HEPATOLOGY

AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

HEPATOLOGY, VOL. 73, NO. 5, 2021

Gallstones, Body Mass Index, C-Reactive Protein, and Gallbladder Cancer: Mendelian Randomization Analysis of Chilean and European Genotype Data

Carol Barahona Ponce,[1,2] Dominique Scherer,[1] Regina Brinster,[1] Felix Boekstegers,[1] Katherine Marcelain,[2] Valentina Gárate-Calderón,[1,2] Bettina Müller,[3] Gonzalo de Toro,[4,5] Javier Retamales,[3] Olga Barajas,[2,6,7] Monica Ahumada,[2,6,7] Erik Morales,[8,9] Armando Rojas,[10] Verónica Sanhueza,[11] Denisse Loader,[11] María Teresa Rivera,[12] Lorena Gutiérrez,[13] Giuliano Bernal,[14] Alejandro Ortega,[15] Domingo Montalvo,[16] Sergio Portiño,[6,7] Maria Enriqueta Bertrán,[17] Fernando Gabler,[18] Loreto Spencer,[19] Jordi Olloquequi,[20] Christine Fischer,[21] Mazda Jenab,[22] Krasimira Aleksandrova,[23] Verena Katzke,[24] Elisabete Weiderpass,[25] Catalina Bonet,[26] Tahereh Moradi,[27] Krista Fischer,[28] Willem Bossers,[29] Hermann Brenner,[30-32] Kristian Hveem,[33,34] Niina Eklund,[35] Uwe Völker,[36] Melanie Waldenberger,[37] Macarena Fuentes Guajardo,[38] Rolando Gonzalez-Jose,[39] Gabriel Bedoya,[40] Maria C. Bortolini,[41] Samuel Canizales-Quinteros,[42] Carla Gallo,[43] Andres Ruiz-Linares,[44-46] Francisco Rothhammer,[38] and Justo Lorenzo Bermejo [1]

BACKGROUND AND AIMS: Gallbladder cancer (GBC) is a neglected disease with substantial geographical variability: Chile shows the highest incidence worldwide, while GBC is relatively rare in Europe. Here, we investigate the causal effects of risk factors considered in current GBC prevention programs as well as C-reactive protein (CRP) level as a marker of chronic inflammation.

APPROACH AND RESULTS: We applied two-sample Mendelian randomization (MR) using publicly available data and our own data from a retrospective Chilean and a prospective European study. Causality was assessed by inverse variance weighted (IVW), MR-Egger regression, and weighted median estimates complemented with sensitivity analyses on potential heterogeneity and pleiotropy, two-step MR, and mediation analysis. We found evidence for a causal effect of gallstone disease on GBC risk in Chileans ($P = 9 \times 10^{-5}$) and Europeans ($P = 9 \times 10^{-5}$). A genetically elevated body mass index (BMI) increased GBC risk in Chileans ($P = 0.03$), while higher CRP concentrations increased GBC risk in Europeans ($P = 4.1 \times 10^{-6}$). European results suggest causal effects of BMI on gallstone disease ($P = 0.008$); public Chilean data were not, however, available to enable assessment of the mediation effects among causal GBC risk factors.

CONCLUSIONS: Two risk factors considered in the current Chilean program for GBC prevention are causally linked to GBC risk: gallstones and BMI. For Europeans, BMI showed a causal effect on gallstone risk, which was itself causally linked to GBC risk. (Hepatology 2021;73:1783-1796).

Each year, gallbladder cancer (GBC; *International Classification of Diseases*, Tenth Revision, diagnosis code C23) kills more than 70,000 people worldwide (globocan.iarc.fr).

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; GBC, gallbladder cancer; IVW, inverse variance weighted; LD, linkage disequilibrium; MR, Mendelian randomization; OR, odds ratio.

Received December 21, 2019; accepted July 31, 2020.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.31537/suppinfo.

Supported by the German Federal Ministry of Education and Research (grants 01DN15021 and 01KT1510); 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 European Union's Horizon 2020 research and innovation program (grant 825741); Germany's Excellence Initiative of Heidelberg University within the program "Mobilitaetsmaßnahmen im Rahmen internationaler Forschungskooperationen 2015–16"; the support program "Stiftungen und Preise" of the Heidelberg University, and the Heidelberg University within the funding program Open Access Publishing; the Deutsche Forschungsgemeinschaft (grant LO 2061/1); and the Biobank of the University of Chile. Part of the research was conducted using the UK Biobank Resource (application number 58030). The funders had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; the preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Most GBC diagnoses occur in low-income and middle-income countries, and research into this aggressive disease has been largely neglected.(1)

A strong association between gallstone disease and GBC has been found in observational studies, with a relative GBC risk of 2.4 for gallstones

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.31537

Potential conflict of interest: Nothing to report.

ARTICLE INFORMATION:

From the [1]Statistical Genetics Group, Institute of Medical Biometry and Informatics, University of Heidelberg, Heidelberg, Germany; [2]Department of Basic and Clinical Oncology, Medical Faculty, University of Chile, Santiago de Chile, Chile; [3]Servicio de Oncología Médica, Instituto Nacional del Cáncer, Santiago, Chile; [4]Escuela de Tecnologia Medica, Universidad Austral de Chile sede Puerto Montt, Puerto Montt, Chile; [5]Servicio de Anatomía Patológica, Hospital de Puerto Montt, Puerto Montt, Chile; [6]Department of Basic and Clinical Oncology, Medical Faculty, University of Chile, Santiago, Chile; [7]Oncology Department, Hospital Clínico Universidad de Chile, Santiago, Chile; [8]Facultad de Medicina, Universidad Católica del Maule, Talca, Chile; [9]Unidad de Anatomía Patológica del Hospital Regional de Talca, Talca, Chile; [10]Laboratorio de Investigaciones Biomédicas en la Facultad de Medicina, Universidad Católica del Maule, Talca, Chile; [11]Servicio de Anatomía Patológica, Hospital Padre Hurtado, Santiago, Chile; [12]Servicio de Anatomía Patológica, Hospital del Salvador, Santiago, Chile; [13]Servicio de Anatomía Patológica, Hospital San Juan de Dios, Santiago, Chile; [14]Laboratory of Molecular and Cellular Biology of Cancer (CancerLab), Department of Biomedical Sciences, Faculty of Medicine, Universidad Católica del Norte, Coquimbo, Chile; [15]Servicio de Anatomía Patológica, Hospital Regional, Arica, Chile; [16]Department of Surgery, Hospital Juan Noe Crevani, Arica, Chile; [17]Secretaría Regional Ministerial de Salud, Ministerio de Salud, Valdivia, Chile; [18]Servicio de Anatomía Patológica, Hospital San Borja Arriarán, Santiago, Chile; [19]Servicio de Anatomía Patológica, Hospital Regional Guillermo Grant Benavente, Concepción, Chile; [20]Laboratory of Cellular and Molecular Pathology, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile; [21]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany; [22]International Agency for Research on Cancer, Lyon, France; [23]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany; [24]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany; [25]International Agency for Research on Cancer, World Health Organization, Lyon, France; [26]Unit of Nutrition and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology, Barcelona, Spain; [27]Division of Epidemiology, Department of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [28]Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia; [29]The Lifelines Cohort Study, Groningen, Netherlands; [30]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany; [31]Division of Preventive Oncology, German Cancer Research Center, National Center for Tumor Diseases, Heidelberg, Germany; [32]German Cancer Consortium, German Cancer Research Center, Heidelberg, Germany; [33]The Nord-Trøndelag Health Research Centre, Norwegian University of Science and Technology, Trondheim, Norway; [34]Department of Public Health and Nursing, K.G. Jebsen Centre for Genetic Epidemiology, Norwegian University of Science and Technology, Trondheim, Norway; [35]Genomics and Biobank, National Institute for Health and Welfare, Helsinki, Finland; [36]Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Universitätsmedizin Greifswald, Germany; [37]Research Unit of Molecular Epidemiology and Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; [38]Instituto de Alta Investigación, Tarapacá University, Arica, Chile; [39]Centro Nacional Patagónico, Instituto Patagónico de Ciencias Sociales y Humanas, CONICET, Puerto Madryn, Argentina; [40]Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín, Colombia; [41]Instituto de Biociências, Universidad Federal do Rio Grande do Sul, Puerto Alegre, Brazil; [42]Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, México; [43]Unidad de Neurobiología Molecular y Genética, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru; [44]Ministry of Education Key Laboratory of Contemporary Anthropology and Collaborative Innovation Center of Genetics and Development, School of Life Sciences and Human Phenome Institute, Fudan University, Shanghai, China; [45]Aix-Marseille Université, CNRS, EFS, ADES, Marseille, France; [46]Department of Genetics, Evolution and Environment, and UCL Genetics Institute, University College London, London, UK.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Justo Lorenzo Bermejo, Ph.D.
Statistical Genetics Group
Institute of Medical Biometry and Informatics
University of Heidelberg
Im Neuenheimer Feld 130.3
Heidelberg 69120, Germany
E-mail: lorenzo@imbi.uni-heidelberg.de
Tel.: +49-6221564180

2.0-2.9 cm in diameter and 9.2-10.1 for gallstones >3 cm.(2) Potential confounding by female gender and other GBC risk factors, however, makes it difficult to infer causality. Around 20% of the GBC burden can be attributed to excess body weight, with a substantial risk increase of 25%-31% for every five body mass index (BMI) units.(1,3-5) To date, however, very little is known about the causal mechanisms that underlie the association between body fatness and GBC. Obesity is linked to chronic inflammation, as reflected in elevated levels of C-reactive protein (CRP); and strong associations between the CRP concentration in serum and the risk of GBC have been reported for Chileans (odds ratio [OR] for the fourth versus the first concentration quartile, 18.6) and Chinese (OR, 7.6).(6-9) The strong observed associations could, however, be related to reverse causation (elevated CRP concentration caused by GBC tumors rather than CRP → GBC). Women are at higher risk of developing GBC, in particular those with early age at menarche, early age at first childbirth, and high numbers of pregnancies and childbirths.(10-12) Additional risk factors include advanced age, a family history of GBC or gallstones, chronic inflammatory conditions affecting the gallbladder, diabetes, a low educational level, and chronic infections with *Helicobacter* and *Salmonella* spp.(2) Lifestyle factors such as cigarette smoking and alcohol consumption as well as environmental pollution (waste gas emission and pollutant plants) also seem to increase GBC risk.(13-15)

Current GBC care, from prevention and early detection to diagnosis and therapy, does not take full account of ethnic, cultural, environmental, and health care system disparities. The identification of possible differences in GBC etiology between regions of high and low incidence could potentially translate into more efficient prevention policies. The genome of modern Chileans is a genetic admixture of Europeans, Native Americans from two major indigenous peoples (Mapuche and Aymara), and Africans.(16) It is well established that individuals with a high proportion of Mapuche ancestry are at high risk of developing GBC: we found that each added 1% of Mapuche ancestry represents a 3.7% increase in the GBC mortality risk.(16) The Chilean government currently supports prophylactic cholecystectomy for men and women aged 35-49 years; and multiparous women with BMI > 27 kg/m^2, 8 years' education or less, and at least one Mapuche surname are considered to be at a particularly high risk of developing GBC.(17) Each year 50,000 gallbladders are removed in Chile, at an average cost of $1,000 per cholecystectomy, in the framework of this prevention policy.(18)

GBC is rare in most countries, and publicly available genotype data from genetic association studies are sparse. We conducted a retrospective Chilean (277 patients, 2,107 controls) and a prospective European (103 cases, 168 controls) study on GBC and applied Mendelian randomization (MR) to investigate the causal relationship between risk factors considered in the current Chilean GBC prevention program, CRP concentration as a marker of chronic inflammation, and GBC risk. The available sample size was small compared with traditional MR studies, but the strong associations found in observational studies—gallstones increase the GBC risk by up to 10-fold, and the ORs associated with increased serum CRP levels vary from 7.6 to 18.6—and the urgent necessity to optimize GBC prevention motivated this study. We used genetic variants robustly associated with gallstone disease, BMI, CRP concentration, age at menarche, and age at first childbirth as instrumental variables and tested the causal effect of these risk factors on GBC risk. Our ultimate goal is to unravel the complex etiology of GBC and discriminate between noncausal and causal risk factors, striving to improve the efficiency of current GBC prevention programs in regions of high and low GBC incidence.

Materials and Methods

All Chilean and European cases were patients with a diagnosis of gallbladder cancer (*International Classification of Diseases*, Tenth Revision, diagnosis code C23). The majority (79%) of Chilean GBC patients were diagnosed incidentally after a prophylactic cholecystectomy to treat gallstone disease. Population controls included individuals affected by gallstone disease. European controls did not include individuals affected by any type of cancer, but information on cancer history was not available for Chilean controls. The proportions of Chilean controls affected by gallstone disease and cancer should, however, be representative of the corresponding proportions in the general population that gave rise to the cases. Informed consent in writing was obtained from each study participant and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval the appropriate institutional review committees. Please see the Supporting methods for details on the ethics approval. The gender and age distributions of the investigated cases and controls are shown in Supporting Table S1. Additional details on the recruitment strategy in Chile are available in our recent publication.(16) Supporting Table S2 describes the arrays used for genotyping of Chilean and European study participants.

We used two-sample MR with genetic variants—specifically, single-nucleotide polymorphisms—as instrumental variables to investigate the causal effects on the risk of GBC exerted by (1) risk factors considered in the current Chilean GBC prevention program (gallstone disease, BMI, and age at menarche) and (2) CRP level as a marker of chronic inflammation.(9,17,18) Summary statistics on the association between the genetic variants and GBC risk adjusted for age, gender, and the first five genetic principal components were obtained using our own demographic and genotype data from the retrospective Chilean (277 cases, 2107 controls) and the prospective European (103 cases, 168 controls) study (Supporting Table S1). Summary statistics on the association between the genetic variants and the risk factors were retrieved from the studies listed in Table 1, while detailed information on the original genetic association analyses including investigated phenotypes, association models and covariates, and analysis tools is provided in the Supporting Methods. In addition to overall analyses, calculations for Chileans were stratified by the median proportion of Mapuche ancestry in the Chilean study (34%).

The underrepresentation of non-European populations is an important problem in current human genetic research.(19) To assess the potential impact of this limitation on our results, we compared the variance explained by the genetic instruments in different populations. We also calculated the F statistic as a measure of the strength of the instrumental variables, with a low value (i.e., <10) indicative of possible weak instrument bias. Please see the Supporting Methods for details on these calculations. Our primary objective was to identify causal associations (to test causality), which requires weaker modeling assumptions than estimation of the magnitude of the causal effects.(20) We calculated Cochran's Q statistic using first-order inverse variance weights (IVWs) to detect heterogeneity, which indicates a possible violation of the instrumental variable or modeling assumptions, of which pleiotropy is a likely major cause.(21) We visually inspected scatter and funnel plots and removed genetic variants with outlying MR estimates in the case of excessive heterogeneity.(22) More precisely, when the initial Q P value was <0.10, genetic variants with departing MR estimates were removed one after another until heterogeneity disappeared (Q P value > 0.10). As a secondary objective, we estimated the causal effect sizes and assessed their robustness by comparing the IVW, MR-Egger regression and weighted median estimates for BMI and age at menarche. MR analyses were conducted using the R version of MR-Base, which provides convenient tools for the harmonization of the summary statistics, including standardization of the effect alleles and removal of the problematic palindromic genetic variants, and implements a random-effect IVW model by default.(23)

We also conducted comprehensive sensitivity analyses, which are described in the Supporting Methods. For the risk factors that showed a causal effect on GBC ($P < 0.05$ and no evidence of violation of instrumental variable assumptions), two-step MR was applied to assess mediation.(24) In the first step of the procedure, genetic instruments for the exposure were used to estimate the causal effect of the exposure on the potential mediator. In the second step, genetic instruments for the mediator were used to assess the causal effect of the mediator on GBC risk. Evidence of association in both steps (for example, BMI → gallstone disease and gallstone disease → GBC) implies some degree of mediation between the exposure and the outcome by the intermediate trait. We also applied bootstrapping for testing the hypothesis that gallstone disease is a mediator which explains the underlying mechanism of the relationship between BMI and GBC risk. We considered the three regression models BMI → GBC, BMI → gallstone disease, and BMI + gallstone disease → GBC with age, gender, and the first five genetic principal components as adjustment covariates and used the CAUSALMED procedure in SAS, version 14.3, to perform causal mediation analyses.

TABLE 1. Studies Used to Retrieve Summary Statistics for the Two-Sample MR Analyses

Trait	Outcome/ Exposure	Study Population	Study Size	Gender	Number of Variants	Explained Variance	*F* Statistic/Detectable OR*		Authors	Year	Ref
							Chileans	Europeans			
GBC	Outcome	Admixed Chilean	277 cases and 2,107 controls	Both	—				—	—	—
	Outcome	European	103 cases and 168 controls	Both	—				—	—	—
Gallstone disease	Exposure	European	15,209 cases and 117,949 controls	Both	6	4%		12.3/3.70	Joshi et al.	2016	(25)
	Exposure	Admixed Chilean	529 cases and 566 controls	Both	6	7%	180/1.71		Bustos et al.	2019	(26)
	Outcome	European	361,194 individuals	Both	—				UK Biobank, Neale	2018	†
BMI	Exposure	Europeans	334,487 individuals	Both	289	4%	100/1.95	12.3/3.70	Hoffmann et al.	2018	(27)
	Outcome	Europeans	359,983 individuals	Both	—				UK Biobank, Neale	2017	‡
CRP	Exposure	Europeans	727 individuals	Both	4	3%	74.7/2.11	9.38/4.20	Nimptsch et al.	2015	(29)
	Exposure	Europeans	12,400 individuals	Both	18	5%		15.3/3.37	Kocarnik et al.	2014	(28)
	Exposure	Hispanic Americans	15,895 individuals	Both	9	4%	100/1.96		Kocarnik et al.	2018	(30)
	Outcome	Europeans	3,301 individuals	Both	—				Sun et al.	2018	(57)
Age at menarche	Exposure	Europeans	329,345 individuals	Women	389	7%	99.9/1.85	15.6/3.50	Day et al.	2017	(31)
Age at first birth	Exposure	Europeans	250,941 individuals	Parous women	10	4%	14.6/4.20	5.29/10.11	Barban et al.	2016	(32)

*Detectable true OR with 80% statistical power and a type I error rate of 5% considering the actual study size, proportion of cases, and proportion of explained variance.
†http://www.nealelab.is/uk-biobank/.
‡http://www.nealelab.is/blog/2017/7/19/rapid-gwas-of-thousands-of-phenotypes-for-337000-samples-in-the-uk-biobank.

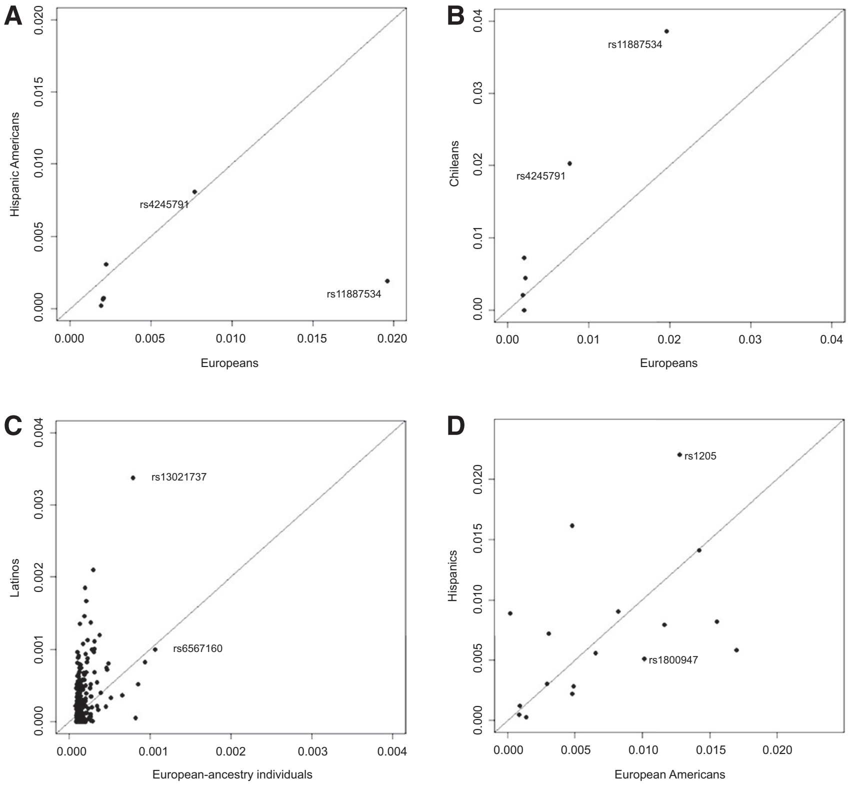

FIG. 1. Variance in liability to gallstone disease explained by the considered instruments in Europeans, Hispanic Americans, and Chileans (A, B), explained variance in BMI in Europeans and Latinos (C), and explained variance in CRP in European Americans and Hispanics (D).

Results

We used the six genetic variants robustly associated with the risk of gallstone disease in the study by Joshi et al. as instrumental variables, under the assumption that they act on GBC risk only through the conferred susceptibilities to gallstone development, and used the heterogeneity between the six MR estimates as a proxy for pleiotropy.(21,25) Summary statistics on the association between the six instruments and the risk of gallstone disease were obtained from the studies described in Table 1 and are provided in Supporting Table S6.(25,26)

Figure 1A,B shows the variance in liability to gallstone disease explained by the genetic instruments used in Europeans, Hispanic Americans, and Chileans. The variance explained by the missense variant rs11887534 at the adenosine triphosphate binding cassette subfamily G member 8 (*ABCG8*) locus was 0.02 for Europeans, 0.002 for Hispanic Americans, and 0.04 for Chileans. The variance attributable to rs4245791—noncoding transcript exon variant in *ABCG8*—was similar for Europeans and Hispanic Americans but twice as high for Chileans. The total variance explained by the six genetic instruments for gallstone disease was 0.04 for Europeans, 0.01 for Hispanic Americans, and 0.07 for Chileans. We found no heterogeneity among instruments (IVW Q $P = 0.56$ for Chileans and 0.47 for Europeans; Fig. 2 and Table 2). Evidence for a causal effect of gallstones on GBC was detected in Chileans (OR, 1.97, $P = 9 \times 10^{-5}$) and Europeans (OR, 5.02, $P = 9 \times 10^{-5}$). The estimated causal effect sizes were higher for Europeans than Chileans, but the difference between the ORs did not reach statistical significance (overlapping 95% confidence intervals [CIs]). Stratified results for Chileans according to the median proportion of Mapuche ancestry (34%) were consistent with the causal effect of gallstone disease on GBC risk increasing with a decreasing proportion of Mapuche

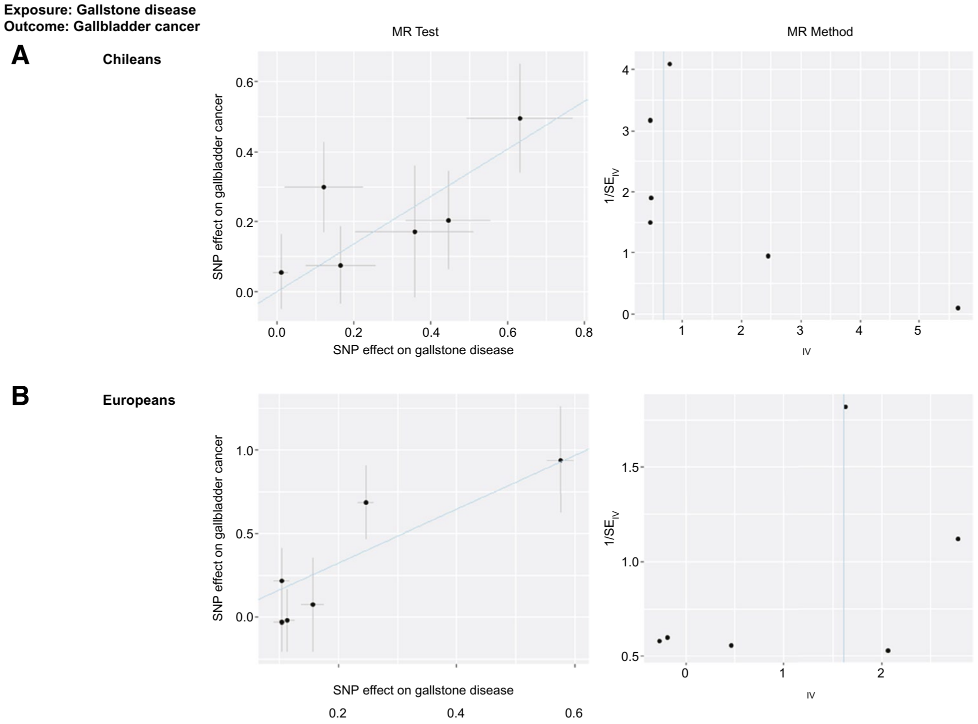

FIG. 2. Scatter and funnel plots for the association between gallstone disease and GBC in Chileans (A) and Europeans (B). Abbreviation: SNP, single-nucleotide polymorphism.

TABLE 2. MR Results for GBC (Outcome) Using Genetic Variants as Instrumental Variables for Established Risk Factors (Exposures)

		IVW				
Exposure	Population	*Q P* Value	***β*** *P* Value	OR*	95% CI	
Gallstone disease	Chileans	0.56	9×10^{-5}	**1.97**	1.40	2.77
	Chileans (Mapuche > 34%)	0.45	0.03	**1.60**	1.04	2.46
	Chileans (Mapuche ≤ 34%)	0.23	0.002	**2.88**	1.49	5.58
	Europeans	0.47	9×10^{-5}	**5.02**	2.23	11.3
BMI (per unit)	Chileans	0.14	0.03	**2.47**	1.10	5.54
	Chileans (Mapuche > 34%)	0.53	0.007	**3.83**	1.46	10.1
	Chileans (Mapuche ≤ 34%)	0.20	0.86	1.13	0.30	4.27
	Europeans	0.18	0.89	0.91	0.22	3.78
CRP (per mg/L)	Chileans	0.42	0.99	1.00	0.71	1.41
	Chileans (Mapuche > 34%)	0.98	0.50	1.16	0.75	1.79
	Chileans (Mapuche ≤ 34%)	0.19	0.72	0.87	0.41	1.85
	Europeans	0.64	4×10^{-6}	**4.44**	2.35	8.37
Age at menarche (per year)	Chileans	0.11	0.79	0.94	0.61	1.46
	Chileans (Mapuche > 34%)	0.47	0.85	0.95	0.56	1.60
	Chileans (Mapuche ≤ 34%)	0.15	0.53	0.79	0.38	1.64
	Europeans	0.69	0.33	0.70	0.34	1.43

*Bold type for IVW OR denotes *Q P* value > 0.10 and *β P* value < 0.05.

ancestry (OR, 1.60 for Mapuche ancestry > 34% versus OR, 2.88 for Mapuche ancestry ≤ 34%).

For the two-sample MR of the impact of BMI on GBC risk, we used the 289 genetic variants identified by Hoffmann et al. and their reported summary statistics on the association with BMI (Table 1).(27) Figure 1C depicts the variance in BMI explained by the genetic instruments in Europeans and Latinos. The variance explained by the intergenic variant rs13021737 was 0.003 for Latinos but only 0.0008 for Europeans, whereas rs6567160 explained 0.001 of the BMI variance in both Latinos and Europeans. The total variance explained by the considered instruments for BMI was 0.04 for Europeans and 0.07 for Latinos. The lower sample size and incomplete parameter information for Latinos, however, motivated us to use exclusively European summary statistics in this study. We excluded instruments with a P for the association with BMI higher than 5×10^{-8} or a low imputation accuracy, resulting in selection of 202 variants. The harmonization of publicly available summary statistics and our own data using MR-Base resulted in 192 instrumental variables for Chileans and 199 instruments for Europeans. We detected slight heterogeneity among the instruments for Chileans (IVW Q P = 0.08); this decreased to P = 0.14 after the visual inspection of scatter and funnel plots and removal of one outlying variant (rs3783890; Supporting Table S5 and Fig. S1A). No heterogeneity (IVW Q P = 0.18) and no directional bias (MR-Egger intercept P = 0.65) were detected for Europeans. Evidence for a causal effect of BMI on GBC was detected in Chileans

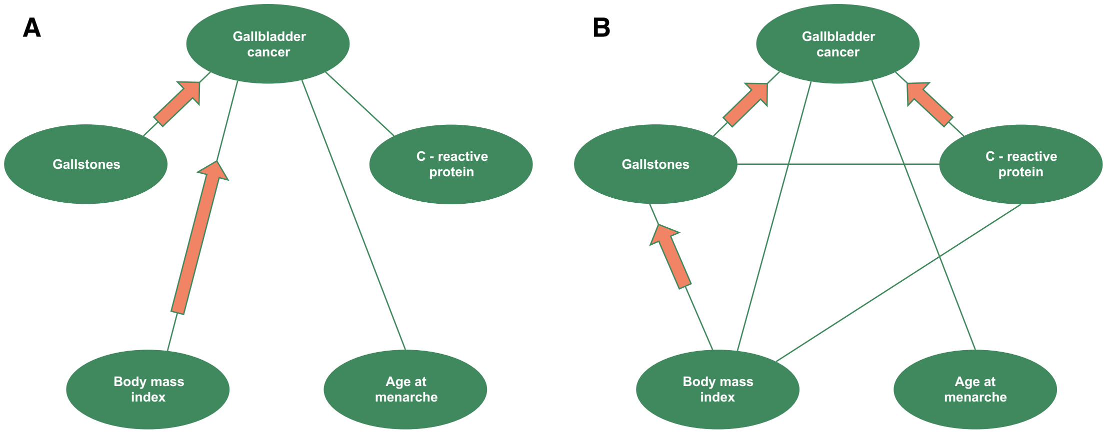

FIG. 3. Causal effects of established risk factors on GBC for Chileans (A). Causal effects of established risk factors on GBC and mediation effects of gallstone disease, BMI, and CRP for Europeans (B). Thin lines depict the investigated causal associations, while orange arrows show the identified causal effects.

TABLE 3. Results from Two-Step MR to Assess the Potential Mediation Effects of Gallstone Disease, BMI, and CRP on GBC in Europeans

		IVW				
Exposure	Potential Mediator	*Q P* Value	*β P* Value	OR/*β**	95% CI	
Gallstone disease†	CRP	0.97	0.54	0.96	0.86	1.08
	BMI	0.14	0.27	0.99	0.98	1.01
BMI (per unit)	Gallstone disease	0.11	0.008	**1.01**	1.00	1.01
	CRP	0.37	0.07	0.17	−0.01	0.36
CRP† (per mg/L)	Gallstone disease	0.37	0.82	1.00	0.99	1.00
	BMI	0.99	0.09	−0.01	−0.01	0.00

*Bold type for IVW β denotes Q P value > 0.10 and β P value < 0.05.
†Data for women only were not available.

(P = 0.03), with consistent causal effect estimates using IVW (OR per inverse normally transformed BMI unit, 2.47) and weighted median (OR, 2.70). Stratified results for Chileans were compatible, with a stronger causal effect of BMI on GBC risk with increasing proportion of Mapuche ancestry (OR, 3.83 for Mapuche proportion > 34% versus OR, 1.13 for Mapuche proportion ≤ 34%).

We found no evidence for a causal effect of BMI on GBC risk in Europeans, but BMI showed a causal effect on gallstone risk according to IVW, MR-Egger regression, and weighted median estimates; and gallstone risk was in turn causally associated with GBC risk in Europeans, as described (Fig. 3 and Tables 2 and 3; Supporting Table S3 and Fig. S1B). Results from causal mediation analysis using our own European data did not indicate any effect of BMI on GBC risk through gallstones: the estimated OR for the natural indirect effect was 1.001 (P = 0.60). This was an expected result given the absence of a direct causal effect of BMI on GBC risk for Europeans, the limited size of the European cohort, and the low quality of available gallstone data (self-reported gallstone history, which was missing for 47% of the study participants). These limitations motivated us to analyze UK Biobank data, which include complete BMI and gallstone information for 271,539 study participants of white British ancestry, 22 GBC cases, and 271,517 histories of self-reported gallstone. Consistent with our results from two-step MR, causal mediation analysis using UK Biobank data identified an indirect effect of BMI on GBC risk through gallstones (OR natural indirect effect, 1.03; P = 0.004). The OR natural direct effect was 0.98 (P = 0.75), and the adjusted OR of GBC associated with a gallstone history was 12.3 (P = 0.0001).

Figure 1D shows the variance in log-transformed CRP levels in serum explained by the genetic instruments in European Americans and Hispanics relying on Kocarnik et al. (2014).[28] For example, the variance explained by the 3' untranslated region variant rs1205 was 0.01 in European Americans and 0.02 in Hispanics. The splice region variant rs1800947 explained 0.01 for European Americans and 0.005 for Hispanics. In our main analyses, we used four tagging genetic variants in the *CRP* gene (rs1205, rs1800947, rs1130864, rs2808630) as instrumental variables to infer causality between CRP as a marker of chronic inflammation and GBC risk. Summary statistics on the association between the four instruments and CRP concentrations were obtained from the study by Nimptsch et al. (Table 1).[29] Evidence for a causal effect of CRP on GBC risk was detected in Europeans (OR, 4.44 per mg/L, $P = 4 \times 10^{-6}$) but not in Chileans (Table 2). As an alternative to the four variants in the *CRP* gene, for Chileans we also used the summary statistics recently reported by Kocarnik et al. (2018) for Hispanic Americans. We found no heterogeneity among the instruments (IVW Q P = 0.29) and no evidence for a causal effect of CRP on GBC risk (P = 0.11; OR, 0.55; 95% CI 0.27-1.14).[30]

To test causality between age at menarche and GBC risk in women, we used the 389 genetic variants identified by Day et al. and their reported summary statistics for the association with age at menarche (all $P < 5 \times 10^{-8}$; Table 1).[31] Exclusion of the variants with low imputation accuracy and problematic palindromic polymorphisms using MR-Base resulted in selection of 339 instruments for Chileans and 342 instruments for Europeans. Heterogeneity as a proxy for pleiotropy was evident for Chileans (IVW Q P = 0.02); this decreased to P = 0.11 after removal of three outlying genetic variants. No heterogeneity and no directional bias were detected for Europeans (Table 2; Supporting Table S3). No evidence for a causal effect of age at menarche on GBC was detected either in Chileans or in Europeans.

We used the 10 genetic variants and the summary statistics reported by Barban et al. to test the causal effect of age at first childbirth on GBC risk.[32] The limitation of the study to parous women only translated into F statistics of 14.6 for Chileans and 5.29 for Europeans; the detectable ORs were 4.2 for Chileans and 10.1 for Europeans (Table 1). Due to the substantially lower statistical power for age at first childbirth compared to the other investigated exposures, age at first childbirth was not considered further.

Results from the sensitivity analyses were consistent with robust rejections of the causal null hypotheses and with robust estimates of the causal effect sizes (Supporting Tables S4 and S5). The estimated causal effect of gallstone disease on GBC for Chileans (OR, 1.97) varied from an OR of 1.95 (adjustment for the first 20 genetic principal components) to an OR of 2.16 (linkage disequilibrium [LD] clumping). The estimated causal effect of BMI on GBC risk for Chileans varied from an OR of 1.70 (radial MR) to 2.79 (LD clumping). The Wald ratios for the genetic variant rs9939609 in the fat mass and obesity–associated gene as an instrumental variable of BMI were an OR of 3.73 (association statistic for Latinos in Hoffmann et al.,(27) n = 8,322) and an OR of 1.18 (statistic for Chileans in Petermann et al.,(33) n = 409). In agreement with our primary results in Table 2, the corresponding Wald ratio for Europeans was an OR of 0.40 and a 95% CI of 0.14-1.12 (data not shown). The estimated causal effect of gallstone disease on GBC for Europeans (OR, 5.02) varied from an OR of 2.47 (integration of summary statistics from UK Biobank) to an OR of 5.66 (exclusion of instruments associated with multiple risk factors). The OR for the causal effect of CRP concentration on GBC risk for Europeans varied from 2.41 (summary statistics for CRP reported by Dehghan et al.(34)) to 5.91 (LD clumping).

Discussion

GBC is a very aggressive disease with considerable potential for prevention. The tumor develops over a period of 10-20 years, and preventive gallbladder removal (prophylactic cholecystectomy) can be offered to individuals at high GBC risk. Maintenance of an ideal body weight by means of a healthy diet and regular physical activity may prevent gallstone formation in the general population, and treatment with ursodeoxycholic acid can be recommended for patients at high risk of gallstones, for example, obese patients during rapid weight loss after bariatric surgery and patients on long-term therapy with somatostatin.(35) Despite the poor prognosis and the substantial prevention potential, research on the disease has been largely neglected, and the mechanisms underlying GBC etiology are not yet well understood. The present study takes advantage of MR to assess the causal relationship between established risk factors and GBC risk.

Gallstones are found in up to 90% of neoplastic gallbladders, and their presence is a major risk factor for developing GBC.(36) The co-occurrence of gallstone disease and GBC differs strongly by ethnicity. East Indian women and Native American Mapuche and Pima often develop both gallstones and GBC. In contrast, north Indian women are at high risk of developing GBC but are rarely affected by gallstones. Around 15% of Caucasian women carry gallstones, but they are at low risk of GBC.(37) Our data provide evidence for a causal association between gallstone disease and GBC risk in Europeans and Chileans. Furthermore, MR results suggest that gallstones mediate the effect of body fatness, marked by BMI, on GBC risk in Europeans. We found a causal effect of BMI on GBC risk in Chileans, and the effect seemed more pronounced in those with a large proportion (>34%) of Mapuche ancestry. Two recent meta-analyses reported an increased risk of GBC for overweight (OR, 1.10-1.14) and obese (OR, 1.56-1.58) individuals, with a 4% risk increase per BMI unit.(38,39) Accordingly, the World Cancer Research Fund concluded that there is strong evidence for a causal role of body fatness on GBC development, which we were able to confirm in the present study.

The association between elevated BMI and gallstone formation has long been established from observational studies. Compared with normal-weight people (BMI < 25 kg/m^2), individuals with a BMI between 25 and 30 kg/m^2 are at 20% increased risk of developing gallstones, while the risk excess increases to 73% for obese individuals (BMI > 30 kg/m^2).(40,41) In agreement with our study, a causal association between BMI and the risk of gallstone disease was recently identified in a large population-based European MR study; the estimated size of the causal effect on gallstone disease was an OR of 1.17 per BMI unit.(42) The formation and growth of cholesterol-based gallstones is a multifactorial process resulting from the complex interplay between systemic factors (age, gender, genetic predisposition, chronic inflammation) and gallbladder-related factors accompanying cholesterol supersaturation of the bile (hypomotility of the gallbladder, hypersecretion of mucin in the gallbladder with local inflammation, rapid precipitation of solid cholesterol crystals).(43,44) Biliary cholesterol supersaturation could also be partially related to poor dietary habits, hyperinsulinemia, and insulin resistance, which are in turn associated with body composition. Hyperinsulinemia promotes two conditions predisposing to cholesterol-supersaturated lithogenic bile: hepatic uptake of cholesterol resulting in an increased secretion of biliary cholesterol and decreased secretion of bile acids.(35) While it is not yet possible to separate the direct and gallstone-mediated effects of body fatness on GBC risk, the present study adds to the current understanding of GBC development, suggesting that the relative contributions of obesity and gallstones to GBC risk depend on ethnicity.(45,46) Taking full advantage of these differences may translate into more efficient GBC prevention.

Despite the evidently strong association between obesity and GBC risk, very little is known about the causal mechanisms that underlie this association. Obesity is causally linked to chronic inflammation, as reflected by increased levels of circulating inflammatory proteins such as CRP.(6-8) Elevated circulatory levels of inflammatory markers are also associated with an increased risk of GBC, as was recently shown in a study of Chinese and Chilean individuals.(9) The study investigated immune-related markers in GBC and patients with gallstone disease from China and validated associated markers in serum samples from Chilean patients. Six inflammation markers, including CRP, were associated with an increased risk of GBC in the two study populations; the estimated OR for GBC associated with increased CRP levels was 18.6 for Chileans and 7.6 for Chinese. Similar associations were observed in a European study, which did not, however, investigate GBC as a distinct cancer entity; rather, GBC was combined with other biliary tract cancers.(47) The study included 137 cases of biliary tract cancer (among them, 51 of GBC) and found a 22% increased risk of biliary tract cancer for elevated CRP levels. It is well established that GBC often develops along the sequence gallstones and inflammation → dysplasia → GBC. Our data provide evidence that genetically increased CRP levels are associated with GBC risk in Europeans, consolidating the causal role of chronic inflammation in GBC development. The unavailability of summary statistics on CRP for women only was, however, a limitation of our study.

Two-step MR and mediation analyses consistently pointed to an indirect effect of BMI on GBC risk through gallstones for Europeans. According to GLOBOCAN (globocan.iarc.fr), the incidence of GBC is progressively decreasing in most European countries, for example, Germany, Italy, and Spain, where an increase in overweight and obesity has been noted. To examine the discrepancy between our results and the decreasing GBC rates in combination with increasing population BMI, we retrieved and plotted existing data for Germany on GBC incidence (krebsdaten.de), BMI,(48) and cholecystectomy rates (gbe-bund.de). The results are shown in Fig. 4. The incidence of GBC has been decreasing, and the percentage of the population with a BMI of 25 kg/m^2 or more has been increasing among both men and women in Germany. At the same time, and possibly reflecting the identified causal effect of BMI on gallstone disease, the number of cholecystectomies in Germany has been increasing, potentially resulting in

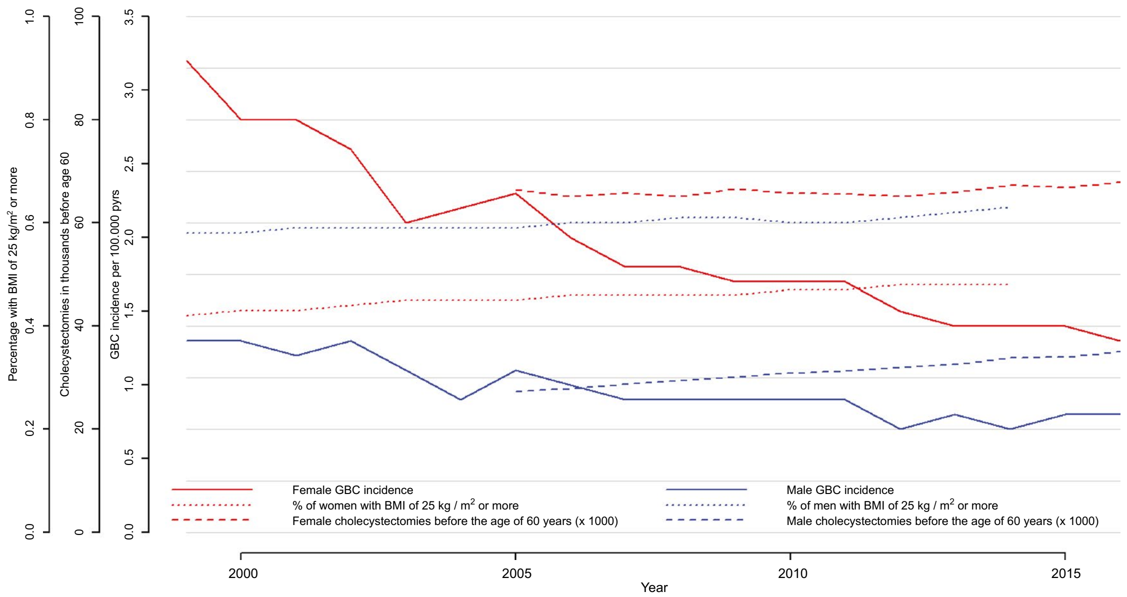

FIG. 4. Incidence of GBC, proportion of persons with BMI ≥ 25 kg/m^2, and number of cholecystectomies in Germany from 1999 to 2016 in women (red lines) and men (blue lines).

the avoidance of some GBC cases. The likely contribution of common external (i.e., environmental) factors that modulate epigenetic mechanisms and the individual inherited predisposition to overweight and obesity, inflammation, gallstones, and GBC, together with the time lag from weight gain to gallstone formation, local and chronic inflammation, and GBC development, add additional complexity to the interdependence among the investigated risk factors and GBC as the final outcome. Besides gallbladder cancer, gallstones and chronic inflammation have been also linked to other types of cancers, including right-sided colon, colorectal, pancreatic, hepatic, prostate, and gastric cancers.(49-53) The development of gallstones and the concomitant elevated CRP levels may thus indicate both local (in the gallbladder) and systemic (through circulating proinflammatory proteins) inflammatory effects of gallstones. However, in contrast to our finding for GBC, a causal effect of CRP on the risk of developing these other types of cancer has not been reported,(54,55) and neither has a causal effect of gallstones on these cancers been investigated by means of MR.

The relatively low numbers of investigated patients with GBC and cases represented a limitation of the study, especially in view of the large sample sizes usually required for MR. For illustration, assuming that 4% of the variation in BMI is explained by the genetic variants used as instruments, the present study had 80% statistical power to reject the causal null hypothesis for a true OR of GBC per standard deviation of the BMI higher than 1.95 for Chileans and 3.70 for Europeans (type I error rate of 5%).(27) The difference in statistical power between the Chilean and the European study was probably larger because women are at a higher risk of GBC than men, and Latino women show a higher proportion of BMI variation explained by known genetic variants than non-Hispanic white women (4.1% versus 3.2%) and greater BMI variability (standard deviation 6.3 versus 6.0 kg/m^2).(27) On the other hand, the Chilean results were limited by the sparse public data on the association between the instruments used and GBC risk factors for the Chilean population and by the population stratification due to the genetic admixture of Chileans. With the exception of CRP and age at first childbirth for Europeans, *F* statistics were high (>10) for the investigated risk factors. We conducted extensive sensitivity analyses to examine the potential influence of the genetic admixture on the estimated causal effects, but further methodological research is needed to deal adequately with stratification in MR studies of admixed populations.(56) In both high-incidence and low-incidence regions, collaborative research is crucial to maximize sample sizes and fully exploit the potential of MR to investigate GBC risk factors, with weaker associations found in observational studies, such as diabetes, educational level, smoking, and alcohol consumption.

In addition to low statistical power, another major limitation of MR studies is pleiotropy. Regardless of the number and strength of the instrumental variables used, first-order IVWs preserve the type I error rate under the causal null.(21) We calculated Cochran's Q statistic using first-order weights to detect heterogeneity, which often reflects pleiotropy. We applied a rather conservative heterogeneity cutoff (Q *P* value = 0.10) and used a random-effect IVW model, but the results based on a fixed-effect model were identical for gallstone disease and CRP and practically identical for BMI (random-effect OR, 2.47; 95% CI, 1.10-5.54; fixed-effect OR, 2.47; 95% CI, 1.14-5.32). We visually inspected scatter and funnel plots, performed MR-Egger regression for BMI and age at menarche as exposures to quantify the amount of bias due to horizontal pleiotropy, used radial MR, and conducted sensitivity analyses by excluding genetic variants associated with multiple GBC risk factors.

In conclusion, we used MR to study GBC, a neglected disease with considerable potential for individualized prevention. The investigated sample size was limited compared with traditional MR analyses, but it is important to consider that strong associations have been reported for established GBC risk factors in observational studies and that GBC is a rare disease in most countries. To put numbers into context, the present MR results for Europeans rely on 103 GBC cases in comparison with the 22 cases of white British ancestry among the 500,000 participants in the UK Biobank. Other points of note were the investigation of genetically admixed Chileans, the examination of the transferability of the genetic instruments among populations with European and Latin American ancestry, and the examination of ethnic differences in GBC causation. We found that two risk factors currently considered in the Chilean program for GBC prevention are causally linked to GBC risk: gallstones and BMI. For Europeans, the effect of BMI on GBC risk seems to be exerted through gallstones. Further collaborative research is needed to identify and quantify ethnic differences in GBC causation and finally improve the performance of GBC prevention programs.

Acknowledgment: Open access funding enabled and organized by Projekt DEAL.

REFERENCES

1) Wistuba II, Gazdar AF. Gallbladder cancer: lessons from a rare tumour. Nat Rev Cancer 2004;4:695-706.
2) Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut Liv 2012;6:172-187.
3) Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. Lancet 2014;384:755-765.
4) Syngal S, Coakley EH, Willett WC, Byers T, Williamson DF, Colditz GA. Long-term weight patterns and risk for cholecystectomy in women. Ann Intern Med 1999;130:471-477.
5) Zakaria D, Shaw A. Cancers attributable to excess body weight in Canada in 2010. Health Promot Chronic Dis Prev Can 2017;37:205-214.
6) Fall T, Hagg S, Ploner A, Magi R, Fischer K, Draisma HH, et al. Age- and sex-specific causal effects of adiposity on cardiovascular risk factors. Diabetes 2015;64:1841-1852.
7) Timpson NJ, Nordestgaard BG, Harbord RM, Zacho J, Frayling TM, Tybjaerg-Hansen A, et al. C-reactive protein levels and body mass index: elucidating direction of causation through reciprocal Mendelian randomization. Int J Obes (Lond) 2011;35:300-308.
8) Welsh P, Polisecki E, Robertson M, Jahn S, Buckley BM, de Craen AJ, et al. Unraveling the directional link between adiposity and inflammation: a bidirectional Mendelian randomization approach. J Clin Endocrinol Metab 2010;95:93-99.
9) Koshiol J, Castro F, Kemp TJ, Gao YT, Roa JC, Wang B, et al. Association of inflammatory and other immune markers with gallbladder cancer: results from two independent case-control studies. Cytokine 2016;83:217-225.
10) Shukla VK, Chauhan VS, Mishra RN, Basu S. Lifestyle, reproductive factors and risk of gallbladder cancer. Singapore Med J 2008;49:912-915.
11) Pandey M, Shukla VK. Lifestyle, parity, menstrual and reproductive factors and risk of gallbladder cancer. Eur J Cancer Prev 2003;12:269-272.
12) Andreotti G, Hou L, Gao YT, Brinton LA, Rashid A, Chen J, et al. Reproductive factors and risks of biliary tract cancers and stones: a population-based study in Shanghai, China. Br J Cancer 2010;102:1185-1189.
13) Liebe R, Milkiewicz P, Krawczyk M, Bonfrate L, Portincasa P, Krawczyk M. Modifiable factors and genetic predisposition associated with gallbladder cancer. A concise review. J Gastrointestin Liver Dis 2015;24:339-348.
14) Unisa S, Jagannath P, Dhir V, Khandelwal C, Sarangi L, Roy TK. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of north India. HPB (Oxford) 2011;13:117-125.
15) Cong X. Air pollution from industrial waste gas emissions is associated with cancer incidences in Shanghai, China. Environ Sci Pollut Res Int 2018;25:13067-13078.
16) Lorenzo Bermejo J, Boekstegers F, Gonzalez Silos R, Marcelain K, Baez Benavides P, Barahona Ponce C, et al. Subtypes of Native American ancestry and leading causes of death: Mapuche ancestry–specific associations with gallbladder cancer risk in Chile. PLoS Genet 2017;13:e1006756.
17) Roa I, de Aretxabala X. Gallbladder cancer in Chile: what have we learned? Curr Opin Gastroenterol 2015;31:269-275.
18) De Aretxabala X, Benavides C, Roa I. Gallbladder cancer: preliminary evaluation of the GES program to prevent the disease. [in Spanish] Revista Chilena de Cirugía 2017;69:196-201.
19) Popejoy AB, Fullerton SM. Genomics is failing on diversity. Nature 2016;538:161-164.
20) Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008;27:1133-1163.
21) Bowden J, Del Greco MF, Minelli C, Zhao Q, Lawlor DA, Sheehan NA, et al. Improving the accuracy of two-sample summary-data Mendelian randomization: moving beyond the NOME assumption. Int J Epidemiol 2018.
22) Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. Epidemiology 2017;28:30-42.
23) Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife 2018;7:e34408.
24) Zheng J, Baird D, Borges MC, Bowden J, Hemani G, Haycock P, et al. Recent developments in Mendelian randomization studies. Curr Epidemiol Rep 2017;4:330-345.
25) Joshi AD, Andersson C, Buch S, Stender S, Noordam R, Weng LC, et al. Four susceptibility loci for gallstone disease identified in a meta-analysis of genome-wide association studies. Gastroenterology 2016;151:351-363.e28.
26) Bustos BI, Perez-Palma E, Buch S, Azocar L, Riveras E, Ugarte GD, et al. Variants in *ABCG8* and *TRAF3* genes confer risk for gallstone disease in admixed Latinos with Mapuche Native American ancestry. Sci Rep 2019;9:772.
27) Hoffmann TJ, Choquet H, Yin J, Banda Y, Kvale MN, Glymour M, et al. A large multiethnic genome-wide association study of adult body mass index identifies novel loci. Genetics 2018;210:499-515.
28) Kocarnik JM, Pendergrass SA, Carty CL, Pankow JS, Schumacher FR, Cheng I, et al. Multiancestral analysis of inflammation-related genetic variants and C-reactive protein in the population architecture using genomics and epidemiology study. Circ Cardiovasc Genet 2014;7:178-188.
29) Nimptsch K, Aleksandrova K, Boeing H, Janke J, Lee YA, Jenab M, et al. Association of CRP genetic variants with blood concentrations of C-reactive protein and colorectal cancer risk. Int J Cancer 2015;136:1181-1192.
30) Kocarnik JM, Richard M, Graff M, Haessler J, Bien S, Carlson C, et al. Discovery, fine-mapping, and conditional analyses of genetic variants associated with C-reactive protein in multiethnic populations using the Metabochip in the Population Architecture using Genomics and Epidemiology (PAGE) study. Hum Mol Genet 2018;27:2940-2953.
31) Day FR, Thompson DJ, Helgason H, Chasman DI, Finucane H, Sulem P, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. Nat Genet 2017;49:834-841.
32) Barban N, Jansen R, de Vlaming R, Vaez A, Mandemakers JJ, Tropf FC, et al. Genome-wide analysis identifies 12 loci influencing human reproductive behavior. Nat Genet 2016;48:1462-1472.
33) Petermann F, Villagran M, Troncoso C, Mardones L, Leiva AM, Martinez MA, et al. Association between FTO (ns9939609)

genotype and adiposity markers in Chilean adults. Rev Med Chil 2018;146:717-726.

34) Dehghan A, Dupuis J, Barbalic M, Bis JC, Eiriksdottir G, Lu C, et al. Meta-analysis of genome-wide association studies in %3e80 000 subjects identifies multiple loci for C-reactive protein levels. Circulation 2011;123:731-738.
35) European Association for the Study of the Liver. EASL clinical practice guidelines on the prevention, diagnosis and treatment of gallstones. J Hepatol 2016;65:146-181.
36) Eslick GD. Epidemiology of gallbladder cancer. Gastroenterol Clin North Am 2010;39:307-330.
37) Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol 2014;6:99-109.
38) Tan W, Gao M, Liu N, Zhang G, Xu T, Cui W. Body mass index and risk of gallbladder cancer: systematic review and meta-analysis of observational studies. Nutrients 2015;7:8321-8334.
39) Li ZM, Wu ZX, Han B, Mao YQ, Chen HL, Han SF, et al. The association between BMI and gallbladder cancer risk: a meta-analysis. Oncotarget 2016;7:43669-43679.
40) Figueiredo JC, Haiman C, Porcel J, Buxbaum J, Stram D, Tambe N, et al. Sex and ethnic/racial-specific risk factors for gallbladder disease. BMC Gastroenterol 2017;17:153.
41) Maclure KM, Hayes KC, Colditz GA, Stampfer MJ, Speizer FE, Willett WC. Weight, diet, and the risk of symptomatic gallstones in middle-aged women. N Engl J Med 1989;321:563-569.
42) Stender S, Nordestgaard BG, Tybjaerg-Hansen A. Elevated body mass index as a causal risk factor for symptomatic gallstone disease: a Mendelian randomization study. HEPATOLOGY 2013;58:2133-2141.
43) Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. Lancet 2006;368:230-239.
44) Lammert F, Gurusamy K, Ko CW, Miquel JF, Mendez-Sanchez N, Portincasa P, et al. Gallstones. Nat Rev Dis Primers 2016;2:16024.
45) Pino-Yanes M, Thakur N, Gignoux CR, Galanter JM, Roth LA, Eng C, et al. Genetic ancestry influences asthma susceptibility and lung function among Latinos. J Allergy Clin Immunol 2015;135:228-235.
46) Maher B. Personal genomes: the case of the missing heritability. Nature 2008;456:18-21.
47) Aleksandrova K, Boeing H, Nothlings U, Jenab M, Fedirko V, Kaaks R, et al. Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. HEPATOLOGY 2014;60:858-871.
48) NCD Risk Factor Collaboration. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 2016;387:1377-1396.
49) Shabanzadeh DM, Sorensen LT, Jorgensen T. Association between screen-detected gallstone disease and cancer in a cohort study. Gastroenterology 2017;152:1965-1974.e1.
50) Ward HA, Murphy N, Weiderpass E, Leitzmann MF, Aglago E, Gunter MJ, et al. Gallstones and incident colorectal cancer in a large pan-European cohort study. Int J Cancer 2019;145:1510-1516.
51) Chen CH, Lin CL, Kao CH. Association between gallbladder stone disease and prostate cancer: a nationwide population-based study. Oncotarget 2016;7:64380-64389.
52) Kang SH, Kim YH, Roh YH, Kim KW, Choi CJ, Kim MC, et al. Gallstone, cholecystectomy and risk of gastric cancer. Ann Hepatobiliary Pancreat Surg 2017;21:131-137.
53) Zhao X, Wang N, Sun Y, Zhu G, Wang Y, Wang Z, et al. Screen-detected gallstone disease and risk of liver and pancreatic cancer: the Kailuan Cohort Study. Liver Int 2020;40:1744-1755.
54) Wang X, Dai JY, Albanes D, Arndt V, Berndt SI, Bezieau S, et al. Mendelian randomization analysis of C-reactive protein on colorectal cancer risk. Int J Epidemiol 2019;48:767-780.
55) Allin KH, Nordestgaard BG, Zacho J, Tybjaerg-Hansen A, Bojesen SE. C-reactive protein and the risk of cancer: a Mendelian randomization study. J Natl Cancer Inst 2010;102:202-206.
56) Epstein MP, Allen AS, Satten GA. A simple and improved correction for population stratification in case-control studies. Am J Hum Genet 2007;80:921-930.
57) Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, et al. Genomic atlas of the human plasma proteome. Nature 2018;558:73-79.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.31537/suppinfo.