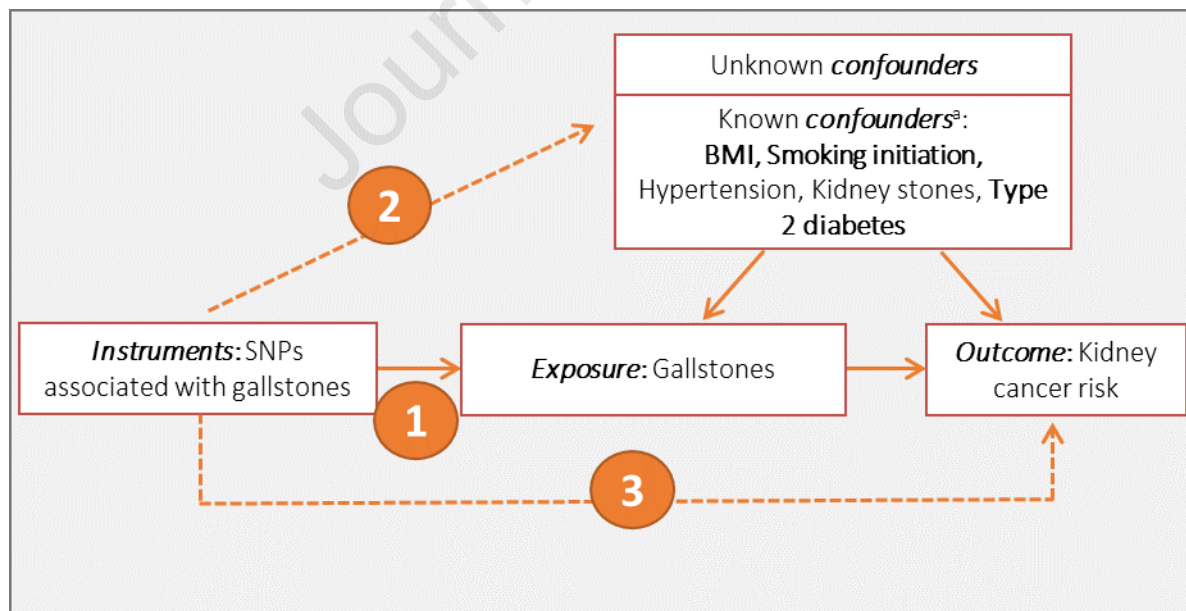
Figure S1: Core assumptions for a valid genetic variant used as an instrumental variable in MR studies. In addition to the main exposure investigated (gallstones), type 2 diabetes, smoking initiation and BMI also showed a causal effect on kidney cancer risk in the present study, and their causal effects on kidney cancer risk were assessed simultaneously with gallstones in multivariate MR.

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 into 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. Multivariable MR allows the causal effect of multiple exposures on an outcome of interest to be investigated simultaneously.

Flowchart describing the MR analyses

The following flowchart (Figure S2) represents the main and sensitivity MR analyses, from the selection of instrumental variables for gallstone disease, to two-sample MR based on 27 selected genetics variants.
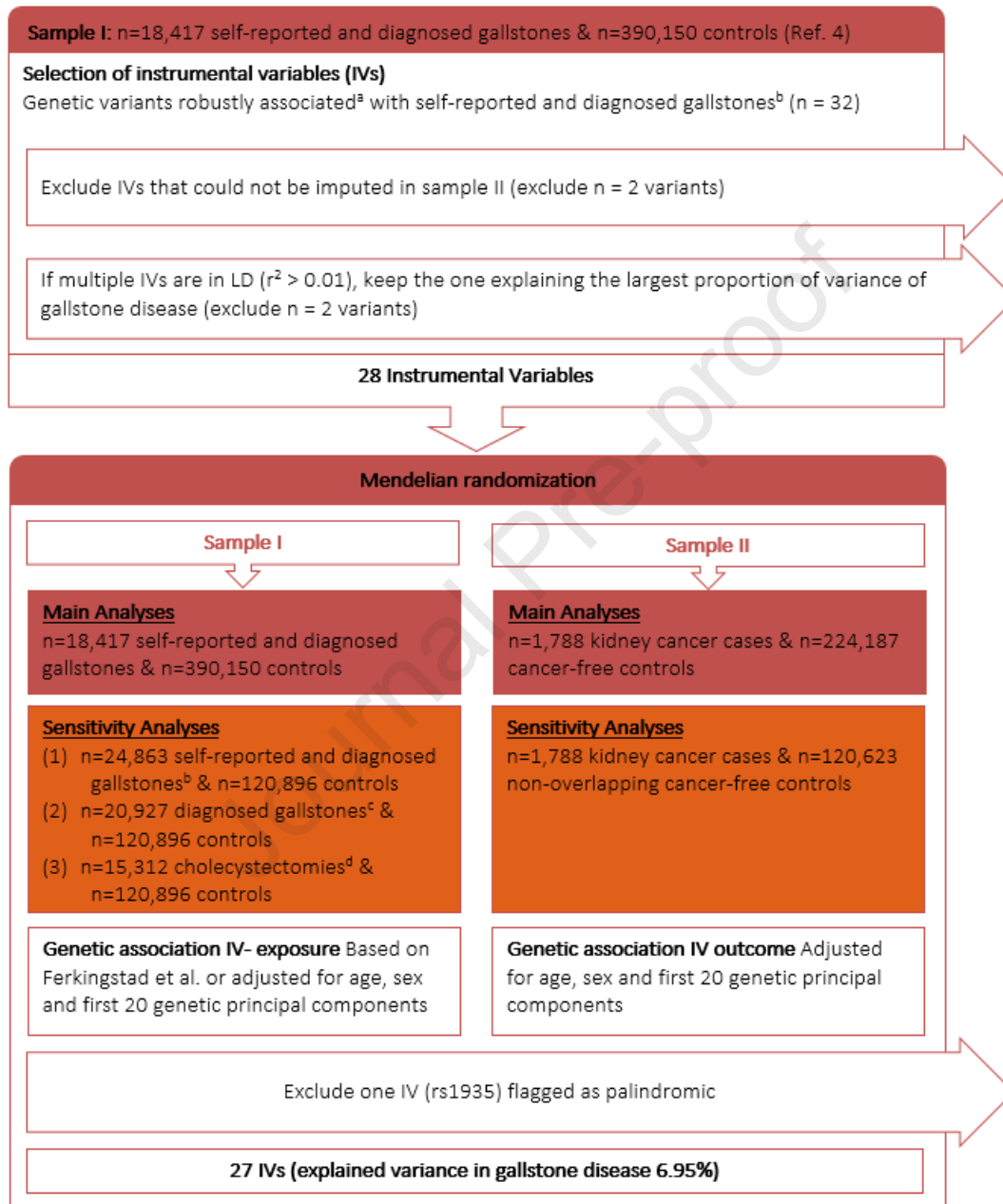
Figure S2: Flowchart representing the main and sensitivity MR analyses

[a]Ferkingstad et al[4] used the significance thresholds 2.0×10^{-7} for high impact variants (including stop-gained, frameshift, splice acceptor or donor), 3.9×10^{-8} for moderate-impact variants (including missense, splice-region variants and in-frame indels), 3.6×10^{-9} for low-impact variants (including upstream and downstream variants) and 5.9×10^{-10} for lowest-impact variants (including intron and intergenic variants).

[b]Self-reported and diagnosed gallstones: The exposure was ascertained in UKBiobank based on self-reported non-cancer illness (Data-Field 20002) and ICD-9 code 574 or ICD-10 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 code 574 or ICD-10 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 the code J18 from the 4th version of the Office of Population Censuses and Surveys Classification of Interventions and Procedures, obtained from primary or secondary diagnoses a participant has had recorded across all their episodes in hospital, considering only patients with previously diagnosed gallstones.

References

1. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ.* 2018;362:k601.
2. Evans DM, Davey Smith G. Mendelian Randomization: New Applications in the Coming Age of Hypothesis-Free Causality. *Annu Rev Genomics Hum Genet.* 2015;16:327-350.
3. Sekula P, Del Greco MF, Pattaro C, Kottgen A. Mendelian Randomization as an Approach to Assess Causality Using Observational Data. *J Am Soc Nephrol.* 2016;27(11):3253-3265.
4. Ferkingstad E, Oddsson A, Gretarsdottir S, et al. Genome-wide association meta-analysis yields 20 loci associated with gallstone disease. *Nature communications.* 2018;9(1):5101.