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Development and internal validation of a multifactorial risk prediction model for gallbladder cancer in a high-incidence country

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Abstract

Since 2006, Chile has been implementing a gallbladder cancer (GBC) prevention program based on prophylactic cholecystectomy for gallstone patients aged 35 to 49 years. The effectiveness of this prevention program has not yet been comprehensively evaluated. We conducted a retrospective study of 473 Chilean GBC patients and 2137 population-based controls to develop and internally validate three GBC risk prediction models. The Baseline Model accounted for gallstones while adjusting for sex and birth year. Enhanced Model I also included the non-genetic risk factors: body mass index, educational level, Mapuche surnames, number of children and family history of GBC. Enhanced Model II further included Mapuche ancestry and the genotype for rs17209837. Multiple Cox regression was applied to assess the predictive performance, quantified by the area under the precision-recall curve (AUC-PRC) and the number of cholecystectomies needed (NCN) to prevent one case of GBC at age 70 years.

Abbreviations: BMI, body mass index; CI, confidence interval; GBC, gallbladder cancer; GES, Chilean gallbladder cancer prevention program; HR, hazard-ratio; IQR, interquartile range; MI, multiple imputation; NCN, and the number of cholecystectomies needed to prevent one case of GBC at age 70 years; NHS, Chilean National Health Survey; *P*val, global probability value; ΔAUC-PRC, change in the area under the precision-recall curve with respect to the Baseline Model; ΔAUC-ROC, change in the area under the receiver operating characteristic curve with respect to the Baseline Model.

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The AUC-PRC for the Baseline Model (0.44%, 95%CI 0.42-0.46) increased by 0.22 (95%CI 0.15-0.29) when non-genetic factors were included, and by 0.25 (95%CI 0.20-0.30) when incorporating non-genetic and genetic factors. The overall NCN for Chileans with gallstones (115, 95%CI 104-131) decreased to 92 (95%CI 60-128) for Chileans with a higher risk than the median according to Enhanced Model I, and to 80 (95%CI 59-110) according to Enhanced Model II. In conclusion, age, sex and gallstones are strong risk factors for GBC, but consideration of other non-genetic factors and individual genotype data improves risk prediction and may optimize allocation of financial resources and surgical capacity.

KEYWORDS
cholecystectomy, gallbladder cancer, gallstones, native American ancestry, non-genetic and genetic risk factors, risk prediction

What's new?

With one of the world's highest incidences of gallbladder cancer, Chile has been implementing a gallbladder cancer prevention program based on prophylactic cholecystectomy for gallstone patients. The effectiveness of this prevention program has not yet been comprehensively evaluated. The findings of this study based on retrospective non-genetic and genome-wide genotype data from patients and controls in Chile suggest that the current implementation of the prevention program, which relies exclusively on symptomatic gallstones, is suboptimal. The non-genetic and genetic factors investigated improve predictive performance, allowing for simple yet more accurate prediction of individual gallbladder cancer risk.

1 | INTRODUCTION

Chile has one of the world's highest incidences of gallbladder cancer (GBC; ICD-10 diagnosis code C23).[1] GBC mortality is also high in neighboring regions such as Bolivia, with poorer data quality than Chile (singular compared with national registry data). Since 2006, the country has been implementing a GBC prevention program (Régimen General de Garantías Explícitas en Salud 26; GES) that rests upon prophylactic gallbladder removal (cholecystectomy) for persons with gallstones between 35 and 49 years of age.[2] Since gallstones are often asymptomatic, the prevention program also recommends gallstone screening by abdominal echography for individuals at high risk of developing GBC, including persons with a body mass index (BMI) greater than 25 kg/m^2, low educational level, at least one Mapuche surname (the Mapuche are the largest Native American group in Chile) and women with more than one child.[3]

The GES prevention program relies on low levels of scientific evidence—mainly case series and reports or expert opinion—and its effectiveness is controversial. The program's initial goal of reducing GBC mortality by 25% has not yet been achieved, and 981 women and 398 men died from GBC in Chile in 2018.[4] Gallstones are a major risk factor for GBC development, and one of the reasons for the high GBC mortality in Chile is the high incidence of asymptomatic and symptomatic gallstones: recent estimates suggest that 26.5% of Chilean women and 17.5% of Chilean men have gallstones by the age of 40 years,[3] which translates into 87 000 newly diagnosed gallstone patients and about 50 000 prophylactic cholecystectomies every year.[4] Abdominal surgery services are overloaded and less than half of the potential beneficiaries of the prevention program are assessed within the expected timeframe—30 days for abdominal echography and 90 days for gallbladder surgery—with significant associated costs to the Chilean health care system—around USD 50 per echography and USD 1050 per cholecystectomy.[4]

The ability of the risk factors included in the current GES prevention program to predict the development of GBC (hereafter "GBC risk") has not been systematically evaluated. To improve this situation and pave the way for future innovations in prevention, we examined retrospective non-genetic and genome-wide genotype data from 473 GBC patients and 2137 population-based controls from Chile to develop three GBC risk prediction models, which we then validated internally to assess model overfitting and overoptimistic predictions. The integration of our data with (1) information from the Chilean National Health Surveys and (2) the effects of genetic variants on gallstone and GBC risk was an important novelty of this study, which we describe following the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) guidelines (Figure S1).[5] We found that the non-genetic and genetic factors investigated improve the predictive performance, allowing for simple yet more accurate prediction of individual GBC risk.

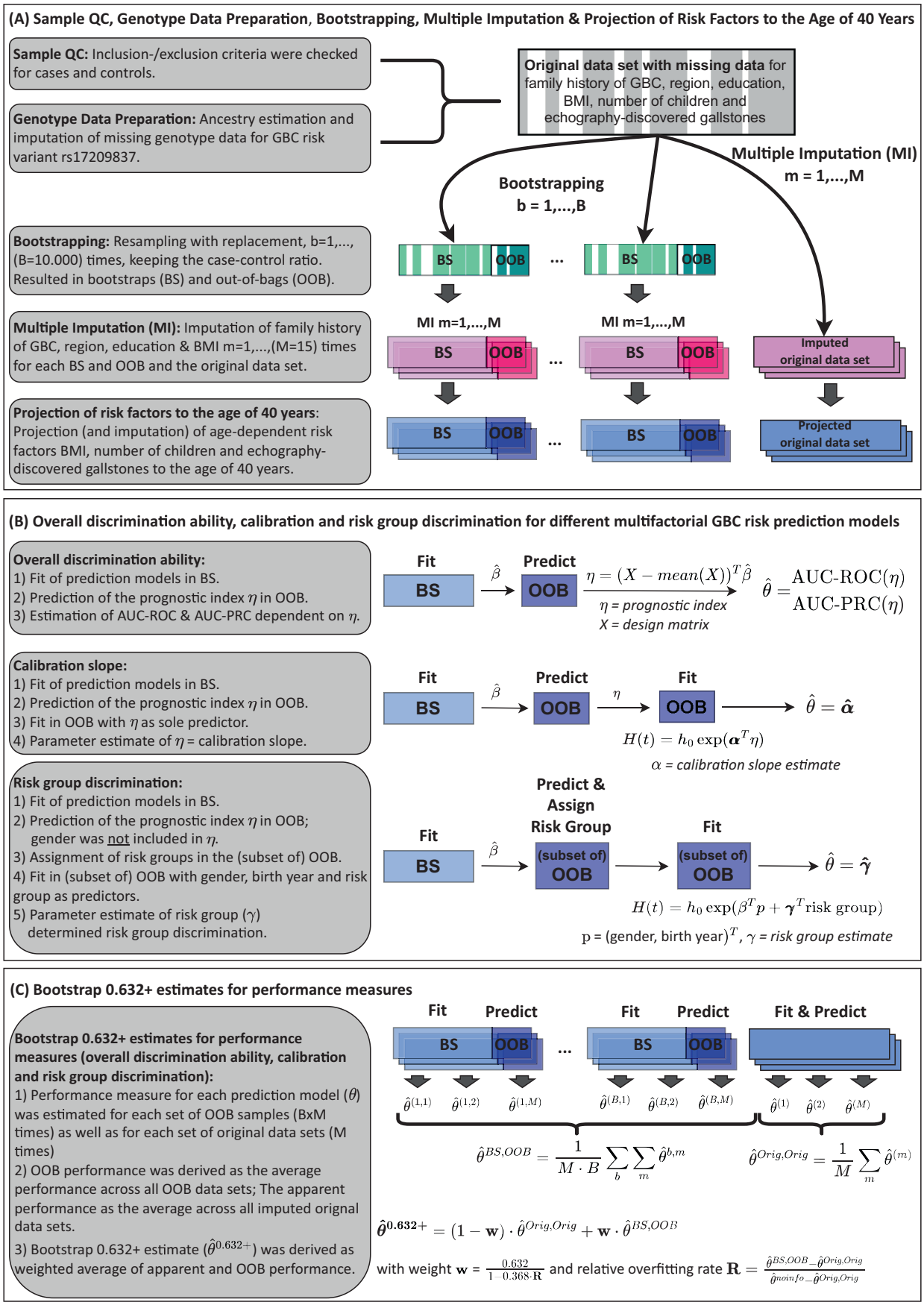

FIGURE 1 Legend on next page.

2 | MATERIALS AND METHODS

2.1 | Study design

Figure 1 depicts the methods applied, including data preparation and estimation of predictive performance. The retrospective study included 473 GBC patients from 16 Chilean hospitals and 2137 population-based controls from all over the country. Among the GBC patients, 363 (77%) were diagnosed with incidental GBC by a pathologist after cholecystectomy and histopathological gallbladder inspection. The remaining 110 patients (23%) were diagnosed with GBC without a previous cholecystectomy and typically at later stages of the disease. Information on non-genetic factors was obtained from clinical records (sex, age, birth year, body mass index (BMI), Mapuche surnames, gallstones) and face-to-face interviews (education, number of children in women and family history of GBC). DNA samples were used for genome-wide genotyping, including gallstone and GBC risk variants, and subsequent estimation of the individual proportions of Mapuche ancestry. All participants gave written informed consent prior to enrolment. Further details on study participants and ethics approval are provided in Data S1. The structured questionnaires administered to study participants are available upon request.

2.2 | Data preparation

Data S2 provides detailed information on data preparation, which included (1) estimation of individual ancestry proportions, (2) multiple imputation of missing data using fully conditional specification (FCS) and (3) projection to age 40 of the age-dependent risk factors BMI, gallstone carriage and parity. The FCS-discriminant function method was used for multiple imputation of missing GBC family history, region and educational level data; the FCS regression method was used for missing BMI data; and the accuracy of imputation was assessed by leave-one-out cross validation (artificial masking of non-missing values, multiple imputation and comparison of actual observed and imputed values).[6,7] As information on the number of children was not available for the majority of female controls (n = 730, 81%), instead of applying multiple imputation, existing information from 485 study women was combined with demographic data from female participants in the Chilean National Health Surveys (NHS; n = 5355), which collected lifestyle, household and medical history information for 11 525 Chileans in 2009-2010 and 2016-2017. The combined data set was then used to estimate and project the number of children for women by age 40, and to set a threshold for low (0-2 children) and high (3+ children) parity. Since abdominal echography data was not available for the controls, their probability of carrying gallstones at age 40 was inferred from echography data available for 3666 NHS participants, complemented by individual genotype data for gallstone risk variants reported for the Chilean population.[8] As the prevalence of gallstones at age 40 years is 26.5% in Chilean women and 17.5% in Chilean men (Figure S4), the 17.5% of male and 26.5% of female controls with the highest estimated risk of gallstones were classified as gallstone carriers.

2.3 | Model development, internal validation and evaluation of model performance

Three prediction models were compared in terms of their ability to correctly infer, for a person aged 40 years, whether or not they will develop GBC by the age of 70 years. The Baseline Model reflected the current implementation of the GES prevention program and took into account only gallstones, sex and year of birth.[9] Enhanced Model I additionally considered the non-genetic risk factors BMI, education, Mapuche surnames, number of children in women (0-2 vs 3+), and family history of GBC. The genetic variant rs17209837 (A > T) is associated with GBC risk in the Chilean population, especially among individuals with a high proportion of Mapuche ancestry,[10] and Enhanced Model II further included the individual proportion of Mapuche ancestry and the rs17209837 genotype as genetic risk factors.

Multiple Cox regression was applied to assess the predictive performance of each model. The MENDEL software was used to conduct survival analyses, as it allowed the integration of the GBC incidence rates from Chile as the baseline hazard.[11,12] To assess model overfitting and overoptimistic predictions through internal validation, all

FIGURE 1 General overview of the data preparation and conducted evaluation of the predictive performance using multiple imputation (MI) combined with the bootstrap 0.632+ approach. In the presence of missing data, the bootstrap 0.632+ estimation technique represents a reliable internal validation strategy to control for overly optimistic estimates of the discrimination performance (overall and for low- and high-risk groups) and the calibration performance. The overoptimistic performance measure ($\Theta^{Orig,\ Orig}$) was estimated relying on the same projected original data sets for fitting and evaluating the prediction model (Panel [A]). Starting point for calculating the optimism-corrected bootstrap 0.632+ performance ($\Theta^{0.632+}$) was the incomplete original data set with missing values for family history of GBC, region, educational level, BMI, parity in women and ultrasound-discovered gallstones. From this incomplete data set, B = 10 000 samples with replacement were drawn such that the new data sets, the bootstraps (BS), contained each the same number of patients and controls as the original data set. For the single BS, some observations were chosen more than once and the bootstrap selection was expected to include 63.2% of all available observations in the original data set. The approximately 36.8% non-selected observations constituted the respective out-of-bag (OOB) data set. Multiple imputation and age-projection to 40 years for each BS and OOB data set resulted in 10 000 · 15 = 150 000 projected BS and OOB data sets. Fitting the prediction model to the BS data set, evaluating the performance in OOB and pooling all estimates yielded the pessimistic performance estimate ($\Theta^{BS,\ OOB}$, Panel [B]). $\Theta^{0.632+}$ was the weighted average of optimistic $\Theta^{Orig,\ Orig}$ and pessimistic $\Theta^{BS,\ OOB}$ (Panel [C]). The weighting required a performance estimate for the prediction model in the absence of an effect (Θ^{noinfo}). This estimate was known a priori for the performance of the overall discrimination (ΔAUC-PRC = ΔAUC-ROC = 0), of the calibration (α = 0) and of the low- and high-risk group discrimination (γ = 0).

TABLE 1 Main characteristics of study participants before/after imputation of missing values and projection of BMI, parity in women and gallstone occurrence to age 40 years.

	Original data set				Imputed and age-projected data set			
	Controls (n = 2137)		GBC patients (n = 473)		Controls (n = 2137)		GBC patients (n = 473)	
Gallstones (n, %)								
No	0	0%	80	17%	1688	79%	149	32%
Yes	0	0%	376	79%	449	21%	324	68%
Missing	2317	100%	17	4%				
Sex and number of children (n, %)								
Male	1238	58%	114	24%	1238	58%	114	24%
Female, 0–2 children	56	3%	121	26%	786	37%	163	34%
Female, 3+ children	113	5%	195	41%	113	5%	196	41%
Missing	730	34%	43	9%				
Birth year								
Median, IQR	1985	1973-90	1954	1947-62	1985	1973–90	1954	1947–62
BMI (kg/m^2)								
Mean, SD	26.4	4.6	28.3	5.8	27.9	4.4	29.5	6.2
Missing (n, %)	0	0%	26	5%				
Education (n, %)								
Primary school	153	7%	228	48%	194	9%	229	48%
Advanced	1878	88%	243	51%	1943	91%	244	52%
Missing	106	5%	2	0%				
Mapuche surname(s) (n, %)								
No	2078	97%	445	94%	2078	97%	450	95%
Yes	59	3%	23	5%	59	3%	23	5%
Missing	0	0%	5	1%				
GBC family history (n, %)								
No	1850	87%	418	88%	2108	99%	425	90%
Yes	26	1%	46	10%	29	1%	48	10%
Missing	261	12%	9	2%				
rs17209837 genotype (n, %)								
Risk allele count (risk allele = Adenine)	3514	82%	811	86%	3516	82%	811	86%
Missing	2	0%	0	0%				
Mapuche ancestry (%)								
Mean, SD	32%	11%	39%	14%	32%	11%	39%	14%

Abbreviations: BMI, body mass index; IQR, interquartile range.

statistical analyses were embedded in the bootstrap 0.632+ algorithm with 10 000 iterations to control for overly optimistic discrimination and calibration estimates.[13] Data S3 describes in detail the Cox regression analyses performed, the discrimination and calibration estimation, and the computation of bootstrap 0.632+ estimates, as illustrated in Figure 1.

The development of GBC was treated as a time-to-event outcome in the Cox regression analyses, but the three prediction models were compared regarding their ability to predict GBC risk by age 70 years, and their predictive performance was determined by a time-invariant prognostic index that depends only on the estimated coefficients of the Cox model (details, including the formula for calculating the prognostic index, are provided in Data S3, "Discrimination performance measures"). Accordingly, the predictive performance was quantified as the change in the area under the receiver operating characteristic curve (ΔAUC-ROC) and in the area under the precision-recall curve (ΔAUC-PRC) with respect to the Baseline Model. ΔAUC-PRC was defined as the primary endpoint because the AUC-ROC has been shown to be less informative in imbalanced case-control settings such as the present scenario, where the ratio of GBC patients to controls was around 1:5.[14]

We also calculated the number of cholecystectomies needed (NCN) to prevent a GBC case at age 70 as the reciprocal of the cumulative GBC risk in the low and high GBC risk groups. The high-risk

group included Chileans with gallstones in the Baseline Model, and Chileans with gallstones and an estimated GBC risk above the median in Enhanced Model I and II. The calibration of the investigated prediction models was examined using the calibration slope, and a simple risk-scoring system was developed to facilitate translation of the present findings to future GBC prevention programs.[15,16] Further details on the statistical analyses performed can be found in Data S3.

2.4 | Sensitivity analyses

The probability of carrying gallstones in the control group at age 40 years was estimated using NHS echography data and individual genotypes for gallstone risk variants; these variants are also associated with GBC risk.[8] The analyses were also performed with random assignment of gallstone carrier status in controls, taking into account only the observed prevalence of gallstones at age 40 years (26.5% in Chilean women and 17.5% in Chilean men), to examine the potential overestimation of the hazard ratio for gallstones in the investigated Cox regression models due to the association of the genetic variants with both gallstone and GBC risk.

3 | RESULTS

3.1 | Characteristics of study participants and results of cox regression

Table 1 shows the main characteristics of the study participants before and after imputation of missing values, as well as projection to age 40 years of BMI, carriage of gallstones, and, in women, number of children. The agreement between artificially masked and multiply imputed values for the categorical variables education, GBC family history and Mapuche surname(s) was high, although most matches were due to chance (Table S9). Pearson correlation between masked and multiply imputed BMI values was 0.05, but the proportion of missing values for BMI was only 1% (n = 26 GBC patients). The mean age at GBC diagnosis was 60.2 years, and 76% of GBC patients were women.

Table 2 shows the results from the multiple two-step Cox regression analysis; univariate results can be found in Table S2. Carriers of gallstones at age 40 years showed a 5.74 times higher risk of GBC than non-carriers (95% confidence interval 5.17-6.37). Women with 0 to 2 children at age 40 years showed a 2.16 times higher GBC risk (95% CI 1.90-2.45), and the risk of GBC was 2.50 times higher (95% CI 2.23-2.81) for women with three or more children than for men. We found that GBC patients were born on average three decades earlier than population-based controls (1954 vs 1985), and thus included year of birth as a continuous explanatory variable in the investigated prediction models to account for the earlier age at diagnosis (and potentially higher hazard ratios) of Chileans born in later calendar years.

BMI at age 40 years was associated with a 3% increase in GBC risk per each kg/m^2 (95% CI 2%-4%), and education level, family history of GBC, and the rs17209837 AA genotype were also associated with GBC risk (*P*val <.001). Collinearity between the investigated risk factors was generally low (variance inflation factor ≤ 1.20; additional information is provided in Tables S10-11). The association between Mapuche surname(s) and GBC risk did not reach the 0.05 significance level after inclusion of Mapuche ancestry in the multiple Cox regression model (Table 2), but the hazard ratio for Mapuche surname/s in Enhanced Model I was 2.65 (95% CI 2.10-3.34). Figure 2 shows the regional distribution of risk factors included in the investigated prediction models after projection to age 40 years, along with regional GBC mortality rates.

TABLE 2 Results from multiple Cox regression after imputation of missing values, as well as projection to age 40 years of BMI, carriage of gallstones, and, in women, number of children.

	HR[a] (95% CI)	*P*val
Gallstones		
No	Ref.	9 10^{-28}
Yes	**5.74** (5.17–6.37)	
Sex and number of children		
Male	Ref.	10^{-9}
Female, 0–2 children	**2.16** (1.90-2.45)	
Female, 3+ children	**2.50** (2.23–2.81)	
Birth year (Per year)	**1.09** (1.09-1.10)	2 10^{-81}
BMI	**1.03** (1.02-1.04)	.0004
Education		
Primary school	**1.41** (1.25-1.59)	4 10^{-5}
Advanced	Ref.	
Mapuche surname(s)		
No	Ref.	.27
Yes	0.85 (0.65-1.11)	
GBC family history		
No	Ref.	.001
Yes	**1.39** (1.18-1.63)	
rs17209837 genotype (risk allele = Adenine)	**1.42** (1.22-1.65)	3 10^{-5}
Mapuche ancestry (Per 1%)	**1.04** (1.03-1.04)	7 10^{-15}

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard-ratio; *P*val, global probability value; Ref., Reference category.
[a]Survival analysis was carried out with the Mendel software, which allowed the integration of the 2012 GBC incidences from Chile as the baseline hazard function.[11,12] Bold type denotes associated 95% confidence intervals that do not include 1.

3.2 | Prediction ability and calibration of investigated GBC risk prediction models

Table 3 shows the bootstrap 0.632+ estimates for the prediction ability and calibration of the investigated prediction models. The Baseline Model showed an AUC-PRC of 0.44, an AUC-ROC of 0.79 and was

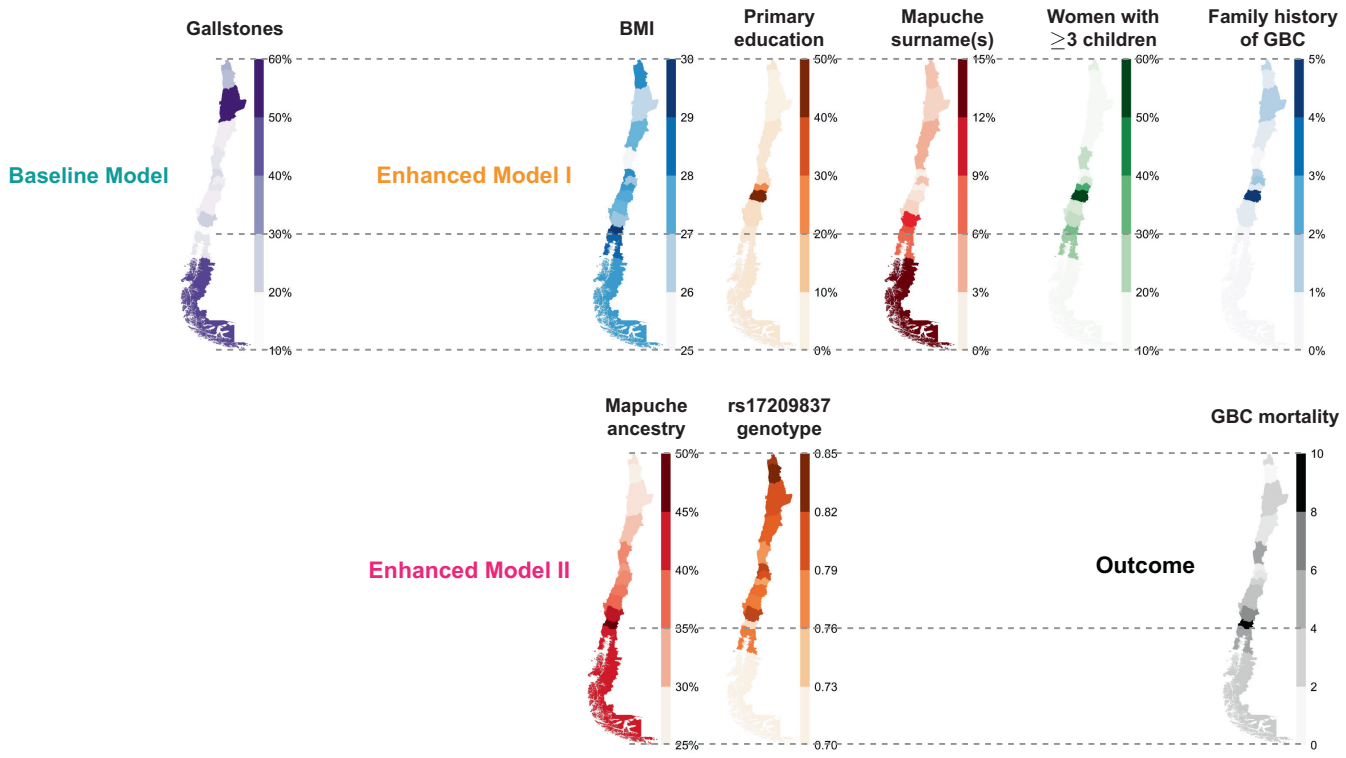

FIGURE 2 Chilean maps with regional distributions of investigated non-genetic and genetic risk factors, and regional age-standardized mortality rates due to gallbladder cancer. Gallstones: Proportion of population-based controls with gallstones projected to age 40 years; BMI: body mass index of population-based controls projected to age 40 years; Education: proportion of population-based controls who completed primary school projected to age 40 years; Mapuche surname(s): proportion of population-based controls with one or two Mapuche surnames; Number of children: proportion of female population-based controls with three or more children projected to age 40 years; Family history of GBC: proportion of population-based controls with family history of GBC projected to age 40 years; Mapuche ancestry (%): average Mapuche ancestry proportion of population-based controls; rs17209837 genotype: risk allele frequency in population-based controls (risk allele = Adenine); GBC mortality: age-standardized mortality rates due to gallbladder cancer in Chile in 2014 (population-based controls recruited between 2010 and 2018).

TABLE 3 Discrimination ability and calibration of the assessed GBC risk prediction models.

GBC risk prediction model	ΔAUC-PRC[a] (95% CI)	ΔAUC-ROC[a] (95% CI)	Calibration slope[a] (95% CI)
Baseline model	0.44 (0.42-0.46)	0.79 (0.77-0.81)	1.15 (0.98, 1.27)
Gallstones + Sex + Birth year	Ref.	Ref.	Ref.
Enhanced model I: Baseline model + BMI + Education + Mapuche surname(s) + Number of children + Family history of GBC	+0.22 (0.15-0.29)	+0.06 (0.04-0.09)	1.04 (0.92, 1.23)
Enhanced Model II: Enhanced Model I + Mapuche ancestry (%) + rs17209837 genotype	+0.25 (0.20-0.30)	+0.08 (0.06-0.10)	1.02 (0.93, 1.19)

Abbreviations: ΔAUC-PRC, area under the precision-recall curve for the Baseline Model (reference) or AUC-PRC improvement for the Enhanced Models compared with the Baseline Model; ΔAUC-ROC, area under the receiver operating characteristic curve for the Baseline Model (reference) or AUC-ROC improvement for the Enhanced Models compared with the Baseline Model; BMI, body mass index; CI, confidence interval; Ref., reference.
[a]Results are based on Cox regression models stratified by region into two categories (high and low GBC mortality rates). Bootstrap 0.632+ estimates rely on 10 000 bootstrap iterations times 15 multiple imputations.

TABLE 4 Estimated cumulative risk of GBC by age 70 years and number of cholecystectomies needed to prevent one case of GBC in the general Chilean population, and according to the assessed GBC risk prediction models.

GBC risk prediction model	Group	Risk category	HR	(95% CI)	Cumulative GBC risk by age 70 years[a] %	(95% CI)	NCN	(95% CI)
General Chilean population	All	All			0.42		239	
		Women			0.57		176	
		Men			0.26		385	
Baseline model: Gallstones + Sex	All	With gallstones	**5.93**	(4.64-7.35)	1.02	(0.90-1.14)	115	(104–131)
		Without gallstones	Ref.		**0.17**	(0.15-0.20)	682	(603-760)
Enhanced model I: Baseline model + BMI + Education + Mapuche surname(s) + Number of children + Family history of GBC	With gallstones	50% at highest risk	**1.58**	(1.04-2.98)	1.29	(0.92-1.97)	92	(60-128)
		50% at lowest risk	Ref.		0.81	(0.52-1.12)	145	(106-225)
Enhanced model II: Enhanced Model I + Mapuche ancestry (%) + rs17209837 genotype	With gallstones	50% at highest risk	**2.09**	(1.41-3.14)	**1.48**	(1.07-2.02)	80	(59–110)
		50% at lowest risk	Ref.		**0.71**	(0.51-0.96)	167	(123-321)

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; NCN, number of cholecystectomies needed to prevent one case of GBC.
[a]Results are based on Cox regression models stratified by region into two categories (high and low GBC mortality rates). Bootstrap 0.632+ estimates rely on 10 000 bootstrap iterations times 15 multiple imputations.

well calibrated (the 95% CI of the calibration slope included 1.00). Compared with the Baseline Model, inclusion of gallstone risk factors in the current GES prevention program plus GBC family history (Enhanced Model I) improved the prediction ability (ΔAUC-PRC +0.22; 95% CI 0.15-0.29). Further inclusion of the proportion of Mapuche ancestry and rs17209837 genotype as genetic risk factors (Enhanced Model II) led to an additional improvement in predictive performance (ΔAUC-PRC, +0.25; 95% CI 0.20-0.30). Similar conclusions were drawn on the basis of AUC-ROC, and the enhanced prediction models also showed calibration slopes close to 1.

The prediction models were also compared in terms of their ability to discriminate between low- and high-risk individuals (Table 4). According to GLOBOCAN, the cumulative risk of GBC by age 70 years in Chileans in 2020 was 0.42%, translating into an NCN of 239.[17] The cumulative risk of GBC by age 70 years was two times higher for women (0.57%) than for men (0.26), resulting in NCN = 176 for women and NCN = 385 for men.

Relying on the investigated prediction models, the risk of GBC was 5.93 higher in individuals with gallstones at age 40 years than in those without gallstones. The cumulative risk of GBC by age 70 was 1.02% and the NCN was 115 for individuals with gallstones at age 40. The NCN was 92 for individuals with gallstones at age 40 years with a higher than average GBC risk according to Enhanced Model I, and NCN = 80 according to Enhanced Model II.

The estimated hazard ratios were transformed into GBC risk scores to facilitate translation of the study results. For example, the total GBC risk score for a woman aged 40 years with three children (1 point), gallstones (8 points) and a BMI of 30 kg/m^2 (2 points), advanced education (0 points), family history of GBC (1 point), Mapuche ancestry proportion of 35% (4 points), and rs17209837 genotype "AA" (3 points) amounts to 19, which corresponds to an estimated cumulative GBC risk by age 70 years of 1.23%. Further details on the proposed risk scores and a script to facilitate their calculation can be found in Tables S3-S5 and SourceCode S1.

3.3 | Results from sensitivity analyses

As expected, random assignment of gallstone carrier status in controls without considering individual genotypes for gallstone risk variants led to a lower GBC risk estimate for gallstones at age 40 years (HR 4.47, 95% CI 3.77-5.31; Table S6). The risk estimates for the other investigated factors were affected only slightly and, compared with the results of the main analyses, the ΔAUC-PRC for Enhanced Models I-II decreased by two units (Table S7). Cumulative risk and NCN estimates were more sensitive to random assignment of gallstones (Table S8). The lower HR for gallstones translated into a higher NCN for individuals with gallstones in the Baseline Model (129, 95% CI 113-142), and a greater reduction in NCN for individuals with gallstones and higher-than-average GBC risk according to Enhanced Model I (98, 95% CI 67-135) and Enhanced Model II (90, 95% CI 67-121).

4 | DISCUSSION

Unlike other hepatobiliary tumors, individuals at high risk of GBC may be offered prophylactic cholecystectomy to prevent this aggressive

disease. Faced with one of the highest mortality rates worldwide, especially among women, 17 years ago the government of Chile initiated the GES prevention program, which subsidizes gallbladder removal in gallstone patients aged 35 to 49 years.[2] Since most gallstones are asymptomatic, the GES prevention program recommends that additional risk factors are considered to help identify silent gallstones that may lead to the development of GBC. In practice, however, the current implementation of the GES prevention program focuses solely on the clinical manifestation of gallstone disease and does not consider other risk factors in making decisions on financial assistance.[9] 84% of the gallstones found in abdominal echography in Chile are asymptomatic, and the overload of surgery services translates into only 60% of gallstone carriers undergoing cholecystectomy, with significant regional and socioeconomic disparities in access to the current GBC prevention program.[3] We assessed the predictive performance of established and novel GBC risk factors in the largest retrospective GBC case-control study in Chile to date: in persons aged 40 years, the potential GBC development over the subsequent 30 years was considered by integrating Chilean GBC incidence rates as the baseline hazard in Cox regression. A GBC risk model that considered only presence of gallstones at age 40, sex and year of birth resulted in 115 cholecystectomies needed to prevent one case of GBC. Taking into account all risk factors included in the current GES prevention program plus family history of GBC increased the predictive performance and reduced the NCN to 92. The additional consideration of two established genetic factors for GBC risk (individual proportion of Mapuche ancestry and individual rs17209837 genotype) further decreased the NCN to 80.

The gallstone carrier status of Chilean controls in the main analyses was assigned on the basis of two gallstone risk variants. Both of these variants have recently been validated for the Chilean population and are also associated with GBC risk, suggesting a genetic link between gallstone disease and GBC in Chileans.[8] This motivated the sensitivity analyses performed with random assignment of gallstone status, which resulted in a HR of subsequent GBC of 4.47 for gallstones at age 40, in contrast to HR = 5.74 for gallstone assignment based on gallstone risk variants. However, this had only a minor effect on the comparison of the investigated models in terms of predictive performance and NCN improvement. Please also note the common background of assigning gallstone carrier status based on gallstone risk variants in the present study and mendelian randomization, which utilizes genetic variants robustly associated with an exposure of interest (gallstones in this study) to assess the causal effect of the exposure on a particular phenotype (GBC development in this study). Assuming a gallstone prevalence of 22% at age 40 years, the two variants considered in this study explained a variance in gallstone susceptibility of 5%, translating into 80% statistical power to detect a true causal OR of GBC higher than 1.7 (type I error rate of 5%), which was substantially lower than the estimated HR.[18]

An important limitation of the present study was the quality of the available data. Missing values were imputed, and values for the age-related risk factors BMI, gallstones and, for women, number of children were projected to age 40 years, introducing uncertainty in subsequent statistical analyses. We examined the accuracy of MI and conducted sensitivity analyses to evaluate the impact of the assignment of gallstone carrier status in controls, which was the greatest source of uncertainty. In this context, it is also important to consider the practical implications of gallstone screening in populations with a high incidence of GBC, including the representativeness of healthy controls consenting to abdominal echography, and the ethical and financial issues related to the clinical management of gallstone carriers (additional pressure on the health care system, adequacy of prophylactic cholecystectomy taking into account the current weak scientific evidence that we attempted to consolidate in the present study). Another limitation of this study is the lack of an independent cohort for external validation of the prediction performance measures and the scoring system developed. The potential bias towards overfitting was addressed by an extended internal validation, but large collaborative studies are urgently needed to collect high-quality information in large numbers of GBC and gallstone patients to optimize GBC prevention in high-incidence regions.

To our knowledge, the NCN has previously been estimated in African Americans, Swedes and Northern Native Americans with gallstone disease aged 40 years and older.[19] The NCN was defined as the reciprocal of the estimated percentage of gallstone patients who developed GBC in the subsequent 20 years—we considered a 30-year period and used the reciprocal of the cumulative GBC risk at age 70 years. The lowest NCNs were estimated for Northern Native Americans (females: NCN 67, 95% CI 57-76; males: NCN 106, 95% CI 79-141). In comparison, our NCN estimates were similar for genetically admixed Chilean women with gallstones at age 40 years (NCN 72, 95% CI 65-82; data not shown) and approximately 50% higher for Chilean men (NCN 158, 95% CI 142-179; data not shown).

Despite its challenges, primary GBC prevention by prophylactic cholecystectomy has good potential for persons at high risk of GBC. Most GBC patients are diagnosed incidentally after cholecystectomy for treatment of symptomatic gallstones.[20] If the incidental tumor is still at an early stage, patient survival rates are much higher for non-incidental GBC.[21] However, if the tumor is advanced or lymph nodes are involved, low survival rates have been reported. The current GES prevention program could be improved by considering a GBC risk scoring system. This would enable simple but more accurate estimation of individual GBC risk, and definition of finer GBC risk groups to prioritize prophylactic cholecystectomy. To illustrate this possibility, we developed two risk-scoring systems. The first considered only non-genetic risk factors. The second also included genetic risk factors: it replaced "Mapuche surname(s)" with the estimated proportion of Mapuche ancestry, in addition to the individual rs17209837 genotype. The second scoring system enables more accurate prediction of GBC risk and reduces by 12 the number of cholecystectomies needed to prevent one GBC case, but is associated with costs and infrastructure needed for individual genotyping. Although the cost of genotyping has dropped rapidly in recent years—currently around USD 40 per sample including DNA extraction and genome-wide genotyping—dedicated cost-benefit analyses will be needed in the future to evaluate the benefit of considering genetic risk factors.

Our findings suggest that the current implementation of the GES prevention program, which relies exclusively on symptomatic gallstones, is suboptimal. Gallstones are a key GBC risk factor in Chileans, but the performance of GBC risk prediction can be improved significantly by considering both established and newly identified GBC risk factors. In particular, consideration of non-genetic factors and individual genotype data may lead to an important reduction in the number of unnecessary cholecystectomies, while simultaneously predicting GBC development with high sensitivity.

AUTHOR CONTRIBUTIONS

Conceptualization: Lorenzo Bermejo, Boekstegers. Resources: Lorenzo Bermejo, Scherer, Barahona Ponce, Gárate-Calderón, Marcelain, Waldenberger, Morales, Munoz, Rojas, Retamales, de Toro, Barajas, Teresa Rivera, Cortés, Loader, Saavedra, Gutiérrez, Ortega, Enriqueta Bertrán, Bartolotti, Gabler, Campos, Alvarado, Moisán, Spencer, Nervi, Carvajal-Hausdorf, Losada, Almau, Fernández, Olloquequi, Fuentes-Guajardo, Gonzalez-Jose, Cátira Bortolini, Acuña-Alonzo, Gallo, Ruiz Linares, Rothhammer. Investigation: Waldenberger. Data curation: Boekstegers, Waldenberger. Methodology: Lorenzo Bermejo, Boekstegers. Validation: Lorenzo Bermejo, Boekstegers. Formal Analysis: Boekstegers. Writing-original draft: Boekstegers, Lorenzo Bermejo. Writing-review and editing: Boekstegers, Lorenzo Bermejo, Scherer, Barahona Ponce, Gárate-Calderón, Marcelain, Waldenberger, Morales, Munoz, Rojas, Retamales, de Toro, Barajas, Teresa Rivera, Cortés, Loader, Saavedra, Gutiérrez, Ortega, Enriqueta Bertrán, Bartolotti, Gabler, Campos, Alvarado, Moisán, Spencer, Nervi, Carvajal-Hausdorf, Losada, Almau, Fernández, Olloquequi, Fuentes-Guajardo, Gonzalez-Jose, Cátira Bortolini, Acuña-Alonzo, Gallo, Ruiz Linares, Rothhammer. Visualization: Boekstegers. Supervision, Lorenzo Bermejo. Project administration: Barahona Ponce, Gárate-Calderón, Scherer. Funding acquisition: Lorenzo Bermejo, Scherer. All authors have read and agreed to the published version of the manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. To gain access, data requestors will need to sign a data access agreement.

ETHICS STATEMENT

The study protocol for the recruitment of Chilean GBC patients conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committees of Servicio de Salud Metropolitano Oriente, Santiago de Chile (#06.10.2015, #08.03.2016 & #12.11.2019), Servicio de Salud Metropolitano Sur Oriente, Santiago de Chile (#15.10.2015 & #05.04.2018), Servicio de Salud Metropolitano Central, Santiago de Chile (#1188-2015), Servicio de Salud Coquimbo, Coquimbo, Chile (#01.04.2016), Servicio de Salud Maule, Talca, Chile (#05.11.2015), Universidad Católica del Maule, Talca, Chile (#102-2020), Ser-vicio de Salud Concepción, Concepción, Chile (ID: 16-11-97 & ID:19-12-111), Servicio de Salud Araucanía Sur, Temuco, Chile (#10.02.2020), Servicio de Salud Valdivia, Valdivia, Chile (ID:438), Centro de Bioética, Universidad del Desarrollo, Clínica Alemana de Santiago, Santiago de Chile (#2018-97, ID 678) and Unidad de Investigación Hospital San Juan de Dios, Santiago de Chile (#6182), the Medical Faculties of Universidad de Chile (approval #123-2012 & #11.10.2012) and Pontificia Universidad Católica de Chile (#11-159). Regarding the controls, ethics approvals for the CANDELA subjects were obtained from the Universidad de Tarapacá and University College London, and for the COPD study from the Ethics Committees of Maulean Health Service and Universidad Autónoma de Chile. Written informed consent was obtained from all subjects involved in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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