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BRIEF ARTICLE

# Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women

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## Abstract

**AIM:** To determine the effects of genetic variants associated with gallstone formation and capsaicin (a pungent component of chili pepper) metabolism on the risk of gallbladder cancer (GBC).

**METHODS:** A total of 57 patients with GBC, 119 patients with gallstones, and 70 controls were enrolled in this study. DNA was extracted from their blood or paraffin block sample using standard commercial kits. The statuses of the genetic variants were assayed using Taqman® SNP Genotyping Assays or Custom Taqman® SNP Genotyping Assays.

**RESULTS:** The non-ancestral T/T genotype of apolipoprotein B rs693 polymorphism was associated with a decreased risk of GBC (OR: 0.14, 95% CI: 0.03-0.63). The T/T genotype of cholesteryl ester transfer protein (CETP) rs708272 polymorphism was associated with an increased risk of GBC (OR: 5.04, 95% CI: 1.43-17.8).

**CONCLUSION:** Genetic variants involved in gallstone formation such as the apolipoprotein B rs693 and CETP rs708272 polymorphisms may be related to the risk of developing GBC in Chilean women.



**Key words:** Genetic risk factor; Gallbladder cancer; Gallstone; Genetic polymorphism; Apolipoprotein B; Cholesteryl ester transfer protein

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## INTRODUCTION

There is a prominent worldwide geographical and racial variability in the incidence of gallbladder cancer (GBC), which correlates with the prevalence of cholelithiasis[1]. High incidences of GBC are observed in specific countries and in confined areas. For example, the incidence of GBC is very high in northern Indian cities (5-7 per 100 000 women) and low (0-0.7 per 100 000 women) in southern India, possibly reflecting the different ethnic origins of these populations[2]. The evidence suggests that the incidence of GBC is associated with the presence of both geographically-specific environmental factors and environmental factor-related genetic factors.

Recent studies have shown that the incidence rate for GBC is higher in Chile than in other countries[3-5]. According to a previous epidemiological study, the consumption of high levels of red chili pepper has been identified as an important risk factor for GBC in Chilean women who carry gallstones (GS)[6]. However, the pathogenic mechanism by which GBC occurs *via* chili pepper consumption in the presence of GS remains uncertain.

Although GS is the main cause of GBC, not all patients with GS develop GBC. While the standard mortality rates for GBC between 1985 and 2002 remained unchanged at 11.3 per 100 000 (0.0113%) in Chile[7], 38.8% of adult women and 14.9% of adult men, which are staggeringly high rates, were GS carriers between 1972 and 1995[8]. Red chili pepper is a widely consumed spice among the Chilean population. Therefore, the development of GBC in Chilean women cannot be completely explained by the presence of GS and chili pepper consumption alone.

In some but not all studies, lipid metabolism-related gene variants have been associated with GS formation. Apolipoprotein (apo) B, apo E, and cholesteryl ester transfer protein (CETP) polymorphisms have been associated with increased risk for cholelithiasis[9-11]. On the other hand, capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active ingredient that makes chili peppers pungent. Although previous findings regarding the potential genotoxicity of capsaicin are inconsistent[12-15], it is possible that GBC can be caused by high consumption of red chili pepper that contains capsaicin. Therefore, genes involved in the metabolism of capsaicin, such as cytochrome P450 (CYP) 2E1, CYP2C9, and CYP3A4, may be related to increased risk for GBC[16-18]. However, no study has examined the association between the risk of GBC in Chile and the genetic variants involved in GS formation and capsaicin metabolism.

We hypothesized that individuals with a genotype promoting greater lipid metabolism/capsaicin metabolism would be more prevalent among the GS and GBC patients than among healthy subjects. We conducted a hospital-based case-control study in a Chilean population with special reference to polymorphism-polymorphism combination.

## MATERIALS AND METHODS

### Study subjects

A total of 57 female patients with GBC who had been diagnosed by histological examination of tissue at Sótero del Río Hospital in Santiago, Chile, between January 2007 and February 2008 were enrolled in this case-control study. A total of 119 female patients with GS who were diagnosed by an ultrasonic diagnostic method were also enrolled in the study. Seventy controls, who were patients with hernia or varicose veins of the legs who had no history of GS or any cancer and who were diagnosed by an ultrasonic diagnostic method, were selected randomly at the same hospital over the same period.

All patients gave their written informed consent, and our study protocol was approved by the Ethics Committee at Sótero del Río Hospital.

### DNA extraction and storage

Samples collected in the hospital were sent to Niigata University, Japan, for DNA extraction and genotyping assay. Genomic DNA was extracted from the blood or paraffin block samples using standard commercial kits for blood samples (DNA Extractor WB-rapid, WAKO Pure Chemicals Industries, Ltd., Osaka, Japan) and for paraffin block samples (Dexpat, Takara Bio Co. Ltd., Tokyo, Japan). The extracted DNA samples were stored in a freezer at -80℃ until genetic polymorphism analyses were performed.

### Genotyping assay

The statuses of the genetic variants of apo B rs693, apo E rs7412, rs429358, CETP rs708272, CYP2C9 rs1057910, CYP2C9 rs1799853, and CYP3A4 rs12721627 were assayed using TaqMan® SNP Genotyping Assays purchased from Applied Biosystems (Foster City, CA, USA). The assay IDs were C_7615420_20 for rs693, C_904973_10 for rs7412, C_3084793_20 for rs429358, C_9615318_10 for rs708272, C_27104892_10 for rs1057910, C_25625805_10 for rs1799853, and C_30634207_10 for rs12721627. Genotyping for the presence of CYP2E1 polymorphisms (rs2031920, rs6413432) was performed using the Custom Taqman® SNP Genotyping Assays purchased from Applied Biosystems. The reaction components for a single 10 μL reaction (using a 96-well plate) included sample genomic DNA, TaqMan® Genotyping Master Mix (Applied Biosystems), SNP Genotyping Assay-Mix (Applied Biosystems), DNase free water was used. The thermal cycler (PE 9700, Applied Biosystems) conditions were as follows: 95℃ for 10 min, followed by 40 cycles of 92℃ for 15 s and 60℃ for 1 min. After the PCR reaction, the plate read-out and allele discrimination were analyzed using a multiplex real-time QPCR system (Mx3000P, Stratagene Japan, Tokyo).

For quality control, all genotyping assays were reconfirmed according to the PCR-restriction fragment length polymorphism method and the replicates were 100% concordant.

**Table 1 Demographic characteristics and food intake frequency of study subjects (mean ± SD)**

| | Controls *n* = 70 | GS patients *n* = 119 | GBC patients *n* = 57 | *P* value | | |
|---|---|---|---|---|---|---|
| | | | | Control *vs* GS | GS *vs* GBC | Control *vs* GBC |
| Age (yr) | 45.8 ± 14.1 | 42.7 ± 9.2 | 56.5 ± 11.2 | NS | $P < 0.001$ | $P < 0.001$ |
| Height (m) | 1.56 ± 0.07 | 1.57 ± 0.07 | 1.54 ± 0.06 | NS | NS | NS |
| Weight (kg) | 72.1 ± 15.5 | 69.3 ± 12.8 | 64.1 ± 12.8 | NS | NS | NS |
| BMI (kg/m$^2$) | 29.5 ± 6.1 | 28.2 ± 4.8 | 27.3 ± 5.6 | NS | NS | NS |
| Chili pepper | 0.8 ± 1.0 | 0.9 ± 1.1 | 1.7 ± 1.4 | NS | $P < 0.05$ | $P < 0.01$ |
| Beef | 1.2 ± 0.6 | 1.5 ± 0.7 | 1.8 ± 0.5 | NS | NS | $P < 0.01$ |
| Pork | 0.8 ± 0.6 | 1.0 ± 0.8 | 1.4 ± 0.8 | NS | $P < 0.05$ | $P < 0.01$ |
| Chicken | 1.6 ± 0.6 | 2.0 ± 0.8 | 2.0 ± 0.5 | $P < 0.01$ | NS | NS |
| Fish | 1.0 ± 0.6 | 1.1 ± 0.6 | 1.5 ± 0.7 | NS | $P < 0.05$ | $P < 0.01$ |
| Fried food | 1.0 ± 0.9 | 1.3 ± 1.1 | 2.2 ± 1.1 | NS | $P < 0.001$ | $P < 0.001$ |
| Butter | 2.3 ± 1.5 | 2.6 ± 1.6 | 3.2 ± 1.1 | NS | NS | $P < 0.05$ |
| Cheese | 1.1 ± 1.0 | 1.1 ± 0.9 | 2.0 ± 1.0 | NS | $P < 0.001$ | $P < 0.001$ |

Food intake was grouped into the following 5 categories, and a sequence number was assigned to each category: (0) never; (1) 1-3 times/mo or less; (2) 1-3 times/wk; (3) 4-6 times/wk; and (4) everyday. Data on height, weight, and food intake was collected from 70 controls, 119 GS patients, and 26 GBC patients. Data were evaluated by one-way analysis of variance (ANOVA) followed by Scheffe's multiple comparison test. GS: Gallstone; GBC: Gallbladder cancer; BMI: Body mass index; NS: No significant difference.

### Measurement of dietary intake

An interviewer-administrated food-frequency questionnaire was used in the present study. Our subjects were asked to report the frequencies of their long-term intake of red chili pepper, vegetables, fruits, beef, pork, chicken, fish, milk, butter, cheese, and fried foods. Food intake was grouped into the following 5 categories, and a sequence number was assigned to each category: (0) never; (1) 1-3 times/mo or less; (2) 1-3 times/wk; (3) 4-6 times/wk; and (4) every day. Each dietary intake assessment was made according to the score obtained from each subject.

### Statistical evaluation

Statistical analyses were performed using SAS software (Release 6.12, SAS Institute Inc., Cary, NC, USA) and STATA software (SE 8.0, STATA Corporation, TX, USA). Fisher's exact probability test was used to assess the association between the genotypes or alleles and GBC risk. The age-adjusted odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated from logistic regression coefficients. Data on demographic characteristics and food intake frequencies were evaluated by one-way analysis of variance (ANOVA) followed by Scheffe's multiple comparison test. *P* values of less than 0.05 were considered to indicate statistical significance. The genotypic distribution of the polymorphisms in the controls was compared with that expected based on Hardy-Weinberg equilibrium (HWE) by the $\chi^2$ (Pearson) test. When the *P* values exceeded 0.05, we estimated that the sample was under the HWE.

## RESULTS

Table 1 shows the demographic characteristics and food intake frequencies of our subjects. The GBC patients had the highest mean age among the three groups, showing significant differences from the other two groups. No significant differences were found in the height, weight, or body mass index (BMI) among the three groups.

Significantly higher consumption of red chili pepper was observed in the GBC patients compared with the controls ($P < 0.01$) and the GS patients ($P < 0.05$). This difference in the consumption of red chili pepper between the GBC and GS patients was in agreement with the result of a previous study[6]. In the GBC patients, the consumption of pork, fish, fried foods, and cheese were significantly higher than in the controls and the GS patients. Overall, higher consumption of the foods we asked about was observed in the GBC patients.

Table 2 shows the GBC risk associated with the apo B rs693 polymorphism. The genotype distributions were consistent with HWE in the controls ($P = 0.44$). The frequency of the T/T genotype was significantly lower in the GBC patients than in the controls; the age-adjusted OR for the GBC risk was 0.14 (95% CI: 0.03-0.63, $P = 0.0099$). The T allele was associated with a decreased risk of GBC (OR: 0.49, 95% CI: 0.29-0.84, $P = 0.010$). Based on these results, we designated the C allele that is presumed to increase the risk of GBC as the "at-risk" allele. No significant differences in the genotypic and allelic distribution were detected between the controls and the GS patients.

Table 3 shows the risk of GBC associated with the CETP rs708272 polymorphism. In the GBC patients, the frequencies of the C/C, C/T and T/T genotypes were 28.1%, 40.3% and 31.6%, respectively. The frequencies of the C/C, C/T, and T/T genotypes in the controls were 25.7%, 57.2% and 17.1%, respectively, and in the patients with GS they were 35.3%, 47.9% and 16.8%, respectively. The distribution of the genotypes of the rs708272 polymorphism agreed with HWE in the controls ($P = 0.20$). The frequency of the T/T genotype was significantly higher in the GBC patients than in the GS patients ($P = 0.012$), though no significant differences were found between the controls and the GS patients, or between the controls and the GBC patients. Based on these results, we designated the T allele that is presumed to increase the risk of GBC as the "at-risk" allele.

**Table 2 Association of the apo B rs693 polymorphism with gallbladder cancer risk**

| Genotypes and alleles | Frequency (%) | | Age-adjusted OR | 95% CI | *P* value |
|---|---|---|---|---|---|
| | Controls | GS patients | | | |
| C/C | 25 (35.7) | 41 (34.5) | 1.0 | | |
| C/T | 31 (44.3) | 65 (54.6) | 1.27 | 0.66-2.47 | NS |
| T/T | 14 (20.0) | 13 (10.9) | 0.59 | 0.24-1.45 | NS |
| C | 81 (57.9) | 147 (61.8) | 1.0 | | |
| T | 59 (42.1) | 91 (38.2) | 0.85 | 0.56-1.30 | NS |
| | $P_{HWE}$ = 0.442 | $P_{HWE}$ = 0.088 | | | |
| | Controls | GBC patients | | | |
| C/C | 25 (35.7) | 30 (52.6) | 1.0 | | |
| C/T | 31 (44.3) | 24 (42.1) | 0.67 | 0.30-1.51 | NS |
| T/T | 14 (20.0) | 3 ( 5.3) | 0.14 | 0.03-0.63 | 0.010 |
| C | 81 (57.9) | 84 (73.7) | 1.0 | | |
| T | 59 (42.1) | 30 (26.3) | 0.49 | 0.29-0.84 | 0.010 |
| | | $P_{HWE}$ = 0.516 | | | |
| | GS patients | GBC patients | | | |
| C/C | 41 (34.5) | 30 (52.6) | 1.0 | | |
| C/T | 65 (54.6) | 24 (42.1) | 0.70 | 0.31-1.59 | NS |
| T/T | 13 (10.9) | 3 ( 5.3) | 0.14 | 0.02-1.05 | NS |
| C | 147 (61.8) | 84 (73.7) | 1.0 | | |
| T | 91 (38.2) | 30 (26.3) | 0.58 | 0.35-0.94 | 0.028 |

NS: No significant difference; $P_{HWE}$: *P* for Hardy-Weinberg equilibrium test.

**Table 3 Association of the CETP rs708272 polymorphism with gallbladder cancer risk**

| Genotypes and alleles | Frequency (%) | | Age-adjusted OR | 95% CI | *P* value |
|---|---|---|---|---|---|
| | Controls | GS patients | | | |
| C/C | 18 (25.7) | 42 (35.3) | 1.0 | | |
| C/T | 40 (57.2) | 57 (47.9) | 0.60 | 0.30-1.19 | NS |
| T/T | 12 (17.1) | 20 (16.8) | 0.68 | 0.27-1.70 | NS |
| C | 76 (54.3) | 141 (59.2) | 1.0 | | |
| T | 64 (45.7) | 97 (40.8) | 0.82 | 0.54-1.24 | NS |
| | $P_{HWE}$ = 0.204 | $P_{HWE}$ = 0.930 | | | |
| | Controls | GBC patients | | | |
| C/C | 18 (25.7) | 16 (28.1) | 1.0 | | |
| C/T | 40 (57.2) | 23 (40.3) | 0.75 | 0.30-1.93 | NS |
| T/T | 12 (17.1) | 18 (31.6) | 1.80 | 0.62-5.26 | NS |
| C | 76 (54.3) | 55 (48.2) | 1.0 | | |
| T | 64 (45.7) | 59 (51.8) | 1.27 | 0.78-2.09 | NS |
| | | $P_{HWE}$ = 0.147 | | | |
| | GS patients | GBC patients | | | |
| C/C | 42 (35.3) | 16 (28.1) | 1.0 | | |
| C/T | 57 (47.9) | 23 (40.3) | 1.31 | 0.52-3.28 | NS |
| T/T | 20 (16.8) | 18 (31.6) | 5.04 | 1.43-17.8 | 0.012 |
| C | 141 (59.2) | 55 (48.2) | 1.0 | | |
| T | 97 (40.8) | 59 (51.8) | 1.56 | 0.99-2.44 | NS |

CETP: Cholesteryl ester transfer protein.

The apo E and capsaicin metabolism-related gene variants were not associated with either GBC or cholelithiasis risk.

Table 4 shows the risk of GBC associated with the combined "at-risk" genotypes of the apo B rs693 and CETP rs708272 polymorphisms. The frequencies of the combined "at-risk" genotypes of the C/C genotype of the apo B rs693 polymorphism and the T/T genotype of the CETP rs708272 polymorphism were 4.3% in the controls, 6.7% in the GS patients, and 19.3% in the GBC patients. Compared with all remaining combinations combined, the frequency of the C/C plus T/T genotypes was significantly higher in the GBC patients than in the controls; the age-adjusted OR for the GBC risk was 4.75 (95% CI: 1.16 -19.4, $P = 0.030$).

## DISCUSSION

In this hospital-based case-control study, the T allele carriers of the apo B rs693 polymorphism were associated with a decreased risk of GBC. In contrast, the T/T genotype of the CETP rs708272 polymorphism was associated with an increased risk of GBC. However, the capsaicin metabolism-related gene variants were not associated with GBC risk.

Singh *et al*[19] found that the frequency of the C allele of the apoB rs693 polymorphism was significantly higher in GBC patients than in GS patients or healthy subjects. Their data also showed that the C/T and T/T genotypes are associated with a lower risk for GBC compared with the C/C genotype (ORs: 0.28 and 0.34, 95% CI: 0.17-0.46, and 0.13-0.89, respectively). They suggested that the apo B rs693 polymorphism confers susceptibility to GBC under specific environmental conditions. Our results were in agreement with their findings, showing an association between the T allele and the lower risk of GBC. Since apo B is a key protein in lipid metabolism[20], the apo B variant may be related to a higher incidence of GS and subsequently GBC. Generally, the T/T genotype has been reported to have significantly higher serum total cholesterol, low density lipoprotein cholesterol (LDL), and apo B levels compared with the C/C genotype[21,22]. Therefore the T/T genotype or the T allele appears to relate to a higher risk of GS or GBC. However, our findings showed that the T allele may work as a preventive factor for GBC. The inverse association between the T/T genotype of the apo B polymorphism and the GBC risk may be explained by structural changes of apo B as proposed by Boekholdt *et al*[23]. Their hypothesis about the inverse association between the T/T genotype and ischemic heart disease (IHD) is as follows: the apo B variant causes hypercholesterolemia, modifies LDL to become a less atherogenic particle, and causes IHD. We could not clarify by what mechanism the T allele decreases the risk of GBC, and the mechanism research will be the topic of our next trial.

Some genetic variants may exert population-specific effects that are independent of the other genetic profiles of the individual and of environmental exposures, while other population-specific effects may be generated under differential gene-gene interactions in different populations, differential gene-environment interactions, or both[24].

As reported by some researchers, GS which is a main risk factor for GBC, has been associated with both decreased levels of high-density lipoprotein (HDL) cholesterol and increased levels of LDL cholesterol and triglyceride[25-27]. CETP has a central role in the metabolism of HDL and therefore might relate to the susceptibility to

**Table 4 Effects of the combined genotypes of the apo B rs693 and CETP rs708272 polymorphisms on the risk of gallbladder cancer**

| Genotypes apo B-CETP | Frequency (%) | | OR | 95% CI | *P* value |
|---|---|---|---|---|---|
| | Controls | GS patients | | | |
| Others | 70 (95.7) | 111 (93.3) | 1.0 | | NS |
| C/C-T/T | 3 (4.3) | 8 (6.7) | 1.64 | 0.42-6.46 | |
| | Controls | GBC patients | | | |
| Others | 70 (95.7) | 46 (80.7) | 1.0 | | |
| C/C-T/T | 3 (4.3) | 11 (19.3) | 4.75 | 1.16-19.4 | 0.030 |
| | GS patients | GBC patients | | | |
| Others | 111 (93.3) | 46 (80.7) | 1.0 | | |
| C/C-T/T | 8 (6.7) | 11 (19.3) | 2.77 | 0.85-9.04 | NS |

Others: All remaining combinations combined.

cholelithiasis[28]. The CETP variant has been reported to be associated with higher plasma CETP levels and lower HDL cholesterol levels[29]. For this reason, we examined the association between the genotypic frequencies of the CETP variant and GBC risk. No significant difference in the CETP variant was found between the controls and the patients with GS or between the patients with GS and those with GBC. However, the frequency of the T/T genotype of CETP rs708272 polymorphism was significantly higher in the GBC patients than in the patients with GS (OR = 5.04, $P = 0.012$). Hassanzadeh *et al*[30] reported that the C allele is associated with higher HDL cholesterol levels and lower CETP activity. Since the C allele is the major allele in GS patients[31], the frequency of the C allele or that of the C/C genotype may have been higher in the GS patients than in the GBC patients. Obesity is one of the risk factors for GBC, and an association between obesity and low HDL cholesterol level has been found[32,33]. Since the CETP variant has been associated with lower HDL cholesterol levels[29], the risk of progression from cholelithiasis to GBC may be increased by obesity through an abnormality in the lipid metabolism of HDL cholesterol. However, the difference in the frequency of the T/T genotype may be caused by the small sample size of the controls and the GBC patients in our study, because the 95% CI was quite broad, ranging from 1.43 through 17.8. Further study in which the numbers of cases and controls are increased is needed to demonstrate our finding more clearly.

We also examined the combined effects of the apo B rs693 and CETP rs708272 polymorphisms on the risk of GBC. As shown in Table 4, the frequency of the combined C/C genotype of the apo B rs 693 polymorphism and the T/T genotype of the CETP rs708272 polymorphism was significantly higher in the GBC patients (19.3%) than in the controls (4.3%, $P = 0.030$). The OR for the "at-risk" T/T genotype of the rs708272 polymorphism alone was 5.04 (95% CI: 1.43-17.8), and that for the combined "at-risk" genotype of the C/C and T/T was 4.75 (95% CI: 1.16-19.4); their 95% CIs were widely overlapping. Thus, the T/T genotype of the CETP polymorphism appeared to be a good candidate gene for the genetic factor independently.

The other genetic variants involved in GS formation that we evaluated in this study did not reach conventional levels of statistical significance. As patients with hernia or varicose veins of the legs who had no history of GS or cancer were used as controls in this study, the association may be attenuated. Since both disease incidences might affect the genotype distribution, healthy subjects having no GS or cancer may be needed to detect significant differences between the controls and cases.

The genotypic and allelic frequencies in the capsaicin metabolism-related gene polymorphisms were not significantly different among the three groups. Previous studies indicated that pure capsaicin was a non-mutagenic substance when tested using the Ames test[12,13], but other studies showed that capsaicin and chili extract both acted as tumor promoters, carcinogens, and potential mutagens[14,15]. Capsaicin is catalyzed by CYP 2E1, CYP2C9, and CYP3A4 to reactive species[16-18]. On the basis of this evidence, we examined the effects of the CYP2E1, CYP2C9, and CYP3A4 variants on the GBC risk. No significant differences in the genotypic and allelic frequencies were found between the three groups. Some other constituents of the chili pepper, e.g., aflatoxin contamination, may be associated with the GBC risk rather than capsaicin itself.

Identification of the high-risk group characterized in terms of genetic measures is important for GBC screening studies.The high-risk group also should be a target of chemoprevention and treatment trials. In addition to genetic association studies of apo B, CETP, CYP2C9 and CYP3A4, further genetic association studies of inflammatory (cyclooxygenase and ATP-binding cassette half-transporters, interleukin-1 beta) genes are needed to help illuminate the complex landscape of GBC risk and genetic variations. We also anticipate that in future genetic association studies of GBC, new approaches will facilitate the evaluation of haplotype effects, either for selected polymorphisms that are physically close to each other or for multiple genes in the overall gallbladder carcinogenesis pathway.

This study had the following limitation. Our sample size was small, and the GBC patients had a bias in age distribution with respect to the controls and the GS patients. Thus, our results may have reduced statistical power to detect a possible association between genetic variants and GBC risk, or they may have failed to reflect precisely the genetic risk factors for the development of GBC. Nonetheless, our finding of the apo B rs693 polymorphism was in agreement with the result of the Indian study[19]. To the best of our knowledge, the present study is the first to demonstrate that the T/T genotype of the CETP rs708272 polymorphism was associated with an increased risk of GBC. An additional study including a greater number of controls and cases is required to clarify the association between these genetic factors and the GBC risk.

In conclusion, the C/C genotype of the apo B rs693 polymorphism and the T/T genotype of the CETP

rs708272 polymorphism were associated with increased risk of GBC in Chilean women. While our findings require further confirmation, they provide evidence that the apo B and CETP genes are associated with a higher risk of GBC in Chilean women.

## COMMENTS

### Background

Gallbladder cancer (GBC) is the most common type of biliary tract cancer which results from a complex interplay of genetic and environmental risk factors, like other common multifactorial diseases such as cardiovascular disease, diabetes mellitus and autoimmune disease. Whether genetic variants involved in gallstone formation and capsaicin metabolism affect the risk of GBC in Chilean women is unknown.

### Research frontiers

In this study, the frequency of the cholesteryl ester transfer protein (CETP) rs708272 T/T genotype was significantly higher in the GBC patients than in the gallstone patients. This is the first analysis of the association between genetic predisposition and GBC risk in Chilean women.

### Innovations and breakthroughs

Variants of gallstone formation-related genes such as apolipoprotein B and CETP were associated with an increased risk of GBC in Chilean women. However, capsaicin metabolism-related gene variants were not associated with GBC risk. To clarify the role of genetic predisposition in the development of GBC, we may have to pay more attention to other genes such as inflammatory genes.

### Applications

The apolipoprotein B rs693 T/T and CETP rs708272 T/T genotypes can be used as biomarkers for selecting patients from the group of individuals at high risk for GBC in Chile. Identifying such susceptibility polymorphisms may lead to the development of tests that allow more focused follow-ups of high-risk groups.

### Terminology

CETP is a protein that facilitates the exchange of cholesteryl esters for triglycerides between high-density lipoproteins (HDL) and triglyceride-rich lipoproteins. Therefore, CETP has a central role in the metabolism of both of these types of lipoproteins. Although the C/C genotype was associated with higher plasma CETP and lower HDL cholesterol levels, there is no consistent result regarding the role of the C/C genotype in the development of gallstones.

### Peer review

This study provided some useful data on genetic predisposition and risk of GBC. Methods used in this study are generally reliable, the results are reasonable and convincing. The manuscript is also well written. Authors also pointed out the shortcoming of this study-small sample size.

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